FACTORS UNDERLYING HETEROGENEOUS INDIVIDUAL GROWTH IN *Macrobrachium rosenbergii* AND THE EFFECT OF SIZE HETEROGENEITY ON ECONOMIC FEASIBILITY OF FARMING IN KUTTANAD (KERALA)

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By

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JANUARY- 2002

Dedicated to My Beloved Parents

DECLARATION

I, Ranjeet K, do hereby declare that the thesis entitled "**Factors underlying heterogeneous individual growth in *Macrobrachium rosenbergii* and the effect of size heterogeneity on economic feasibility of farming in Kuttanad (Kerala)**" is a genuine record of research work done by me under the supervision of **Dr. B. Madhusoodana Kurup**, Professor, School of Industrial Fisheries, Cochin University of Science and Technology and has not been previously, formed the basis for the award of any degree, diploma associateship, fellowship or other similar title of any University or Institution.

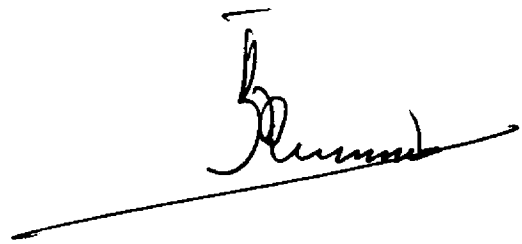
Cochin 682 016,

January 2002.

Ranjeet, K

CERTIFICATE

This is to certify that this thesis entitled "**Factors underlying heterogeneous individual growth in *Macrobrachium rosenbergii* and the effect of size heterogeneity on economic feasibility of farming in Kuttanad (Kerala)**" is an authentic record of research work carried out by Shri. **Ranjeet, K.** under my supervision and guidance in the **School of Industrial Fisheries, Cochin University of Science and Technology** in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** and no part thereof has been submitted for any other degree.



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Ranjeet, K.

Contents

Abbreviations

APD	Average Percent Difference
BEP	Break even price
BEPR	Break even Production
Em	Empty vacuole
ER	Endoplasmic reticulum
FRP	Fibre glass Reinforced Plastic
HIG	Heterogeneous Individual Growth
MT	Microtubules
NR	Net profit
PCR	Polymerase Chain Reaction
PG	Pigment granules
RAPD	Random Amplified Polymorphic DNA
RBD	Random Block Design
SBC	Strong Blue Clawed male
SBF	Strong Blue Clawed female
SF	Small Female
SM	Small Male
SOC	Strong Orange Clawed male
TC	Total Cost
TOF	Strong Orange Clawed female
t-SOC	pre-Transforming Strong Orange Clawed male
TVC	Total variable cost
WBC	Weak Blue Clawed male
WBF	Weak Blue Clawed female
WOC	Weak Orange Clawed male
WOF	Weak Orange Clawed female
Z	Zymogen granule

Section I

General Introduction

General Introduction

1. Background of the study

Aquaculture, the farming of aquatic animals and plants has turned out to be an important industry worldwide. Aquaculture production systems used across the world differ widely depending on the species being cultured and on the geographical location and socio-economic context. Crustacean aquaculture in India has recently been facing severe crisis owing to massive set backs suffered by the shrimp culture industry as rampant out break of viral diseases still persist and also due to the imposition of legal regimes such as Coastal Regulation Zone Act etc. Mismanagement by the farmers, due to noncompliance of prescribed standards of aquaculture, had been attributed as the reason for aggravating the present day crisis. The pursuit for an alternate eco-friendly and sustainable aquaculture has led to the recognition of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man), with the trade name Scampi, as the prime candidate species for freshwater grow-outs. *M. rosenbergii*, has been long recognized as one of the potential aquaculture candidate species for freshwater bodies of different parts of Indo-pacific regions. Fairly good knowledge on the biology, faster growth under captive conditions, bigger size it could attain, greater disease resistance and good demand in both domestic and export markets have made it a prime species for culture in the freshwater grow-outs of India. Although resurgence in the farming of this species has been noticed in recent years all over India with the availability become a reality, the fallow water bodies have not been fully utilized for raising this foreign exchange raising commodity. Kerala is endowed with large freshwater resources in the form of 3000 ha of

tanks and ponds, 30,000 ha of reservoir, 85,000 ha of rivers and 55,000 ha of rice field in Kuttanad and 30,000 ha of rice fields in Kole lands of Trichur. Although paddy cultivation in rice fields has been practicing on a regular basis, the successful integration of scampi in the rice fields of Kuttanad had infact evoked much response among farmers of Kuttanad as a means for improving their economic status. During the past decade, there has been a phenomenal increase in the cost of rice production in Kuttanad could be discernible. The paddy farming has, therefore, become less attractive and due to the diminishing returns, there is a strong tendency among the farmers to abandon rice cultivation and are in the look out for a meaningful utilization of these rice fields. Integration of paddy with prawn/fish has turned out to be the only viable alternative to effectively utilize the vast expanse of fertile derelict water bodies available in Kuttanad.

Development and sustenance of freshwater prawn farming based on *M. rosenbergii* on a commercial scale has now become widespread, especially in South-East Asian countries. However, in India, freshwater prawn culture has not gained much progress until recently. Recent set backs experienced in land based agriculture in Kerala owing to the prevailing socio-economic conditions have further tempted many farmers to switch over to the farming of 'scampi' in derelict water bodies and fallow polders by resorting to either monoculture of this species or polyculture in combination with fresh water fishes. The intricate coconut-garden channels and homestead ponds, which are available in plenty in Kerala also provide conducive grow-out

environment for successful farming operations. Success of freshwater prawn farming, in general, depends greatly on the availability of good quality seeds and nutritionally balanced feed with low FCR, which offer superior growth performance within the stipulated period.

Basically three types of natural grow-outs of *M. rosenbergii* are available in Kuttanad such as 'polders', which are utilized for rice culture mostly only once in an year the 'homestead ponds' and 'coconut garden channels'. Some of the paddy fields are kept fallow, where paddy is not raised in any of the seasons. The extent of the polders generally varies from 0.5 to 100 hectares. Homestead ponds are comparatively smaller and their area ranges from 0.01 to 0.2 hectares. The 'coconut garden channels', on the other hand, usually have a water spread in the range 0.2 to 7 hectares. Paddy cultivation and farming of scampi are carried out on a rotation basis in some of the polders and this water body accounts for 65-75% of scampi farming in Kuttanad, followed by coconut garden channel (15-25%) and homestead ponds (10-15%) (Kurup and Ranjeet, 2001). Agriculture and fisheries provide the main source of income to the people of Kuttanad. *M. rosenbergii*, being a native of this region, is gaining much attention as a candidate species for aquaculture of this region. Despite the fact that it is the mainstay in the capture fisheries of this region over the past four decades, it is now emerging as one of the prime candidate species for freshwater farming in this part of the country. Of the total estimated 5000 ha which are currently being utilised for freshwater prawn farming in Kuttanad (Kurup and Ranjeet, 2001) scampi farming is undertaken in 250 ha under monoculture

system. while in 4750 ha *M. rosenbergii* is raised along with Indian major carps and exotic carps. Of the total farming area in Kuttanad. 4700 ha belong to polders, while 160 ha are of coconut garden channel and 70 ha are Homestead ponds (Kurup and Ranjeet, 2001).

M. rosenbergii is known to exhibit a complex social organizational hierarchy (Ra'anan and Cohen, 1984), comprising morphologically distinct dominant, sub dominant and subordinate animals. Predominance of a definite social hierarchy among the male morphotypes inflict the differential growth pattern within its population. Thus in sexually matured single or multiaged population of *M. rosenbergii*, the males exhibit remarkable heterogeneous individual growth, which allows them to be distinguished as different male morphotypes. On the contrary, the size distribution of female population is almost homogeneous. The three male morphotypes so differentiated are Small Males (SM), Orange Clawed Male (OC) and Blue Clawed male (BC). These morphotypes represent three developmental stages of male maturation pathway and are known to undergo transformation from SM → OC → BC (Ra'anan and Cohen, 1985; Karplus *et al.*, 1992a; Kurup *et al.*, 1996). SM which occupies the initial stage of developmental pathway (Cohen *et al.*, 1981) have translucent body and second cheliped and are subordinate, not territorial in social behavior. OC are subdominant and represent a stage of high somatic growth (Ra'anan, 1982). Depending on the spination and claw colour, the OC morphotype has three different transitional stages, which represent three distinct stages in the transformation pathway. The transitional stages of OC are Weak Orange Clawed

male (WOC). Strong Orange Clawed male (SOC) and transforming Strong Orange Clawed male (t-SOC) males. BC represents the terminal stage in the morphotypic transformation pathway and is characterized by thick and dark-blue claws. The somatic growth at this stage is remarkably low except for the growth of second cheliped. The long chelae and distinct blue colour help it to create a territory of its own. Weak Blue Clawed male (WBC) forms one of the transitional stages of BC (Harikrishnan and Kurup, 1997). The terminal morphotypic stage of BC with relatively small body size in terms of carapace length and body weight disproportionate with claw length is termed as Old Blue Clawed male (OBC). This complex social structure that is basically confined to males of *M. rosenbergii* is termed "Heterogeneous Individual Growth" (HIG). HIG is one of the limiting factors for uniform growth and development of *M. rosenbergii* in the grow-out system. The wide size disparity among the males of the cultured stock and subsequent skewness of their weight distribution, appear to be the most commercial disadvantage of this species.

In grow-outs, generally, 50% of the male population comprises of the small males having a weight range of 5-20 gm, while orange clawed males form about 40% which is characterized with wide variation in weight groups from 30-180 gm, whereas blue clawed males form only 10% which invariably represent the highest weight class reaching up to 250 gm. Hence, the economic viability of farming of this species is profoundly influenced by relative proportion of orange clawed and blue clawed male morphotypes and their allied intermediary stages in the harvested population (Kurup *et al.*, 1996). In general,

the heterogeneous growth shown by the males of *M. rosenbergii* has been associated with: (a) Intrinsic factors such as genetic differences, hatching order or age of metamorphosis (Smith *et al.*, 1978., Sandifer and Smith, 1979), (b) Environmental factors giving rise to competitive situation in cases of limited resources such as space and food (Magnuson, 1962), and (c) Social factors such as position within the size hierarchy, social status and territoriality (Symons, 1972). Most of the studies pertaining to the size heterogeneity in grow-outs have concentrated on social interactions of various morphotypes since it was assumed to be the major factor affecting the differential growth. Among the various intrinsic and extrinsic factors associated with the culture of *M. rosenbergii*, differential growth shown by the male morphotypes appeared as a serious threat to the farmers incurring heavy economic loss. Though the exact cause of size heterogeneity among male morphotypes has not been clearly understood, it could be said that HIG is a cumulative effect of both intrinsic as well as extrinsic factors (Ra'anan and Cohen, 1985). The magnitude of this differential growth is greatly dependent on the management measures that a farmer adopts in his farm. It is now considered in general that one may not be able to deprive this phenomenon from the culture system unless otherwise innovative management strategies are adopted from pre-stocking stage up to harvest. It may, therefore, be concluded that for an economically viable and sustainable aquaculture of fresh water prawn, it is utmost important that the differential growth among the culture stock shall be kept at minimum levels and reciprocally the marketable yield structure can be improved.

Intrinsic factors governing size heterogeneity include the inborn traits associated with ontogenesis along with biological and genetic differentiation. Since all these factors become evident from the larval stage itself, HIG could be traced from the embryonic development phase occurring in the egg mass of *M. rosenbergii* (Malecha *et al.*, 1981a). Similar works linking the embryonic development to the size heterogeneity in different species of *Macrobrachium* have been investigated and the size of egg, utilization of yolk, hatching order, hatching intensity, etc., have been correlated with the phenomenon of differential growth (Kulesh and Guiguinak, 1993). However, very little attention has been given to characterize the intrinsic factors associated with size heterogeneity. One of the most important tools to unravel the mystery of differential growth is by resorting to biochemical and genetical investigations. While there was no attempt to address the genetical basis of size heterogeneity, the proximate composition of various morphotypes have been studied (Sureshkumar and Kurup, 1998a). Success of *M. rosenbergii* culture depends on a number of factors such as standardisation of stocking density and adoption of appropriate pre-stocking and post-stocking management measures. The role of various management strategies useful for increasing the production and mean weight of prawns has not been given much attention till date. Management approaches basically designed to minimize HIG have concentrated on selective harvesting or size grading of pond populations of prawns (Malecha *et al.*, 1981b; Karplus *et* al., 1986a and b, 1987 and D'Abramo *et al.*, 1991). Though management procedures on the whole are targeted to reduce the size disparity prevailing among male morphotypes, due emphasis also needs to be given to find

out ways and means for increasing the survival rate of prawns, reduce the additional expenditure on operational cost and to increase the percentage of marketable prawns in the harvested population. Against this background, a detailed investigation was carried out to unravel the factors associated with size heterogeneity among male morphotypes of *M. rosenbergii* and also to enumerate strategies to reduce the differential growth seen in male population. The results so obtained would be useful in improving the net yield and income from the culture of *M. rosenbergii* in Kuttanad.

The objectives of the present study can be outlined as follows:

(1) To unravel the factors determining the size heterogeneity among male morphotypes of *M. rosenbergii* by resorting to biochemical, genetical and ultrastructural characterization studies.

(2) To assess the extent of size heterogeneity problems in the cultured stock of *M. rosenbergii* in Kuttanad and to develop an eco-friendly, economically sustainable and scientific culture practice incorporating innovative management strategies to reduce the size disparity and to reciprocally improve the marketable yield and income from scampi farming in Kuttanad.

Results of the present study are presented in twelve chapters, which are organized under five sections. A general introduction to the topic is provided in the first section and a brief review of the most relevant literature was also made in this section.

Second section consists of a single chapter that deals with the role of ontogenesis on the size heterogeneity in *M. rosenbergii*. The effect of hatching order and hatching intensity on the growth and metamorphosis of larvae were investigated and the results are presented in Chapter 1. Third section deals with the biochemical characterization of male morphotypes of *M. rosenbergii* and which is presented in chapters 2 to 4. Chapter 2 investigates factors associated with differences in protein synthesis, while chapter 3 emphasizes protein characterization studies as an index for heterogeneous individual growth. Chapter 4 brings out the mechanism of pigment migration and associated colour variation in the second cheliped of *M. rosenbergii* through ultrastructural studies.

Fourth section deals with genetical characterization of male morphotypes of *M. rosenbergii* and comprises two chapters. Chapter 5 brings out the allozymal variations among the male morphotypes, while chapter 6 deals with DNA characterization of *M. rosenbergii* through Random Amplified Polymorphic DNA (RAPD) studies.

Section five consists of six chapters and deals with innovative management strategies advocated for improving the economic viability of *M. rosenbergii* farming in Kuttanad. Chapter 7 embodies the results of standardisation of stocking density in two natural grow-outs of Kuttanad- polders and coconut garden channels, while chapter 8 addresses the importance of a nursery phase for scampi farming and brings out the advantages of a two-phase nursery system for the successful farming of *M. rosenbergii* in Kuttanad. The necessity of incorporation of various pre-stocking management practices to

increase yield and income from *M. rosenbergii* farming in natural grow-outs of Kuttanad is discussed in Chapter 9. Chapter 10 highlights the role of artificial substrate in increasing the survival rate and also envisages the adoption of tiers of net as additional shelters in the polders of Kuttanad. Chapter 11 underlines the necessity of a partial harvest and also explores the possibility of modified cull harvesting strategy through seining in a low lying waterlogged ecosystem like Kuttanad as a means for increasing the marketable yield structure. The role of a steady market structure and micro level economic analysis of the various management strategies in the farming of *M. rosenbergii* is discussed in Chapter 7 to 11 are summarised in Chapter 12. This is followed by summary and references.

2. Physiography of Kuttanad

The area known as Kuttanad which supports a population of one million is a low-lying region extending over 900 Km covering Kottayam, Alleppey and Pathanamthitta districts. This is a deltaic formation of four river systems draining into the Vembanad lake. Much of the area is below the sea level. An intricate polder system has been constructed over the years for agricultural purposes. The whole Kuttanad area is divided into 6 regions namely Vaikom, Upper Kuttanad, Lower Kuttanad, Kayal lands, Purakkad and North Kuttanad (KWBS, 1990). No clear-cut demarcation of the above 6 zones exists at present. However, qualitative and impressionate distinction has been made (Fig. 1). Kayal lands are the most recently reclaimed, low-lying and poorly drained areas, whereas, the Upper Kuttanad includes elevated area. Purakkad region is characterised by of black peaty soils, which possess the characteristic

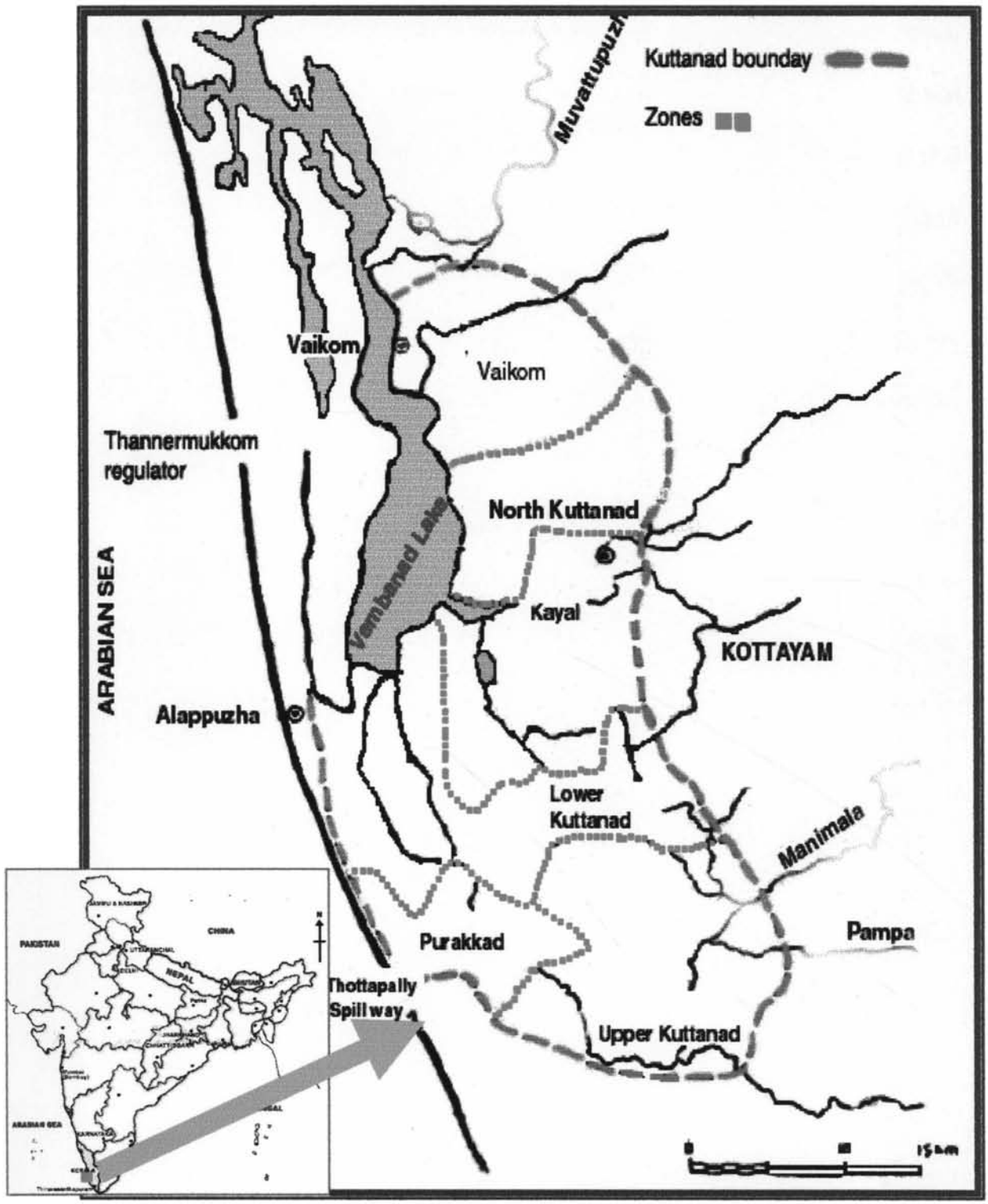


Fig. 1 Map showing the physiography and agroeconomic zones of Kuttanad

of once submerged forests. The region between Upper Kuttanad and Kayal lands is delineated as Lower Kuttanad. Upper Kuttanad, Lower Kuttanad and Kayal lands cut across these regions and are found in pockets in the South-West and Northern part of Kuttanad. North Kuttanad is located North-east of Vembanad lake and a major portion of it falls under Kottayam district. Total area of Kuttanad region is estimated at 1,10,000 ha which comprises of 28% dry lands, 60% wet lands and 12% of other water bodies such as lakes, rivers, channels etc. Wetlands in Kuttanad are mainly used for paddy cultivation with a total extend of 54,000 ha comprising of 'Karapadam' (38,500 ha), 'Kayal lands', which are areas reclaimed from Vembanad lake (9846 ha) and 'Kari lands' which have a sort of high organic content and low pH (6600 ha). Of the total water spread area covered by Kuttanad major portion (60-65%) comes under Alleppey district, 25-30% under Kottayam district and rest under Pathanamthitta district.

Review of literature

The males of *Macrobrachium rosenbergii* in culture systems not only exhibit differential growth but also exhibit variations in morphological features (Fujimura and Okamoto, 1972; Smith *et al.*, 1978; Brody *et al.*, 1980; Malecha *et al.*, 1984). This variability was found to be associated with morphotypic differentiation (Cohen *et al.*, 1981). Adult male population can be differentiated into three morphologically distinguishable morphotypes viz., Small males (SM), Orange clawed males (OC) and Blue clawed males (BC) representing three phases in the developmental pathway (Brody *et al.*, 1980; Cohen *et al.*, 1981; Kuris *et al.*, 1987). The above male morphotypes differ from each other morphologically, anatomically, physiologically and the hierarchy among them are closely associated with social roles and reproductive behaviors (Telecky, 1984; Sagi, 1984; Sagi and Ra'anan, 1988; Barki *et al.*, 1991; Karplus *et al.*, 1990 , 1992 a and b). Two transitional stage of OC viz., weak orange clawed males (WOC) and pre-transforming strong orange clawed males (t-SOC) are also distinguishable and therefore, the fully differentiated OC are known as strong orange clawed males (SOC) (Sagi and Ra'anan, 1988). Harikrishnan and Kurup (1997) brought out the heterogeneous nature of BC population consisting of weak blue-clawed males (WBC) as well as strong blue clawed males (SBC). BC with relatively smaller body size in terms of carapace length and body weight disproportionate with claw length is differentiated as old blue clawed males (OBC) (Sagi and Ra'anan, 1988). Sureshkumar and Kurup (1996) and Kurup (1997) studied the length-weight relationship of different male morphotypes of *M. rosenbergii*. Female population in culture system was known to be rather

homogeneous (Cohen *et al.*, 1981; Karplus *et al.*, 1987) and does not have perceptible size variation. However, Harikrishnan (1997), Harikrishnan and Kurup (1997) and Harikrishnan *et al* (1998) distinguished the female morphotypes in the natural population and characterized them allometrically.

Though morphotypic differentiation could be established in the grow-out and natural population of *M. rosenbergii* (Cohen *et al.*, 1981; Ra'anan and Cohen, 1985; Sagi and Ra'anan, 1988; Harikrishnan and Kurup, 1997), however, no concerted attempts have so far been made to bring out the factors underlying size disparity among the male morphotypes of *M. rosenbergii*. The differences in fecundity of *Macrobrachium rosenbergii* has been reported to range from 60,000 to 1,30,000 by several authors (Ling, 1969; Jinadasa, 1985; New and Singholka, 1985; Patra, 1976; Sureshkumar and Kurup, 1998b). The difference in the fecundity has been attributed to the size of the berried female (Rao, 1965, Ang and Kok., 1991, Shakuntala, 1977, Manesveta *et al.*, 1994). Intraspecific variations in egg size in crustaceans have been recognized as geographic clines, and their ecological significance was investigated in connection with continuously changing environmental factors such as water temperature (Patel and Crisp, 1960; Efford, 1969; Nishino, 1980). Lindley (1997) reported that the incubation period in crustaceans is directly dependant on the yolk contents in the egg. Mashiko (1983, 1986, 1990) however, found that large and small eggs in a single clutch gave birth to large and small zoeal larvae. Kulesh and Guiguinyak (1993) have pointed out that quantitative parameters of growth and reproduction in prawns are directly related to heterogeneity of certain individuals in a sample population. Mashiko (1987) registered that the duration

of embryogenesis in fresh water prawns at steady temperature is directly proportional to egg volume, i.e. the larger the egg volume, the longer females carry their eggs. Differential pattern of hatching seen among Palaemonid prawns has been well documented by Davis (1963), Yeh and Rouse (1994), Anson and Rouse (1994) and the resulting difference in growth pattern for the larvae were traced by Tayamen and Brown (1999), Kulesh and Guiguinyak (1993). Barnes (1965), Pandian (1970) and Pandian and Katre (1972) have worked out the dynamics and energy budget involved during hatching and corresponding changes in larval metamorphosis. Sureshkumar and Kurup (1998 a and b) brought out the differences in the reproductive capability of different male morphotypes and also established an index on fecundity for various female morphotypes. Development and growth heterogeneity in oriental prawn *M. nipponense* during ontogenesis was studied by Kulesh and Guiguinyak (1993). The role of differential hatching order and hatching intensity in determining the extent of HIG among *M. rosenbergii* was however brought out by Ranjeet and Kurup (1999).

Growth in animals has been attributed to change in either length or weight or increase of dry weight body mass with reference to time (Buckley, 1979). Somatic growth in animals has been directly related to the anabolism associated with protein synthesis (Trudier, 1978). Significant difference in muscle protein, DNA and RNA can be taken as an index of high cellular activity (Lemmens, 1995). Anger and Hirche (1990) who reported that an increase of DNA could well be taken as an indicator of faster growth of fishes and crustaceans. Amino acid composition in collagen, muscle tissue of *M.*

rosenbergii, *Penaeus japonicus* and *Penaeus* monodon have been studied by Nip *et al.* (1981), Sarac *et al.* (1994) and Fang *et al.* (1992). No attempts have so far been made to relate the changes in the biochemical composition to the differential growth seen among male morphotypes of *M. rosenbergii*. Sherief *et al.* (1992) studied the biochemical composition of pond reared *M. rosenbergii* giving due emphasis to fast growing bulls and stunted runts. An increase in the protein, glycogen and lipid contents was observed in the testes and ovary of *M. kistnensis*. Joseph *et al.* (1991) studied the variations in the proximate composition of *M. idella* from three different biotopes of Kerala and observed that there exist no significant variations in protein and fat contents among them. Fatty acid and amino acid composition of eggs, muscle and midgut glands of freshwater prawns, *M. rosenbergii* raised in fertilized and unfertilised ponds were studied by Tidwell *et al* (1998a), while that for developing larvae were estimated by Roustaian *et al* (2000). Individual heterogeneity levels and relative growth performance in fishes have been studied by Appleyard *et al.* (2001). Relationship between growth rate, RNA and DNA contents in *P. indicus* were studied by Thomas and Diwan (1990, 1993), while Nip *et al* (1981) brought out the amino acid profile for the insoluble collagen in *M. rosenbergii*. Muscle protein characterization through fractionisation and quantitative estimation of various sub units has been documented (Kimura and Tanaka, 1986; Kye *et al.*, 1988; Mizuta *et al*, 1992). While resorting to tool of electrophoresis Sriraman *et al.* (1995) An *et al.* (1988) investigated the differences in protein banding nature of muscle tissue of *M. rosenbergii*.

Structural changes are the visual proof for the morphotypic differentiation in *M. rosenbergii*. Although the changing colouring pattern of the second cheliped in adult males have been reported (Govind and Pearce, 1993), ultrastructural modifications involved in the development and aggregation of pigments in chromatophore, especially that pertaining to *M. rosenbergii* is apparently lacking. It is well known that the crustacean chromatophore includes a stellate pigment-containing cells along with spatially fixed tubes through which pigment granules move either centrifugally or centripetally (McNamara, 1981a). The origin and development of these multicellular pigmentory effectors, however, remains unestablished. Earlier attempts were made to unravel the mechanism of colour change by studying the chromatophoric pattern in crabs (Quackenbush, 1980), other palaemonids (McNamara, 1979), post larvae of *M. rosenbergii* (Hiramatsu *et al*, 1985) and embryos and adults of *M. olfersii* (McNamara, 1979 and McNamara and Taylor, 1987), however, the phenomenon of changing colour pattern of the claw commensurating with the transformation of morphotypes of *M. rosenbergii* has not yet been addressed so far. Lambert and Fingerman (1977, 1978) proposed a model for the action of red-pigment-concentrating-hormone (RPCH) and red-pigment-dispersing-hormone (RPDH) in *Palaemonetes spp.* The former was mediated through the action of cAMP while the latter acts via Ca^{2+} influx. Ultrastructural studies have revealed the presence of well-developed micro-tubular and microfilamentous systems within crustaceans (Elofsson and Kauri, 1971; Robinson and Charlton, 1973; McNamara and Taylor, 1987). Though many studies have focused on the possible relationships between microtubules, microfilament and pigment

migration (Robinson and Charlton, 1973; McNamara and Sesso, 1982), the micro-anatomy of the crustacean chromatosome is still not well known and data on structural rearrangements associated with pigment movements are lacking in particular. Robinson and Charlton (1973) who stated that alternately, microtubules by undergoing reversible cycles of polymerisation and depolymerisation might aid in pigment dispersion and pigment concentration respectively. Role of pigment dispersing hormones and their chemistry has been elucidated by Rao and Riehm (1989) and Nakamura (1978), while the pigment dispersing pattern in various crustacean have been reported by Miyawaki and Taketomi (1984), McNamara (1979, 1981a), Klove (1995) and McNamara and Taylor (1987).

The population genetic structure of only a few species belonging to the genus of Caridean prawn *Macrobrachium* has been studied so far. Basically, the genetic difference within a species has been assessed based on their geographical distribution (Benzie *et al.*, 1992; Berglund and Lagercrantz, 1983; Koh *et al.*, 1999; Hedgecock *et al.*, 1979; Duda and Palumbi, 1999; Velez *et al.*, 1999) or for breeding experiments (Garcia and Benzie, 1995; Malecha, 1983; Maclean and Penman, 1990), no concerted attempts have so far been made to link phenotypic difference to genetical variations through allozymal or DNA sequencing techniques. Although sufficient data on the electrophoretic and allozymal variations among the members of this genus has been documented (Sriraman *et al.*, 1995; Chow and Fugio, 1985 a and b; Hedgecock *et al.*, 1979; Malecha *et al.*, 1983; Heath *et al.*, 2002), very scant information is available on their genetic differences especially from the molecular level. Genetic variability

data have proved to be useful in identifying genetic compatibility among populations of a species or between species in hybridization programme. Though sufficient data on the population structure of penaeid prawns are available (Benzie *et al.*, 1992; Benzie, 2000), only very few reports are available for Palaemonids especially *M. rosenbergii.* This disparity in somatic growth has been ascribed to environmental impacts (Wong and McAndrews, 1990); ontogenic causes (Mashiko, 1983); social hierarchy (Cohen and Ra'anan, 1985) and biogenetic (Malecha *et al*, 1980). The limited works on allozymes pertaining to the geographical variations among populations in *Macrobrachium spp.* were reported by Hedgecock *et al.* (1979) and Wong and McAndrews (1994). Quantification of allozymal variations has been a common successful practice to achieve information on genetic variation (Park and Moran, 1994; Velez *et al*, 1999; Shaklee *et al.*,1990). Among *M. rosenbergii* the amount of electrophoretically detectable genetic variability within local breeding populations appears to be slightly below normal value typically found in other decapods. Nelson and Hedgecock (1980) reported average heterozygosities for 50 decapod species with a mean value of nearly 5.5%. Trudier (1978) reported a mean heterozygosity of 17.1% over 6 populations of *M. ohione.* High allozyme variation and genetic similarity within two populations of penaeid prawns were observed by Velez *et al.* (1999), while that among coral pit crabs were reported by Blecher *et al.* (1999). RAPD and AFLP techniques for the analysis of genetic relationship in two genera of Decapoda were performed by Lu *et al.* (2000), while resorting to restriction endonuclease analysis of mt-DNA, Anitha *et al.* (1997) described the genetic difference between two populations of *P. indicus.*

Farming of *M. rosenbergii* has become popular globally and research and development activities in these lines are still going on mainly aimed at in improving production from grow-outs of this species and also to minimizing the size disparity (New, 1995). Economic success of prawn culture in any locality is governed by the proper selection of stocking density and stocking size (D'Abramo *et al.*, 1989). The available reports suggest that females invariably dominate in grow-outs of *M. rosenbergii* (Smith *et al.*, 1978; 1981; Sandifer and Smith, 1975). The positive correlation between prawn stocking rate and yield and negative correlation between stocking rate and individual weight have previously been noted by Sandifer and Smith (1975), Smith *et al.* (1976), Willis and Berrigan (1977) and Brody *et al.* (1980) for a variety of prawn culture conditions. Effect of density on food intake for fishes is well documented (Petit *et al.*, 2001). Effects of initial stocking density on the appearance of size variation in *M. rosenbergii* juvenile population have been reported by other investigators (Willis and Berrigan, 1977; Sandifer and Smith, 1975; Malecha, 1977). Effect of stocking density on the production and population structure of *M. rosenbergii* either in monoculture (Siddiqui *et al.*, 1997, Kurup *et al.*, 2000), polyculture farms (Sadek and Moreau, 1998, Garcia *et al.*, 2000), laboratory (Lobao *et al.*, 1994), rice field (Janssen *et al.*, 1988) and earthen ponds (D'Abramo *et al.*, 1989; Reddy *et al.*, 1996, Valenti *et al.*, 1993) have been well documented (Daniels *et al*, 1985). Karplus *et al.* (1986a) studied the effect of stocking density on the population structure and weight distribution of *M. rosenbergii* raised in earthen pond, while the production under different levels of stocking densities of *M. rosenbergii* along with fish species have been reported

by Cohen and Ra'anan (1983); Pavel *et al* (1986); Costa-Pierce *et al*.(1984); Wohlfarth *et al.* (1985) and Miltner *et al.* (1983). The performance of larvae of *M. rosenbergii* under different stocking densities have been monitored by many investigators (Sarver *et al.*, 1980; Ra'anan and Cohen, 1984; Willis *et al.*, 1977; Lobao *et al.*, 1994), while the effect of density on survival and production of *M. rosenbergii* been documented by Janssen *et al* (1988), D'Abramo *et al.* (1989); Alekhnovich and Panyushkin (1991) and Perez *et al.* (2000), while the effect of density on population dynamics of crayfish have been investigated by Fidalgo *et al.* (2001).

During the past few decades, considerable attention has been given in developing a threshold grow-out strategy of *M. rosenbergii*. Among the standardisation procedures much attention has been given to rearing the post larvae in nurseries to ensue better survival, higher mean weight and hence higher production from its culture (de Marques, 2000). Most works on prawn nursery rearing deals with intensive indoor nursery systems under temperate regions (Kneale and Wang, 1979; Smith and Sandifer, 1979; Smith *et al.*, 1983). Mulla and Rouse (1986) compared four different techniques of nursery rearing and found that nursery ponds, either in the form of plastic nursery pools or earthen ponds when supplied with additional substrate showed highest net production. Although concerted attempts to standardize the rearing techniques in closed nursery systems have been previously attempted (Smith *et al.*, 1983; Bindu *et al.*, 1999; Arieli and Rappaport, 1982), not much effort have been taken to develop the potential of outdoor nursery system for the farming of *M. rosenbergii*. Another important area, which needs much emphasis as far as optimization of

grow-out technology for *M. rosenbergii* is concerned, is the incorporation of additional substrates to the culture ponds for enhancing survival rate and growth of the prawns. Tidwell *et al.* (1998a, 1999) studied the effect of artificial substrates on the ponds for increasing the production. Cohen *et al.* (1983) reported that adding substrate to ponds allowed for an increase in prawn production of 14%, while Tidwell *et al.* (1998b) assessed the effect of added substrate under temperate conditions and reported that in prawns stocked at relatively low densities, production and average size were increased @ 20 and 23% respectively. Ra'anan *et al.* (1984) reported that substrate was more effective in intensely stocked systems. However, Tidwell *et al.* (1999) added fixed amount of substrate to ponds stocked at different densities and found no significant interactions between stocking density and presence of substrate, though substrate did significantly increase production without decreasing the average weight. Keshavanath *et al.* (2001) reported the increase in net production with inclusion of additional substrates for fishes. Ra'anan *et al.* (1985) used horizontally hung nets as artificial substrates, while Tidwell *et al.* (1999) used horizontal tiers of nets to increase the surface area in pond. Ra'anan *et al.* (1984) reported that yields of marketable prawns were 24% higher in ponds with substrates. The percentage of marketable prawns was 17.7% higher in the treatment with substrates and the overall survival also increased by about 10%. Cohen *et al.* (1983) observed that the introduction of substrates resulted in increase in the total marketable yield from 2,500 kg/ha in six months when grow-out with no added substrates was provided to 2,850 kg/ha within a similar period in the presence of substrates.

One management approach designed to minimize HIG has been the size grading of nursery populations prior to stocking. Karplus *et al* (1986b, 1987) proportionately size graded nursery raised juveniles into two and three size groups. Similar studies on size grading were also attempted by D'Abramo *et al.* (1991) and Daniels and D'Abramo (1994). Daniels *et al.* (1995) proved that size grading produced stocking population that structurally differed from the original and achieved an increase in mean weight and yield at harvest. Grading at stocking affects both mean weight and the population structure. *M. rosenbergii* exhibits territorial behavior (Cohen *et al.*, 1981) and greater energy expenditure due to the disputes for territory and its defense at higher densities may eventually reduce growth rates (Celia and Valenti, 1996). Interactions among individuals from different size subpopulations have been found to affect both growth and survival already during early juvenile stages (Ra'anan, 1982). Different batch grading practices designed to control HIG and, thereby, to reduce the percentage of low value prawns in populations of *M. rosenbergii* harvested from production ponds have been investigated (Karplus *et al.*, 1987). In most cases, this stock manipulation procedure has resulted in substantial increases in mean harvest weight and yield (Daniels and D'Abramo, 1994). Malecha *et al.* (1981a) suggested a multi-staged, rotational stocking and harvesting system, for year round monoculture. Ra'anan and Cohen (1983) divided juvenile prawns, after secondary nursing, into two fractions and stocked the juveniles on the basis of their size. Other studies have evaluated the potential for increasing yields by size grading nursery populations prior to stocking (Karplus *et al.*, 1986b, 1987; Hulata *et al.*, 1990). The diverse pattern of growth at the final harvest among

different sets of adaptive trials denotes the effect of social interactions within prawns under different management practices. The final population density affects mean harvest weight of *M. rosenbergii* (Brody *et al.*, 1980). It seems as if the moult increment of the larger individuals is associated the different growth strategies assumed by the Jumpers and Laggards within a given population at the initial stage of the ontogeny of social structure (Cohen *et al.*, 1981; Ra'anan, 1983).

Various management approaches designed to minimize HIG have concentrated on selective harvesting or size grading of pond population. In temperate climates selective harvest of "market size" prawns prior to a final drain down harvest has been performed (Willis and Berrigan, 1977; Cohen and Ra'anan, 1983). Malecha *et al.* (1981b) proposed a management practice that included pre-harvest size grading and restocking of non-market size prawns after complete harvesting by pond draining. It has been reported by earlier authors that partial harvesting or cull harvesting during the mid of the culture period allows to selectively harvest larger males and low sized undesirable female prawns from the population (Malecha *et al.*, 1981a; Siddiqui *et al.*, 1995; Lin and Boonyaratpalin, 1988). Harvest efficiency for cull harvesting is less when compared with that of selective harvesting (McGinty and Alston, 1993). Siddiqui *et al.* (1995) evaluated the effects of harvesting methods (cull and batch) on population structure, growth, and yield of freshwater prawn, *M. rosenbergii*, cultured at two densities. Economic comparison of stocking and marketing strategies for aquaculture of *M. rosenbergii* has been evaluated by Sandifer *et al.* (1982). Liao *et al.* (1982), Liao and Smith (1983), Robert and Bauer (1978), and

Shang and Fugimura (1977) have assessed cost and returns from *M. rosenbergii* farming under different marketing strategies. Various management strategies comparing the economic influence of pre and post stocking management practices to improve the yield and therefore the profit from *M. rosenbergii* farming has been discussed by Sadek and El Gayar. (1995), Liao and Smith (1982), Avault and Granados (1995), Lacroix *et al.* (1995), Engle (1987), Fitzgerald (1988) and Sastradiwirja (1986). Shang (1982) and Shang *et al.* (1983) reported the economic comparison of freshwater prawn farming under intensive as well as with intergration with agriculture. The net revenue incurred from *M. rosenbergii* culture indicate that freshwater prawn aquaculture has potential to become a source of supplemental income to farmers who are relying directly on paddy for their livelihood (Shang and Fujimura, 1977; Kurup *et al.*, 2000; Shang, 1986 and Lin and Boonyaratpalin, 1988).

Section II

Role of ontogenesis on the size heterogeneity among *M. rosenbergii*

Chapter 1.

Effect of hatching order and hatching intensity on growth and metamorphosis of larvae of *Macrobrachium rosenbergii* (de Man)

1. Introduction:

Most crustaceans carry their eggs in a marsupium, brood pouch, ovisacs or as attached egg masses (Sastry, 1983). Typically, estuarine and marine species possess relatively small or numerous eggs (Magalhaes and Walker, 1988; Jalihal *et al.*, 1993; Collart and Rabelo, 1996) and undergo a prolonged and complex larval history, comprising even up to 13 stages before reaching the juvenile stage. The number of the eggs in a clutch is indicative of the fecundity of a particular species (Periera and Garcia, 1995). The more the number, lesser will be the percentage survival of larvae emerging out of it. The volume of the eggs produced by crustacean species is at least, in part, under genetic control (Raven, 1961). Fecundity of *M. rosenbergii* has been reported by various authors (Ling, 1969; Jinadasa, 1985; New and Singholka, 1985; Patra, 1976; Ang and Kok, 1991; Sureshkumar and Kurup, 1998b), which ranged from 60,000 to 1,30,000 eggs. During the incubation period of 19 days, part of the egg mass may be shed off and a progressive change in colour could be discernible from orange to yellow, grey and finally black before hatching (Ang and Kok, 1991). In the genus *Macrobrachium*, the planktotrophic larvae pass through ten or more discrete zoeal stages (Gore, 1985; Melo and Garcia, 1999), through metamorphosis over a period of 30 days after hatching (Williamson, 1969; Choudhary, 1970, 1971).

The giant freshwater prawn *Macrobrachium rosenbergii* shows a unique feature of differential growth, known as Heterogeneous Individual Growth (HIG). The final harvested population appears highly skewed and result in a disparate population structure. Therefore, feasibility of its culture directly depends on the extent of minimising the size heterogeneity problems. Earlier reports showed direct impact of extrinsic factors such as stocking density, social structure and hierarchical dominance on the HIG (Cohen *et al.* 1981; Daniels *et al.* 1985; Karplus *et al.* 1986a). However, variation in ontogenic process in a single clutch of eggs, such as delay in hatching, difference in rate of metamorphosis, etc., has not so far been paid much attention as cause of HIG. However, the role of such variations in ontogeny in determining the extent of HIG in prawns even before their exposure to other extrinsic factors have to be traced out (Mashiko, 1986). Hatching invariably takes place in the dark and the duration may be from hours to 2-3 days. Should the hatching continue for two days, approximately half of zoea is released during the first night, while during day time the process is discontinued and is completed in the succeeding nights (Kulesh and Alekhnovich, 1982). The performance of larvae, which are hatched out on the successive days remain unknown. Similarly, in *M. rosenbergii* also, a diverse pattern in the order of hatching of eggs was encountered. In general, the hatching can either be completed fully in a single day (full hatched) or in subsequent days (partial hatched) over a period of 2-3 days. Hence, based on the order and day of hatching, larvae can further be obviously divided into two groups, namely, first hatched and later hatched larvae. Similarly, based on the number of larvae produced at each partial hatching, it can

be divided into major and minor. Hence, the larvae may either belong to a first major group or second minor group or vice versa. In the present study, an attempt was made to unravel the fate of larvae originated from a single clutch and also to bring out variations in size, rate of metamorphosis and heterogeneity of larvae produced from different hatching order and intensities in *M. rosenbergii*.

2. Materials and Methods:

Berries of *M. rosenbergii*, after initial quarantine treatments were kept in tanks of 100-litre capacity. Upon hatching the entire clutch of larvae were transferred from the hatching tank. First set of experiments was designed to assess the variations, if any, in the rate of metamorphosis, mean stage and body length progression etc., of larvae produced from full and partial hatching. Partial hatchling from a single clutch was segregated further, as 'first hatched' and 'second hatched' based on the day of hatching. Thus treatments OT1 (complete hatching in a single day), OT2 (partially hatched larvae of first day) and OT3 (partially hatched larvae of second or subsequent days after initial partial hatching) were grouped.

In the second set of experiments the role of hatching intensity on metamorphosis, stage progression and growth was studied. Depending on the hatching intensity, the larvae were maintained as different treatments viz., HT1, HT2, HT3, HT4 and HT5. The larvae, which hatched completely on a single day, were transferred to two tanks under treatment HT1, similarly based on the intensity of the larval hatch from a single clutch duplicate tanks were maintained separately for larvae emerging from first 'major' hatch

Plate 1.1

A Experimental set up of hatchery trails conducted in *M.rosenbergii* at the School of Industrial Fisheries

(HT2), second 'minor' hatch (HT3), first 'minor' hatch (HT4) and second 'major' hatch (HT5) groups respectively.

Both sets of experiments were carried out in oxford blue Fibre Reinforced Plastic (FRP) tanks with a capacity of 400 L following randomised block design (RBD) model. Modified clear water system of larviculture (Ang and Kok, 1991) was followed for the rearing of larvae in all the treatments. Salinity in the tanks was maintained at 12 ± 1 ppt throughout the experimental period and pH was kept in the range of 7-8. All tanks were continuously aerated. Larvae were fed initially with newly hatched artemia nauplii and subsequently weaned towards prawn-egg custard. Water was exchanged @ 50% on a daily basis. Stocking density in all the tanks was maintained at 100 larvae/ litre throughout the cycle. Observations on Ammonia, H_2S and Nitrites were done weekly and maintained within limits (New, 1995). Random samples of 30 larvae were taken daily from each tank and preserved in 4% formalin. Larval stages were identified following Malecha (1983). Samples were observed through a zooming stereomicroscope for identifying stages and observing other morphological features. Individual length of larvae was recorded using a stage micrometer to nearest mm. Moulting frequency was recorded at every 3-days of interval by keeping 20 larvae in 1 litre beaker for 12 hrs and their moults were counted. The experiments were continued till PL-15 stage and thenceforth, were transferred into grow-outs. Regression and analysis of variance of individual length and mean stage of larvae between the treatments were carried out following Mashiko (1987) to find out difference, if any, among treatments. Mean stage, mean length, heterogeneity coefficient (HC) and

coefficient of variation (CV) at an interval of 5 days were calculated (Kulesh and Guiguinyak, 1993) and statistical analysis was done following Snedecor and Cochran (1961).

3. Results

3.1 Hatching order

Results of ANOVA showed that there was no significant difference ($P>0.05$) within the treatments manifesting that mean length of larvae within the treatments did not vary significantly. Mean values of length, coefficient of variation and heterogeneity coefficient of larvae in the three sets of treatments (OT1 to OT3) under differential hatching order are presented in Table 1.1. During the developmental stages of the larvae of *M. rosenbergii* from Zoea I to XI, difference in metamorphosis, growth and Scout PL time with reference to hatching order was observed. Among them, faster metamorphosis and growth in terms of length of larvae were recorded in 'late' hatched batch. Coefficient of variation among the three treatments showed low values in OT3, the size differentiation among the larvae in this treatment was remarkably less. On the other hand, variation in OT2 was found very high. Similarly, higher modal lengths were also shown by OT3 and therefore, the mean modal class in this group was conspicuously higher when compared to other two treatments (OT1 and OT2). Heterogeneity coefficient (HC) also complemented these findings. 'HC' was rather low in OT3 and this would manifest the presence of an even population among the later hatched larvae.

The 'first hatched' larvae (OT2) had a faster initial growth rate till the VI larval stage, thereafter, a slight lag in the stage progression could be observed (Table 1.2). The 'second hatched' larvae (OT3), on the other hand, had an initial lag but during the end of larval metamorphosis, the stage progression became steadfast especially from IX to XI larval stages. Mean length of post larvae emerged from the 'first hatched' larvae were remarkably on a higher side when compared with that of 'second hatched' larvae. Scout-PL time for OT3 was 22 days whereas it prolonged even up to 31 days for the OT1 and OT2. Standard deviation showed higher values on the 11^{th}, 16^{th} and 20^{th} days indicating a slight lagging of metamorphosis during these days that coincided with the appearance of VI, IX and XI stages. Mean length and weight of post larvae released from the early hatched batch was recorded to be 6.4 mm and 6.0 mg respectively and this was higher when compared to that of later hatched batch, the latter had only a length of 5.3mm and weight of 4.8 mg. Moulting frequency did not show and particular pattern in either set of treatments. But it was faster during the initial days of stage transformation in the 'first hatched' batch which appeared to be corroborating with their faster stage progression, however, the moulting intensity was considerably slow during the 11^{th}, 16^{th} and 20^{th} days for all the treatments. At PL-15 stage, prior to stocking, the mean length of the post larvae from the 'second hatched' was 1.68cm which was on the higher side when compared to that of the 'first hatched' (1.46cm). Relationship between mean length and time of metamorphosis among larvae from different hatching orders is depicted in Fig. 1.1. There exists a definite pattern of progression among the three treatments showing faster metamorphosis and

growth in OT2 followed by OT1, whereas, in OT3 metamorphosis and growth were slower. Numerically, their relationship with time can be represented as follows:

$$Y = 1.7785 + 0.2646\ X; \quad (r = 0.9888) \qquad (OT1)$$

$$Y = 0.8022 + 0.2684\ X; \quad (r = 0.9968) \qquad (OT2)$$

$$Y = 1.9308 + 0.3049\ X; \quad (r = 0.9726) \qquad (OT3)$$

The 'b' value was found highest in OT3, which shows that there was faster stage progression and corresponding attainment of high mean length in this treatment.

3.1. Hatching intensity:

In compliance with the hatching order, results of ANOVA for hatching intensity showed no significant difference ($P>0.05$) in the mean length of the larvae within the treatments. Mean values on length, coefficient of variation and heterogeneity coefficient of larvae in the five sets of treatments (HT1 to HT5) under differential hatching intensity are presented in Table 1.3. Mean length of Zoea I of *M. rosenbergii* from Tanks HT1 to HT5 was not uniform and ranged between 1.0 mm to 1.7 mm on the first day, which undoubtedly indicates that the size heterogeneity was evident from one-day-old larvae itself. It is worth noticing the presence of larger sized Zoea I in Tanks HT2 and HT5, which accommodates larvae emerged from 'major hatching'. Their size ranged from 1.20 to 2.01 mm, while in minor cluster (HT3 and HT4) it ranged between 1.08 and 1.92 mm. Coefficient of variation, which expresses the index for diversity from the mean modal length was found higher during the first five days in all the treatments, though their values were higher in T3 and T4. A

diverse pattern of growth could be observed during the initial stages itself in the larvae emerged from a minor hatch. This can well be attributed to the sudden initial spurt in growth of larvae emerged from the minor hatch, which did not follow any definite pattern of growth. On the other hand, larvae originated from major hatch (T2 and T5) showed uniformity in initial growth and consequently their size range was rather homogenous till the end of the 5th day. Heterogeneity coefficient i.e., maximum-to-minimum size ratio for any given stage (Kulesh and Guiguinyak, 1993) recorded in the present study are presented in Table 1.3. Heterogeneous coefficient was more in minor hatched group at the PL stages and this index was glaringly high in respect of first minor hatched group (HT4), which worked out to be 2.63, while it was least in the second major hatch group (HT5) (1.88). It would thus appear that the pattern of heterogeneity among the major hatched larvae did not deviate much from their initial stages. From the results of the modal frequency among the five treatments (Table 1.3), it could be seen that there are possibility of faster and better succession of mean weight in respect of HT1, HT2 and HT5. HT1, HT2 and HT% were thus characterised by faster growth and metamorphosis as well as uniform succession of modal length. On the contrary, in HT4, the modal length class of the final population showed a transitory lag with advancement of stages, indicating possibility of the initiation of size disparity or HIG. Size heterogeneity can further be attributed to the time lag for settling of first post larvae (Scout-PL time) in the minor hatched groups viz., HT3 and HT4.

Pattern of mean stage progression and moulting frequency of larvae from different hatching intensity treatments are given in Table 1.4.

Moulting did not follow a definite pattern in any of the tanks under observation, although hectic moulting was registered in all treatments at the III, VI and XI stages. Among the five treatments, the stage progression in HT5 was fastest and consequently post larval settlement could be observed from the 31st day onwards. Complementary to the results of mean length variations, the stage progression in HT4 was found to be very slow. Patterns of stage progression showed variation among the five treatments. In compliance to this, smaller Zoea in tanks HT3 and HT4 showed rapid transformation to successive larval stages initially when compared to larger ones in tanks HT2 and HT5. In total contrast, larvae of treatments HT3 and HT4 showed a slower rate of progression towards the final stages. As a result, the Scout-PL time extended in the first and second minor hatched groups (34 and 38 days respectively). On the contrary, the larvae from major hatching showed no signs of lagging and consequently had a relatively shorter scout-PL time of 31 days. Irrespective of the treatments a slight lag in stage transformation and diversity in mean length among larvae was more pronounced during the later stages, especially in stages IX and XI. The post larvae settled from the major clutch were slightly larger (11.2 to 12.4 mm) than to their counter parts from minor clutch (9.81 to 10.2 mm). At PL-15 stage the average weight attained by individual larvae were at a rate of 0.048 g for the minor and 0.056 g for major hatched batches.

At the end of the cycle very poor survival rates were recorded in minor hatched larval groups (6%), while a better survival rate was recorded in the first major hatched group (21%). Table 1.4 shows the pattern of dominance of different stages in the five sets of treatments. A lagging in stage

progression could be discernible in first and second minor hatched clutch. This coupled with higher values of coefficient of variation showed a possibility of higher degree of size disparity among minor hatched clutches. Whereas, in major hatched clutch, faster progression of modal stage with low values of coefficient of variation and shorter Scout-PL time confirm lesser extent of HIG among this group. Progression line for mean length of larvae originated from different hatching intensity among the five treatments is shown in Fig. 1.2. The pattern of growth within the five treatments was found to be diverse and their relationship with time can be expressed as follows:

$$Y = 1.655 + 0.3706\ X; \qquad (r = 0.9938) \qquad (HT1)$$

Where Y = length of larvae in mm; X = time from the moment larvae started to metamorphose into successive stages,

$$Y = 1.0939 + 0.3690\ X; \qquad (r = 0.9985) \qquad (HT2)$$

$$Y = 0.6509 + 0.3437\ X; \qquad (r = 0.9951) \qquad (HT3)$$

$$Y = 1.5683 + 0.3140\ X; \qquad (r = 0.9882) \qquad (HT4)$$

$$Y = 1.655 + 0.4099\ X; \qquad (r = 0.9927) \qquad (HT5)$$

Among all the treatments, the b value of HT5 was highest which would manifest the faster stage progression and rapid shift of mean length.

4. Discussion

Intraspecific variations of egg size in crustaceans have been recognised as geographic clines, and their ecological significance was investigated in connection with changing environmental factors such as temperature (Efford, 1969). Kulesh and Guiguinyak (1993) have pointed out that

quantitative parameters of growth and reproduction, in prawns, are directly related to heterogeneity of certain individuals in a sample population. Biology of prawns is associated not only with the fact that during ontogenesis the larvae passes through qualitatively different and substantially lengthy larval stages, but also due to a complex social structure prevailing among the adult population. This is characteristic of *M. rosenbergii*. Although concerted attempts were made in the past for their domestication at the grow-out stage incorporating management measures to reduce heterogeneity, no study have so far been conducted to understand the role of ontogenesis on differential growth (Ra'anan and Cohen, 1985; Karplus *et al*., 1986a). The duration of embryogenesis in fresh water prawns at steady temperature is directly proportional to egg volume, i.e. the larger the egg volume, the longer females carry their eggs (Mashiko, 1987). Thus, it can be inferred that smallest larvae are destined to hatch from smaller eggs first, and the larger larvae will hatch later from larger eggs. Contrary to this, in the present study, the larvae from the minor clutch had a faster initial stage progression when compared to larger larvae from major clutch. Kulesh and Guiguinyak (1993) observed a similar pattern of larval transformation with respect to the egg volume in *Macrobrachium nipponense* wherein, the larvae hatched from the larger eggs had enough yolk to subsist them during their initial stages. As a result, the initial stage advancement in eggs having enormous yolk lagged when compared to larvae from less yolk filled smaller eggs. The results of the present study were complimentary to that of Kulesh and Guiguinyak (1993) on the development and growth heterogeneity in oriental river prawn, *M. nipponense* (de Haan) during ontogenesis. Faster metamorphosis and larger mean

length obtained from the major hatched groups (HT2 and HT5) in the present study can be attributed to the better growth during the initial stages and the resultant larger Zoea larvae. Kulesh and Alekhnovich (1982) showed a better survival ability of subtropical prawn *Macrobrachium nipponense* (de Haan) at early ontogenesis stage especially those hatched from larger eggs. During the early larval stages, a stiff competition for food occurs among the larvae that leads to a greater proportion of larvae to be underfed leading to their weakening. But the larvae (major hatched) emerged from larger eggs have an additional nourishment of yolk during their initial stages as a result they continue to thrive on yolk of the first few days in spite of the struggle for food. This would ultimately increase their chances of survival. The results of the present study show that the larvae from HT2 and HT5 (major hatch) showed relatively less degree of heterogeneity when compared to the treatments on 'minor hatch' groups. It would thus appear that selection of these larvae for stocking would reduce the initial size variation during the nursery stocking. The post larvae (PL-15) from 'major hatching' which could attain larger size have the advantage of reaching adult prawn much earlier due to their faster growth and this would be helpful in enhancing the net yield from the grow-outs of *M. rosenbergii.*

Since, just prior to hatching, a sizable part of energy is expended on embryonic metabolism (Barnes, 1965; Pandian, 1970), larvae released on subsequent nights contain less energy than those released on the first hatching night (Pandian and Katre, 1972). This might be the reason for the slower stage progression in larvae emerged from a major hatch group during initial days, but as and when they started feeding, energy loss is duly

compensated and a shift thenceforth to successive stages could be discernible. On the contrary, Pandian (1970) reported that the crustacean larvae released early have more energy and hence show an initial spurt in growth. Davis (1963) reported that in palaemonids, the larvae hatched out of the eggs through bursting of the outer membrane that occurs through the osmotic swelling of an inner membrane accompanied by the swelling of prolarva through the imbibition of water. Once the yolk content has been exhausted the larvae absorb water from the surrounding medium to its inner membrane through osmotic imbibition and burst the outer membrane. This process helps the larvae to reduce the shock posed by osmotic pressure difference within the egg and outside medium. Hence smaller eggs with less yolk contents hatch first. According to Shakuntala (1977), the egg size of larger females is smaller than those carried by smaller females, but such a phenomenon is absent in *M. rosenbergii* (Sureshkumar, 1998). Mashiko (1990) is of the view that the large and small eggs gave birth to large and small zoeal larvae, respectively, differing in some physiological characteristics and reported that besides the difference in the egg size, the difference in the total amount of egg matter available for one spawning gave rise to the varied clutch sizes among individuals or populations, especially those with similar egg sizes. According to him, the variations in the number of eggs per clutch among the local populations of *M. nipponense* was due to the difference of egg size and fluctuations in the total amount of egg matter produced prior to spawning.

Pandian and Katre (1972) have reported that the larvae released on the second hatching night exhibited a 2% increase in their total body length. Similar observations could be recorded in the present study also, that the mean larval size was remarkably larger in second hatched groups when compared to the first hatched ones. The longer the larvae, the greater is likely to be their oral aperture, and this presumably enables a wider range of food to be consumed at a faster rate by the individual (Blaxter and Hempel, 1963). This may be an adaptation, since these larvae released on the second night contain 10.5% less reserve yolk energy than those hatched on the preceding night (Pandian, 1970). The better growth and metamorphosis recorded in the larvae of second 'major' hatch and later hatched in the present study can be attributed to this. Larvae of *M. idea* released on the first or second hatching nights contain reserve yolk energy of 1.0 or 0.7 cal (Pandian and Katre, 1972). These larvae can use this energy for metabolism and other activities until they consume food and become independent of reserve-yolk-energy. Once the yolk shows depletion in the egg, the chances for the larvae to hatch out are high (Lindley, 1997). In the case of early-hatched larvae, the depletion in yolk allows them to transform faster and attain the successive stages, but since the oral aperture in these larvae could not widen in accordance with the body length, most of the larvae fail to utilise the supplementary feed available in the medium (Davis, 1963). This would lead the larvae unhealthy and consequently mortality among minor hatched groups showed an increase in the present study. Throughout the planktonic stages, feeding regime probably has the greatest influence on metabolic status, especially because rapidly moulting larvae need to utilise

energy quickly (Gore, 1985). According to Lester (1988), hereditary influences the growth of *Penaeus vannamei* and *P. stylirostris* at the beginning of their life cycle. Malecha *et al.* (1981a) are of the view that genetic and environmental factors were probably responsible for the differential growth pattern seen in the cultured population of *M. rosenbergii.* However, heterogeneity in size and weight of the larvae, at any stages of ontogeny, cannot occur due to genetic differences only. It may also be due to the stimulation of growth related to various group effects such as density, metabolites, physiological influences with the group, environmental factors, increasing or reducing genetical heterogeneity, etc., (Efford 1969). Kuris *et al.* (1987) expressed that the principal factor limiting the growth and productivity is the complex social structure precisely seen in males rather than genetical difference. According to Kurup (1996) the dynamics of interaction and transformation of male morphotypes of *M. rosenbergii* (de man) would be a decisive factor governing the size heterogeneity among the male morphotypes in the grow-outs.

Results of the present study reveal that there exists a definite variation in the rate of metamorphosis, stage progression, growth and survival of larvae during ontogenesis, based on the differential hatching order and intensity. It can therefore be concluded that apart from extrinsic factors such as stocking density, physio-chemical parameters prevailing in the grow-outs, social behaviour, ontogeny also contributes significantly towards the development of size disparity in the population of *M. rosenbergii.*

Table 1.1 Mean values of length, coefficient of variation and heterogeneity coefficient of *M. rosenbergii* larvae under differential hatching order

Treatments	Days	Sample number	Min length (mm)	Max length (mm)	Heterogeneity coefficient (HC)	Coefficient of variation (C.V) (%)	Modal length (Mo) (mm)	Percentage of modal class (%)
Full hatched	1 - 5	125	1.40	2.80	2.00	26.28	1.85	21
(OT1)	5 - 10	125	2.90	4.80	1.66	11.60	3.25	28.5
	10 - 15	125	3.39	6.10	1.80	5.37	5.25	14.6
	15 - 20	100	4.46	7.20	1.61	4.92	6.29	15.5
	20 - 25	75	4.63	9.30	2.01	364.00	7.30	18.6
	25 - 30	75	5.77	10.80	1.87	3.11	8.81	21.5
First hatched larvae	1 - 5	150	1.20	3.00	2.50	21.77	2.15	18.5
(OT2)	5 - 10	150	2.30	3.70	1.61	20.00	2.80	22.5
	10 - 15	125	3.15	5.74	1.82	15.26	4.65	12.6
	15 - 20	100	4.35	7.10	1.63	6.06	5.12	26.9
	20 - 25	75	5.60	9.40	1.68	4.35	8.35	17.3
	25 - 30	75	6.30	10.90	1.73	4.31	8.70	15.1
Second hatched larvae	1 - 5	150	1.20	3.40	2.83	24.97	2.40	25.6
(OT3)	5 - 10	150	3.40	5.80	1.71	12.32	4.90	33.9
	10 - 15	125	5.00	6.85	1.37	5.10	5.85	15.4
	15 - 20	100	6.16	7.80	1.27	4.17	7.00	11.5
	20 - 25	75	6.42	10.40	1.62	3.85	8.90	41.5

Table 1.2. Development, mean stage progression and moulting frequency in larvae of *M. rosenbergii* under differential hatching order

Duration of larval development (days)	OT1		OT2		OT3	
	A	B	A	B	A	B
1	I	1.4	I	1.2	I	1.4
2	II	1.6	II	1.8	II	1.8
3	II		II		II	-
4	III	2.7+	III	2.55+	III	3+
5	III		IV	3.2	III	-
6	IV	4.1	IV		IV	4.4
7	IV		IV		IV	-
8	V	4.82+	V	4.7	V	5.8
9	V		V		V	-
10	V		V		VI	6+
11	VI	5.8	V		VI	-
12	VI		VI	5.54+	VII	6.8
13	VI		VI		VII	-
14	VI		VI		VIII	6.9
15	VI		VI		VIII	-
16	VII	6.60+	VII	6.3	IX	7.5+
17	VII		VII		IX	-
18	VIII	7.2	VII		X	8.5
19	VIII		VIII	7.0+	X	-
20	VIII		VIII		XI	9.1+
21	IX	8.305+	IX	8.13+	XI	-
22	IX		IX		PL	11.2
23	IX		IX			
24	IX		IX			
25	IX		X	9.2		
26	X	8.8	X			
27	X		X			
28	XI	9.56+	XI	9.99+		
29	XI	-	XI			
30	XI	-	XI	-		
31	PL	10.4	PL	10.5		
32						
33						

A - Zoea stage; B + - indicates moulting, numerical values denote larval length (mm); PL- post larvae

Expansion of OT1, OT2 and OT3 are given in Table 1.1

Table 1.3 Mean values of length, coefficient of variation and heterogeneity coefficient of *M. rosenbergii* larvae under differential hatching intensity

Treatments	Days	Sample number	Min length (mm)	Max length (mm)	Heterogeneity coefficient (HC)	Coefficient of variation (C.V) (%)	Modal length (Mo) (mm)	Percentage of modal class (%)
Full hatched	1 - 5	125	1.0	2.8	2.80	20.10	1.5	33.5
(HT1)	5 - 10	125	2.6	5.6	2.15	14.43	3.3	28.4
	10 - 15	125	3.4	6.8	2.00	3.91	5.7	39.8
	15 - 20	100	4.1	7.7	1.87	9.19	6.4	15.5
	20 - 25	75	4.5	9.0	1.80	3.38	8.2	22.7
	25 - 30	75	5.2	11.2	2.10	2.19	9.3	18.5
Partial 'first' major	1 - 5	150	1.2	3.6	3.00	23.05	2.2	39.1
(HT2)	5 - 10	150	2.3	5.9	2.56	9.80	3.8	28.5
	10 - 15	125	3.1	7.1	2.29	4.81	5.6	30.6
	15 - 20	100	3.6	8.4	2.30	3.23	6.2	41.4
	20 - 25	75	5.2	9.6	1.80	4.32	7.8	28.5
	25 - 30	75	5.8	14.1	2.40	5.71	8.9	12.9
Partial 'second' minor	1 - 5	150	1	2.3	2.30	31.81	1.3	32
(HT3)	5 - 10	150	2.1	3.8	1.80	9.27	2.7	23
	10 - 15	125	3.45	6	1.74	12.45	4.1	21
	15 - 20	100	5	7.9	1.58	8.38	6.2	17
	20 - 25	75	6.3	9.9	1.57	6.64	8.1	15
	25 - 30	75	6.8	11.4	1.67	5.20	8.7	13
Partial 'first' minor	1 - 5	150	1.2	2.4	2.00	28.68	1.1	18
(HT4)	5 - 10	150	2.4	4.5	1.87	12.36	2.7	21
	10 - 15	125	3.3	7.2	2.18	12.82	4.8	44
	15 - 20	100	3.7	8.1	2.19	15.30	6.1	24
	20 - 25	75	4.3	9.1	2.11	13.70	7.9	33
	25 - 30	75	4.6	12.1	2.63	20.31	8.4	12
Partial 'second' major	1 - 5	150	1.7	3.2	1.88	20.10	2.5	18
(HT5)	5 - 10	150	3.1	5.6	1.80	14.43	4.3	34
	10 - 15	125	4.8	7.2	1.50	3.41	5.7	19
	15 - 20	100	5.5	8.9	1.61	9.19	6.4	21
	20 - 25	75	6.4	10.3	1.61	3.38	8.2	31
	25 - 30	75	7.5	14.3	1.88	2.19	9.3	17

Table 1.4. Development, Mean stage progression and moulting frequency in larvae of *M. rosenbergii* under differential hatching intensity

Duration of larval development (days)	HT1		HT2		HT3		HT4		HT5	
	A	B	A	B	A	B	A	B	A	B
1	I	1.2	I	1.3	I	1.4	I	1.2	I	1.5
2	II	2.1	II	1.7+	II	1.8	II	1.8	I	-
3	II	-	II	-	II	-	II	-	II	2.2+
4	III	2.9+	III	2.4+	III	3+	III	2.2+	II	-
5	III	-	III	-	III	-	III	-	III	2.8+
6	III	-	III	-	III	-	III	-	III	-
7	III	-	IV	4.1	III	-	III	-	III	-
8	IV	5	IV	-	IV	4.4	IV	3.5	IV	4.3
9	IV	-	IV	-	IV	-	IV	-	IV	-
10	V	5.9+	V	5.6+	IV	-	IV	-	IV	-
11	V	-	V	-	V	5.8	IV	-	V	5.7+
12	V	-	V	-	V	-	V	4.1	V	-
13	VI	6.8+	V	-	VI	6+	V	-	VI	5.9+
14	VI	-	VI	6.1+	VI	-	V	-	VI	-
15	VI	-	VI	-	VI	-	VI	5+	VI	-
16	VI	-	VI	-	VI	-	VI	-	VII	6.4
17	VI	-	VI	-	VII	6.8	VI	-	VII	-
18	VII	7.1	VII	7.2	VII	-	VII	5.7+	VII	-
19	VII	-	VII	-	VII	-	VII	-	VIII	7.8+
20	VIII	7.4	VII	-	VII	-	VII	-	VIII	-
21	VIII	-	VIII	7.8	VIII	6.9	VIII	6.6	IX	8.1+
22	VIII	-	VIII	-	VIII	-	VIII	-	IX	-
23	VIII	-	VIII	-	VIII	-	VIII	-	IX	-
24	VIII	-	VIII	-	VIII	-	IX	7.3+	IX	-
25	IX	8.5+	IX	8.8+	IX	7.5+	IX	-	X	8.9
26	IX	-	IX	-	IX	-	IX	-	X	-
27	IX	-	IX	-	IX	-	IX	-	XI	9.8+
28	IX	-	IX	-	IX	-	IX	-	XI	-
29	IX	-	X	9.3	IX	-	X	8.7	XI	-
30	X	9.4	X	-	X	8.5	X	-	XI	-
31	X		X	-	X	-	X	-	PL	12.4
32	XI	9.8+	XI	9.8+	X	-	XI	9+		
33	XI	-	XI	-	XI	9.1+	XI	-		
34	XI	-	XI	-	XI	-	PL	9.8		
35	XI	-	XI	-	XI	-				
36	PL	10.1	PL	11.2	XI	-				
37					XI	-				
38					PL	10.2				

A - Zoea stage; B + - indicates moulting, numerical values denote larval length (mm);
PL- post larvae
Expansion of HT1, HT2, HT3, HT4 and HT5 are given in Table 1.3

Fig 1.2 Progression line for mean length of *M. rosenbergii* larvae originated from different hatching intensity

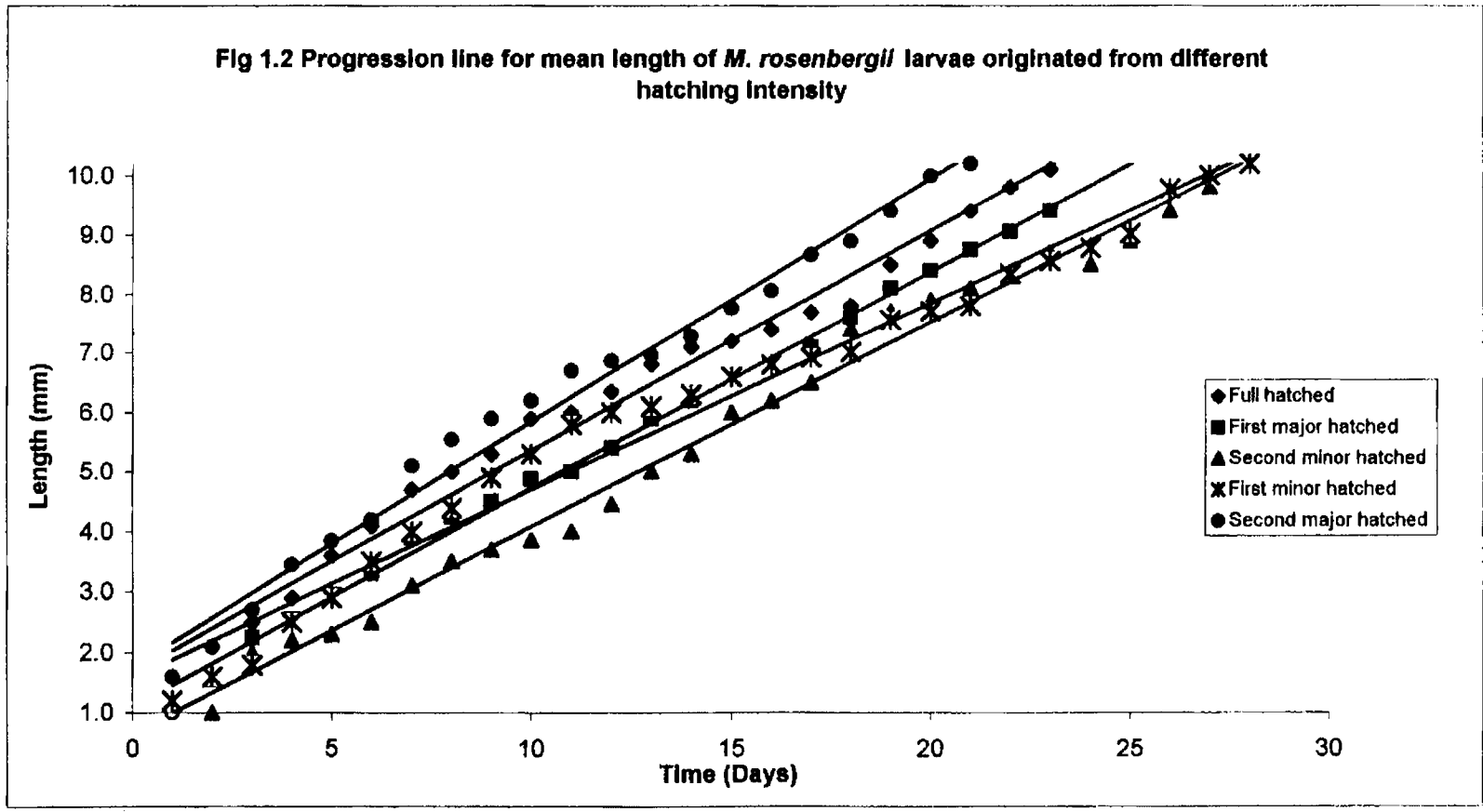
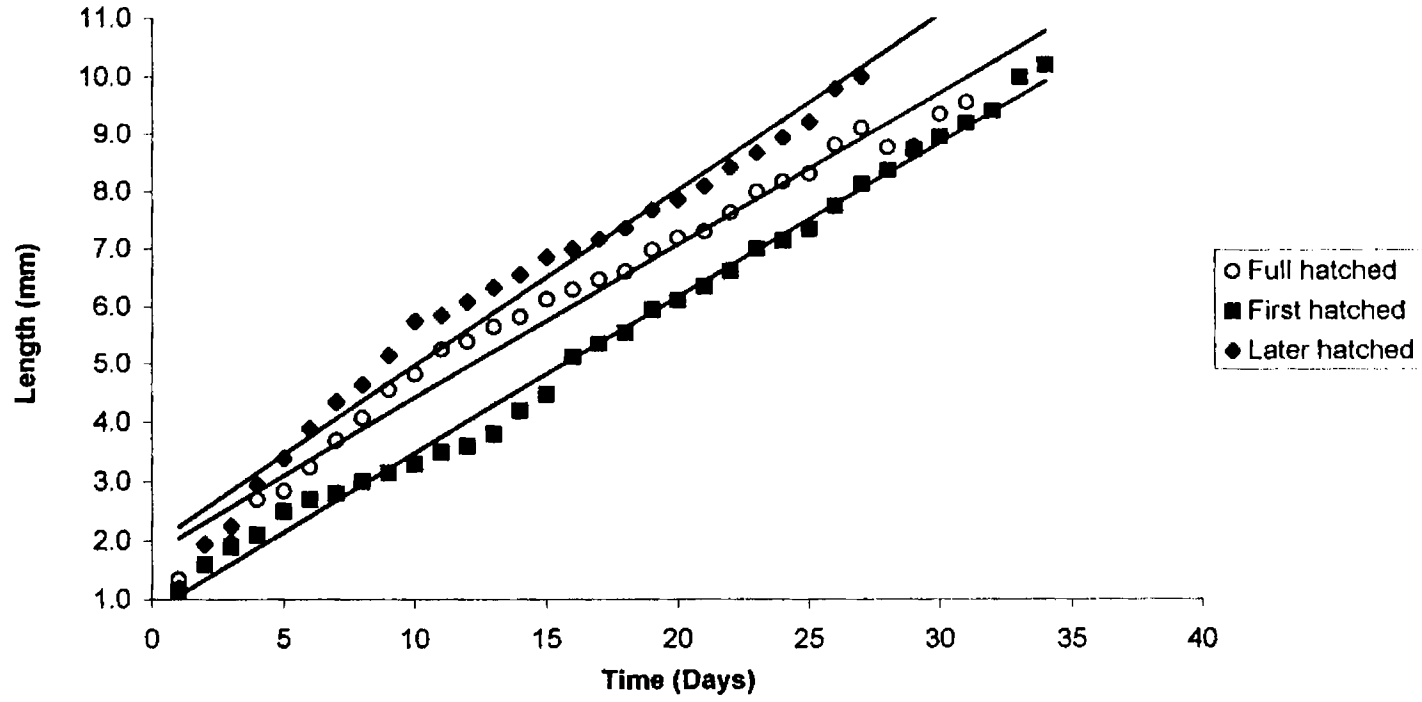


Fig 1.1 Progression line for mean length of *M. rosenbergii* larvae originated from different hatching order



Section III

Biochemical characterization of male morphotypes of *M. rosenbergii*

Chapter 2.

Difference in Protein Synthesis associated with male morphotypes of *Macrobrachium rosenbergii* (de Man) as an Intrinsic factor causing differential growth

1. Introduction

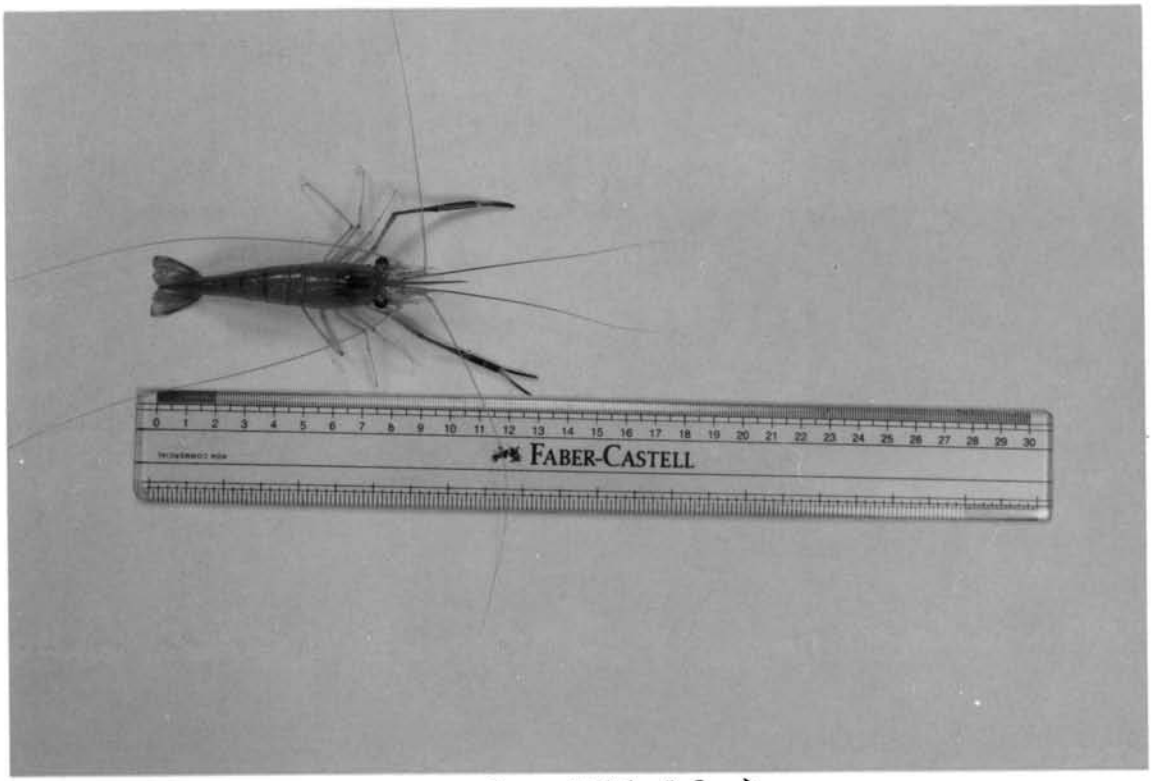
Macrobrachium rosenbergii is known to exhibit a complex social organizational hierarchy (Ra'anan and Cohen, 1985), comprising morphologically distinct dominant, subdominant and subordinate animals. The prevalence of a definite social hierarchy among the male morphotypes is reported to be causing the differential growth among the population. Hence in sexually matured single as well as multi aged population of *M. rosenbergii* (de Man), the size distribution of female population almost appears to be homogeneous, on the contrary, the male shows individual heterogeneous growth and therefore can be differentiated into three distinct morphotypes which show differences among themselves morphologically, anatomically, physiologically and biochemically (Kuris *et al.*, 1987; Sureshkumar and Kurup, 1998a). The three male morphotypes so differentiated are Small males (SM), Orange Clawed Male (OC) and Blue Clawed male (BC). These morphotypes represent three developmental stages of male maturation process and are known to undergo transformation from SM → OC → BC (Ra'anan and Cohen, 1985; Karplus *et al.*, 1992 a and b). SM occupies the initial stage of developmental pathway (Cohen *et al.*, 1981). They are subordinate, non-territorial and the body and second cheliped are translucent. OC are subdominant and represents a stage of high somatic growth (Ra'anan, 1982). Depending on the spination and claw colour OC has three different

transitional stages, which represent three distinct stages in the transformation pathway. The transitional stages of OC are Weak Orange Clawed (WOC), Strong Orange Clawed (SOC) and transforming Strong Orange Clawed (t-SOC) males. Old Blue clawed male (OBC) represents the terminal stage in the morphotypic transformation pathway and is characterized by thick and dark-blue claws. The growth at this stage is least and only the length of second cheliped increases. The long chelae and the distinct blue colour are advantageous to create a territory of its own. Weak Blue Clawed males (WBC) and Strong Blue Clawed males (SBC) form the two transitional stages of BC morphotype (Harikrishnan and Kurup, 1997). The terminal morphotypic stage of BC with relatively small body size in terms of carapace length and body weight, disproportionate with claw length is termed as Old blue clawed (OBC). This pattern of differential growth that is characteristic among males of *M. rosenbergii* is termed "Heterogeneous Individual Growth" (HIG). HIG is one of the limiting factors of farming of this species. The wide disparity in size structure of the cultured stock and the skewness in their weight distribution, which is profoundly influenced by the male morphotypes apparently, appear to be the most commercial disadvantage of this species.

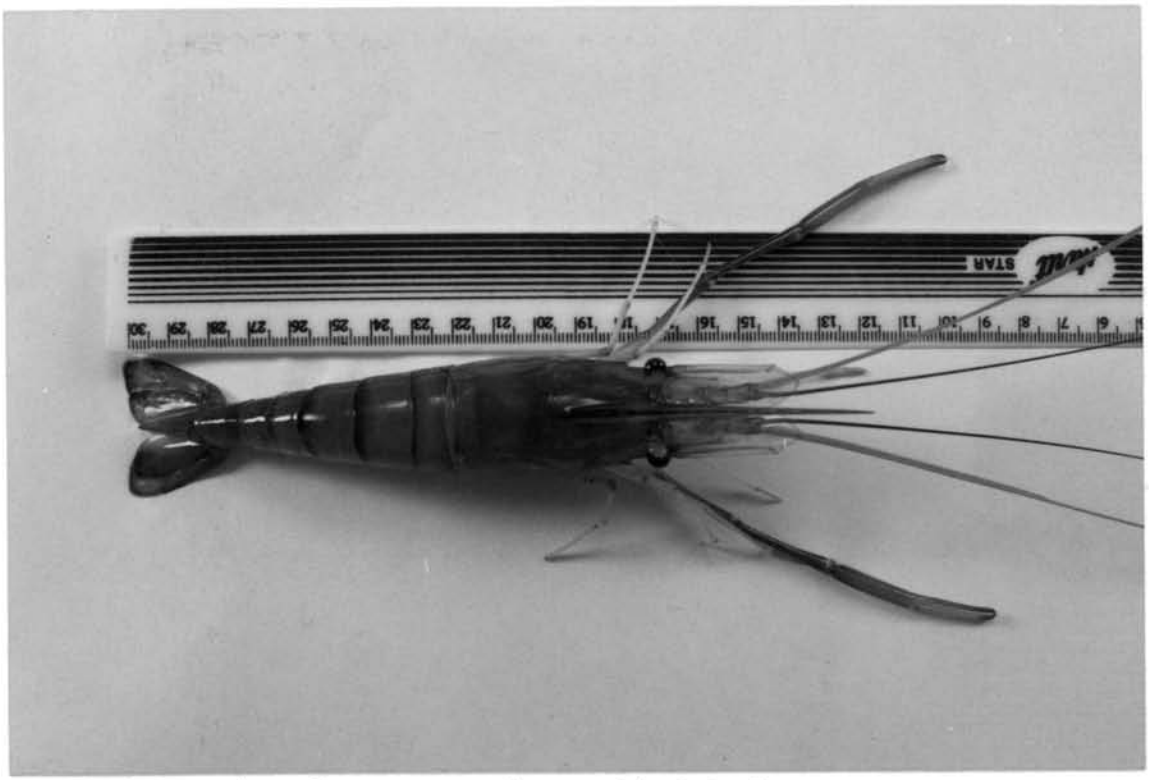
Male Morphotypes of *M. rosenbergii* (De Man)

1) Small Male (SM) (Plate 2.1A)

SM is the first stage of male morphogenesis. Body very small ranging from 75 to 139 mm in total length and the colour pattern is quite variable. Body translucent with light greyish colouration. 2nd cheliped also translucent with bluish hue on the sides of the propodus and fixed finger blue. Carpus may have



A. *Macrobrachium rosenbergii* (de Man)
Small Male (SM)



B. *Macrobrachium rosenbergii* (de Man)
Weak Orange Clawed male (WOC)

a red back on the distal end. A red spot on propodus at the point of articulation with dactylus. Dactylus is slightly yellowish. Chelipeds devoid of spination and the surface is more or less smooth.

2) Weak Orange Clawed Male (WOC) (Plate 2.1B)

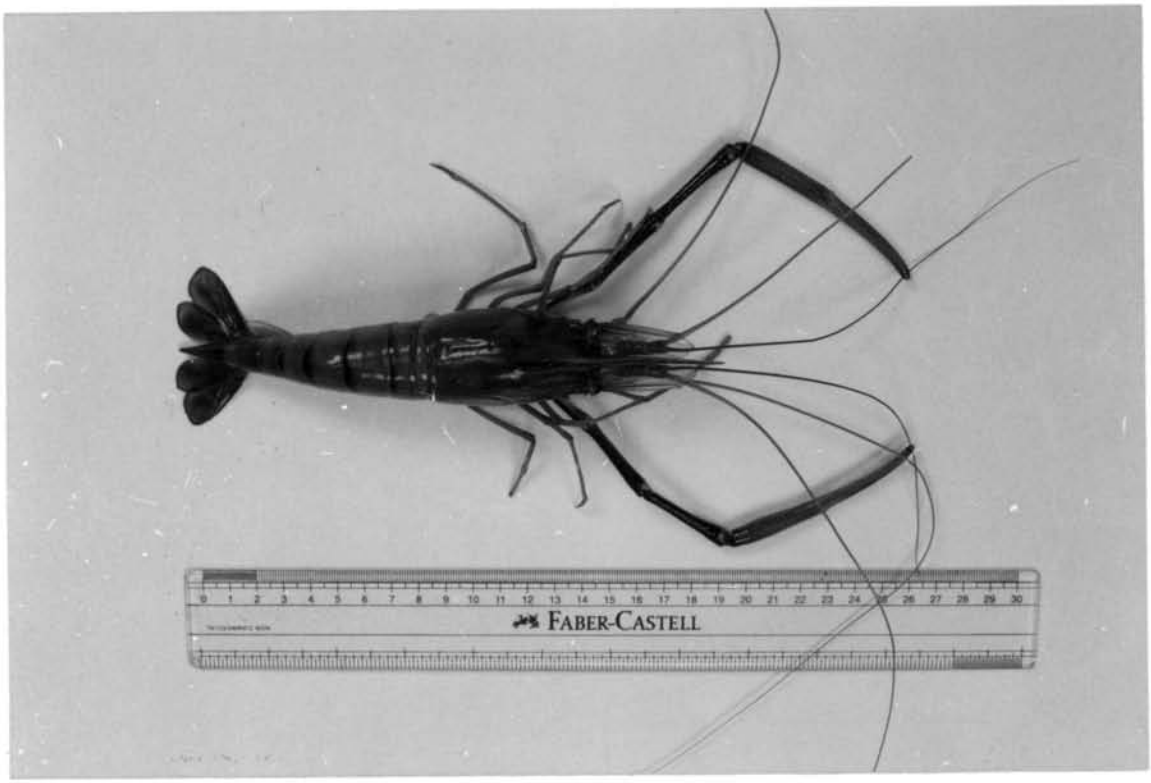
WOC is the transitional stage between SM and SOC. Characterised by weak 2nd cheliped, which is orange in colour. Spination of 2nd cheliped feeble that imparts its surface a rough appearance. Most of the portion of propodus is orange in colour. Inner median sides of the ischium, merus and carpus with orange chromatophore, whereas, outer proximal area suffused with blue pigments. Dactylus yellowish orange and naked.

3) Strong Orange Clawed Male (SOC) (Plate 2.2A)

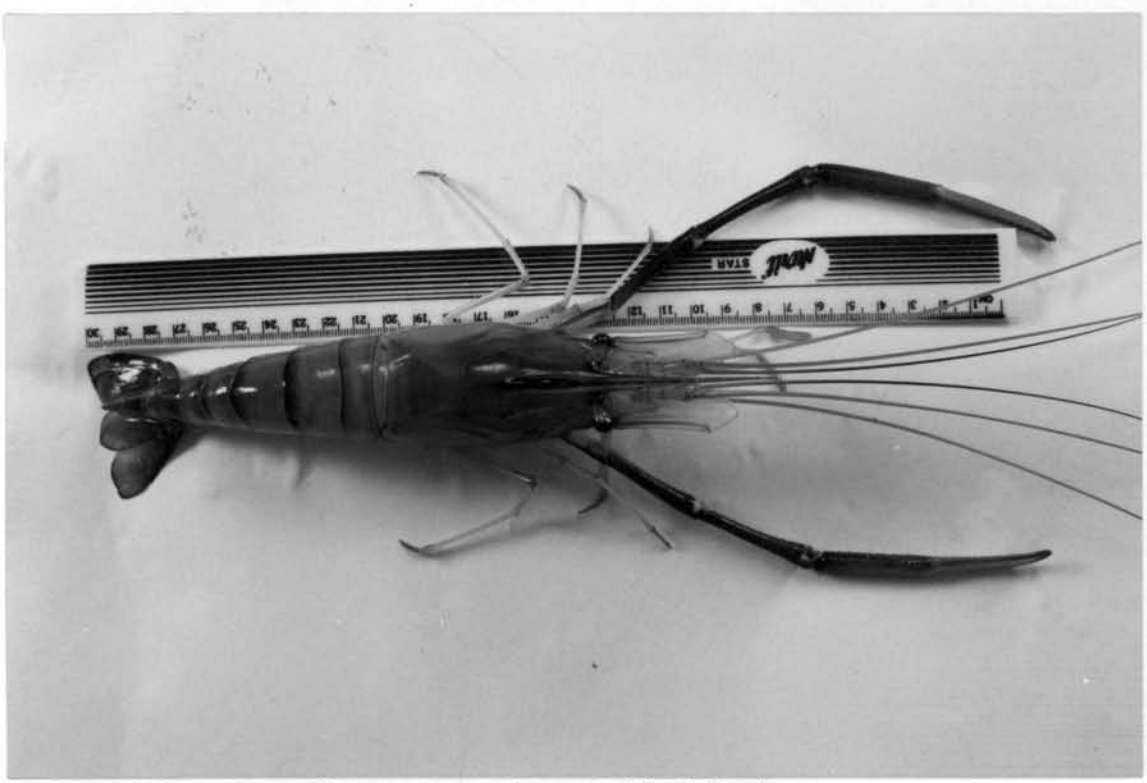
Representing second stage of male morphogenesis characterised with strong chelipeds with orange colouration. Ischium, merus and carpus possess stout spines and the colouration is similar to that of WOC. Propodus orange in colour with whitish medial face. Spines on propodus fragile and orange in colour with a black horny tip. Spines on the cheliped form an acute angle (30-45°) with the surface of the chelipeds. Dactylus fully covered with grayish brown hairs.

4) Pre-transforming Strong Orange Clawed Male (t-SOC) (Plate 2.2B)

This is the transitional stage between OC and BC. This group is found to be more heterogeneous in nature as the perceptible variation could be observed in total length from 106 to 293 mm. Body size and colouration resemble WOC and SOC, but can easily be distinguished by the presence of



A. *Macrobrachium rosenbergii* (de Man)
Strong Orange Clawed male (SOC)



B. *Macrobrachium rosenbergii* (de Man)
Transforming Strong Orange Clawed male (t-SOC)

bluish colouration, which may be replacing orange colouration, which can be taken as the first sign of transformation to BC.

5) Weak Blue Clawed Males (WBC) (Plate 2.3A)

Body size of this group rather heterogeneous. Chelipeds characterised by deep blue colouration with feeble spination and naked dactylus. Inner surface of ischium, merus and carpus are light bluish.

6) Strong Blue Clawed Males (SBC) (Plate 2.3B)

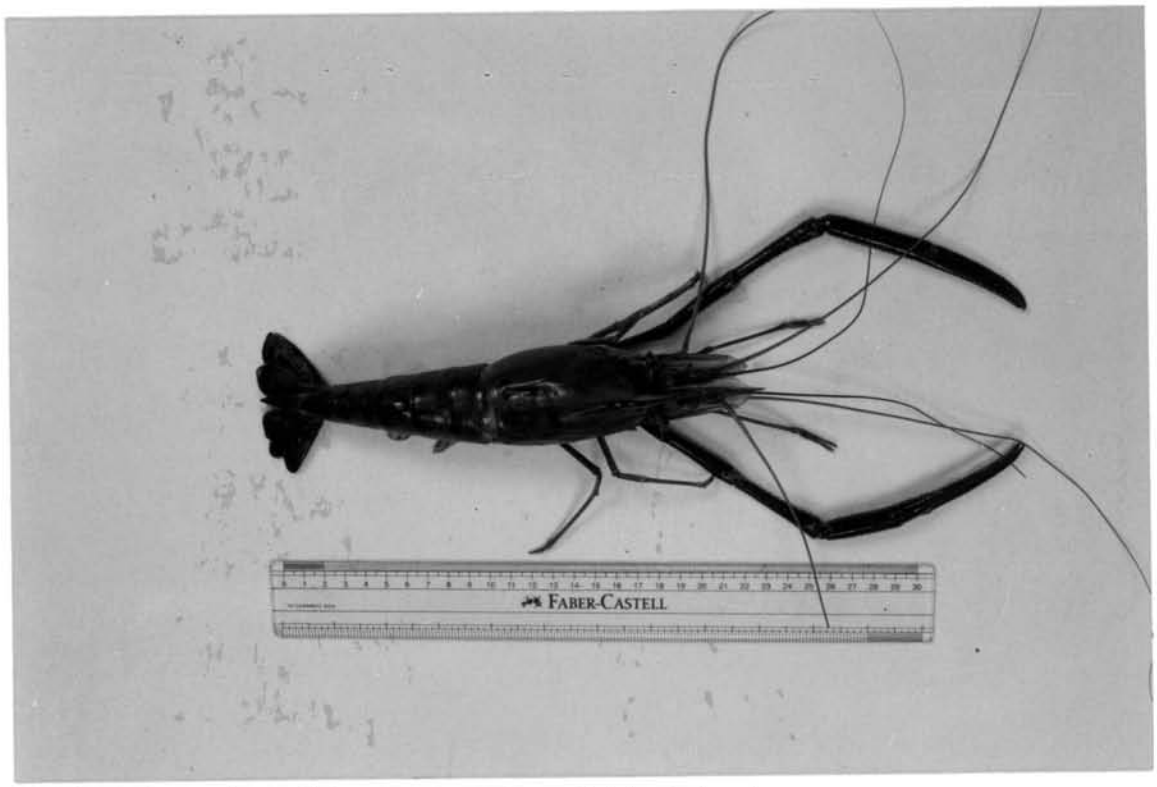
Representing 3rd morphotypic stage of male morphogensis. Large animals with total length ranging from 178 to 289 mm. Characterised by the presence of a deep blue or peacock blue, large and strong cheliped. Stout spination on ischium, merus and carpus and the spines are deep blue in colour and forms an angle 60-75o with the surface of cheliped. Dactylus have a thick covering of grayish brown plumes.

7) Old Blue Clawed Male (OBC) (Plate 2.4)

Largest individuals ranging from 248 to 354 mm in total length, representing the terminal position of male morphotypic transformation pathway. Presence of exceptionally large cheliped longer than total length, which are disproportionate with body length. Colouration and spination similar to that of SBC.

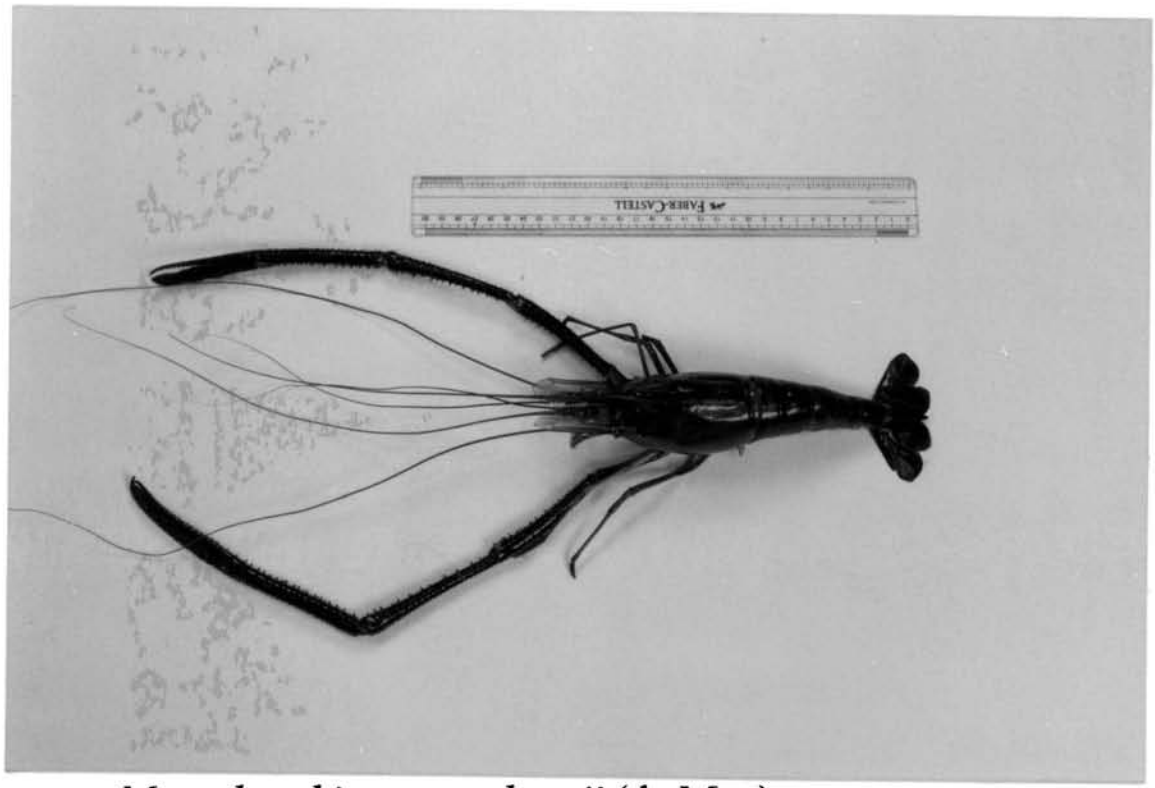
In general, the heterogeneous growth shown by the males of *M. rosenbergii* may be governed by: (a) Intrinsic factors such as genetic differences, hatching order or age of metamorphosis (Smith et. al., 1978., Sandifer and Smith, 1979); (b) Environmental factors responsible for the competitive situation in cases of limited resources such as space and food

A. *Macrobrachium rosenbergii* (de Man)
Weak Blue Clawed male (WBC)



B. *Macrobrachium rosenbergii* (de Man)
Strong Blue Clawed male (SBC)

Plate 2.4



Macrobrachium rosenbergii (de Man)
Old Blue Clawed male (OBC)

(Magnuson, 1962); and (c) Social factors such as position within the size hierarchy, social status and territoriality (Symons, 1972). Inevitably, most of the studies pertaining to the size heterogeneity focussed on social interactions of various morphotypes in natural grow-outs (Cohen *et al.*, 1981; Karplus and Hulata, 1995; Ra'anan and Cohen, 1985; Karplus *et al.*, 1991, 1992 a and b). Though attempts were made to study the biochemical basis of morphotypic differentiation in male morphotypes of *M. rosenbergii* (Sureshkumar and Kurup, 1998a), however, no concerted efforts has so far been made to bring out the molecular differentiation existing among these morphotypes. Against this background, the proposed study was undertaken to understand and to unravel the factors underlying the size heterogeneity in the male morphotypes of *M.rosenbergii.* Since, a clear-cut variation in the somatic growth of individual morphotypes was clearly discernible an attempt was made to bring out relationship if any, between the degree of size heterogeneity and the rate of protein synthesis taking place in different male morphotypes of *M. rosenbergii*.

2. Materials and Methods

Prawns for the present study were collected from a monoculture farm at Vechoor, Kerala, South India in order to ensure that the morphotypes collected for RNA, DNA and Amino Acid studies belonged to the same age groups. Details on the morphotypic composition, individual length and weight were measured on the day of harvest (Brody *et al.*, 1980; Cohen *et al.*, 1981) and the samples were transported to the laboratory in live conditions where they were segregated into male morphotypes such as Small males (SM), Strong Orange clawed (SOC) males and Strong Blue clawed (SBC) males and their intermediate

stages viz. Weak orange clawed (WOC) male, pre-transforming Strong Orange clawed (t-SOC) male and Weak blue clawed (WBC) male and Old Blue Clawed males (OBC) (Harikrishnan and Kurup, 1997). Abdomen muscle tissue (0.5 gm) was suspended approximately in 10 ml of 10% Trichloroacetic acid (TCA) at 0°C and homogenized using a glass tissue homogeniser. The homogenate was centrifuged at 3000 rpm for 10 min and the supernatant poured off, and the sediment was washed once again with 2.5 to 5 ml of ice cold 10% TCA. The tissue was then homogenated and centrifuged at 3000 rpm for 10 min. The supernatant was then discarded. This process was continued for two more successions. The final sediment remaining after removal of acid soluble compounds was extracted twice with 5 ml of 95% ethanol, ethanol-ether mixture along with centrifugation in 3000 rpm for 10 min. Lipid free tissue residue was suspended in 1.3 ml of distilled water and 1.3 ml of 10% TCA and the mixture was heated for 15 min at 90°C with occasional stirring. The residue was washed with 4 ml of 5% TCA and mixed well and centrifuged at 3000 rpm for 10 min. The supernatant was collected and estimated for RNA following Orcinol reagent test and DNA following diphenyl amine reagent test (Schneider, 1957).

Following calculations were made to estimate the total amount of RNA and DNA in the tissue (Schneider, 1957).

$$\text{Amount of DNA} = \frac{\text{O.D of the sample}}{\text{O.D of the standard}} \times \frac{0.001}{0.20} \times \text{weight of the tissue}$$

$$\text{Amount of RNA} = \frac{\text{O.D of the sample}}{\text{O.D of the standard}} \times \frac{0.002}{0.10} \times \text{weight of the tissue}$$

Total and free amino acid concentration in muscle tissue of *M. rosenbergii* was estimated following Ninhydrin method of Yemm and Cocking (1955) and free amino acid through Fluorescence spectrophotometer method of Marc Roth (1971). Amino acid profiling of the muscle tissue was done following Ishida *et al.* (1981). For this about 100 mg of the sample in duplicates was weighed accurately into a heat-sealed test tube. 10 ml of 6 N HCl was added, the tube were heat sealed after filling pure nitrogen gas. Hydrolysis was carried out in hot air oven at 110°C for 24 hrs. Once the hydrolysis was over, the test tubes were broke open, content was removed quantitatively and filtered into a round bottom flask through Whatman filter paper No: 42. The filter paper was washed 2-3 times with distilled water. The content of the flask were flash evaporated to remove traces of HCl, the process being repeated for 2-3 times with added distilled water. The residue was made up to 10 ml with 0.05 M HCl. The sample so prepared was filtered again through a membrane filter of 0.45 µM and 20 µl of this was injected to Shimadzu HPLC LC 10 AS Amino Acid analysis system. The column is of sodium type i.e., ISC-07/ S 1504 Na with a length of 19 cm and diameter 5 mm. The mobile phase of the system consists of two buffers- Buffer A (Sodium citrate, ethanol of 99.55%, perchloric acid 60%, pH- 3.2) and Buffer B (sodium citrate boric acid, 4N NaOH, pH- 10.0). The oven temperature was maintained at 60°C. The amino acids were eluted from the column by stepwise elution i.e., acidic amino acids first followed by neutral and then alkaline amino acids. The analysis was done with non-switching flow method and flourescence detection after post column derivatization with o-phthalaldehyde. In case of proline and hydroxy proline, imino groups were converted to amino group with

sodium hypochlorite. Amino acid standards from Sigma chemical Co. was also run to calculate the concentration of sample amino acid depending on the standard chromatogram.

The percentage of each amino acid in the muscle was calculated using the equation (Ishida *et al.*, 1981) (g Amino acid/ 16 gm Nitrogen) =

$$\frac{\mu M \text{ in standard amino acid x Mol.wt x volume made up x 1000 x 10 x 1}}{\text{Standard area x 20 x wt. Of sample x 1000 x 1000 x N\%}}$$

The total muscle protein in the body was biochemically evaluated following Micro-Kjeldahl method (AOAC, 1990). The results of the biochemical analysis was statistically tested using analysis of co-variance and Duncun's multiple range t-tests at $P<0.05$ using SPSS 7.5 for Windows.

3. Result

3.1 RNA and DNA ratios :

Table 2.1 shows the mean values of RNA, DNA and its ratio in the muscle tissue of the seven different male morphotypes studied. A significant difference ($P<0.01$) in the RNA/DNA ratios of the morphotypes could be recorded. Though a significant difference in the values of DNA among the morphotypes could not be established, the values of RNA and correspondingly its ratio with DNA registered highly significant variations in t-SOC. In contrast, least values were recorded in OBC. The results on quantification of the amount of DNA (Table 2.1) showed slight difference among the different morphotypes, showing highest in t-SOC (0.481±0.15 mg/g) and lowest in SM (0.29±0.05 mg/g). Similarly highest RNA content was recorded in t-SOC (0.893±0.12 mg/g) with least in both SM (0.476±0.1 mg/g) and OBC (0.49±0.05 mg/g). A direct

relation on the change in the concentrations of DNA and RNA was noticed in the present study. A sequential increase in the percentage of RNA and values of RNA/DNA ratios from SM to t-SOC could be discernible followed by a gradual decrease thereafter to OBC. Results of one-way ANOVA using SPSS are given in Table 2.1. The F value was found to be insignificant for DNA (F= 1.6110), but significance at 5% level was found for RNA (F=12.682) and a greater significance (P<0.01) was observed for RNA/DNA ratios (F=17.8739). The results of pair wise comparison through t-test (Table 2.2) showed that a greater degree of heterogeneity in the quantity of RNA and DNA present in SM and SOC and SOC and OBC (P<0.01), while no significant differentiation (P>0.05) could be observed between SM and OBC.

3.2 Protein and Amino acids:

Table 2.1 shows the mean value of protein, free amino acids and total amino acids in the muscle tissue of male morphotypes of *M. rosenbergii*. The concentration of protein among the morphotypes was least recorded in SM (16.28%), while the values were high for t-SOC (22.47%). In contrast to this, the values of amino acids followed an inverse correlation to that of protein. The concentrations of total and free amino acids were least in t-SOC (5.76 and 3.14 %mg respectively) while it was highest in SM (14.56 and 5.76 %mg respectively). The trend seen in the variation of amino acids commensurate with the morphotypic transformation appeared to be inversely related to that of RNA and DNA contents. Highest values were recorded for SM, which sequentially lowered till in t-SOC and thenceforth gradually increased till OBC. Results of one-way ANOVA for protein and amino acids are presented in Table 2.1. The F

value was found to be significant at 5% level for total amino acid (F= 6.2817) and muscle protein (F= 10.734), while for Free amino acids it showed significant difference. (P<0.01) (F= 21.5236). Results of pair wise analysis using multiple comparison of Duncuns t-test through SPSS are given in Table 2.2. Protein levels in muscle tissue of SM showed significant difference from that of SOC, t-SOC and WBC. Similarly, amino acid contents in muscle tissue of t-SOC showed significant difference with all the BC morphotypes especially with that of OBC (Table 2.2). Results of the amino acid profiling of the three main male morphotypes and four intermediary stages are given in Table 2.3. In all the morphotypes and their stages the level of acidic amino acids (Glutamic acid and Aspartic acid) were found to be very high (14.34 to 17.65 G/16g N and 9.38 to 11.32 G/16g N respectively). Among the neutral amino acids Leucine (6.3 to 7.36 G/16g N), Glycine (4.27 to 6.13 G/16g N) and Alanine (5.16 to 6.70 G/16g N) were present in appreciable concentrations, while, the most dominant among the basic amino acid group was Arginine (7.41 to 9.92 G/16g N). Almost all of the 18 amino acids analysed showed a peak in SOC and thereafter showed a gradual decrease till OBC.

4. Discussion

Growth in animals has usually been expressed as a change in either length or weight or increase of dry weight body mass with reference to time (Buckley, 1979). Accompanying and underlying these changes in weight and morphology, changes in biochemistry and chemical composition of the animal can normally take place simultaneously. Since synthesis of protein is ultimately

controlled by DNA, which carries all the information needed to specify the structure of every protein the cell can make, each morphotypes of *M. rosenbergii* is characteristically has a definite size and growth pattern. In this respect it is of paramount importance to quantify the intrinsic factors involved and governing the differential growth of male morphotypes and hence the role of RNA and DNA which are directly involved in the process of protein synthesis and consequently the growth have been quantified. Any relationship arriving on the pattern of variation of RNA and DNA among various morphotypes shall be useful in arriving at meaningful explanation for the difference in pattern of protein synthesis which in turn results in the variation in somatic growth. In the present study the protein content in the muscle tissue of different morphotypes was found to vary from 16.28 to 22.47%. The difference in the protein contents could directly be corroborated to difference in phenotypic traits and the difference in somatic growth seen associated with the male morphotypes. The results of the present study were found comparable to that of Sureshkumar and Kurup (1998a). Pair wise analysis through multiple comparisons of mean values using Duncun's t-test also confirmed the difference among all the morphotypes studied. From the results so arrived at it could be inferred that the rate of protein synthesis is high in SOC and its intermediate stages, while the rate of protein anabolism and consequent somatic growth was less in SM and BC morphotypes. Another salient findings made in the present study was the relationship between protein and amino acids observed in SM, OC and BC morphotypes. In SM although the protein content was less they had a high amino acid content capable of producing larger quantities of protein. In OC, on the other hand, the

percentage of protein and amino acid were high which directly explains the better somatic growth associated with this morphotype. In BC, however, the percentage of protein and amino acids were comparatively less. This would mean that although being deprived of food and space due to the prevailing social hierarchy in the grow-out population, SM remains subordinate but has a potential to transform to successive stages once this hindrance is removed (Karplus *et al.*, 1987). OC has a faster growth rate as a result its head to tail ratio is higher than that of BC. While BC occupies the terminal stage and dominant position in the social hierarchy, thus providing no scope for further somatic growth in the morphotype and their protein synthesis rates were found to be on a lower side.

Similarly, among the various male morphotypes studied, high protein and RNA content were observed in the muscle tissue of SOC and t-SOC and this manifest the possibilities of higher rates of protein synthesis in these morphotypes. Similar finding was reported in the juvenile spider crab, *Hyas araneus* (Anger and Hirche, 1990). Similarly, muscle DNA content was also found higher in SOC and t-SOC and this fully corroborates with that of Bulow (1970) and Anger and Hirche (1990) who reported that an increase of DNA could well be taken as an indicator of faster growth of fishes and crustaceans. Among the various morphotypes, SOC and its transitional stages are reported to be the fastest growing morphotypes (Karplus *et al.*, 1987; Sureshkumar and Kurup, 1998a) and the highest protein, RNA and DNA content of the body muscle recorded among these morphotypes fully explain the biochemical basis of the above biological manifestation. The results directly revealed the fact that being in a faster growth phase RNA produced by SOC was continuously being

utilised for protein synthesis, unlike that of the OBC, which showed poor growth performances. Changes in total amino acids, free amino acids, and RNA and RNA/DNA ratios are measures of metabolic activity during the ontogenesis and stage progression (Zeitoun *et al*, 1977). The pattern of variation of these biochemical components is presumed to be the evidence of difference in protein synthesis (Ikeda, 1989). Since morphotypic development could be directly attributed to difference in somatic growth (Sagi and Ra'anan, 1988), each morphotype characteristically and specifically shows different values for these biochemical constituents. Significant difference in muscle protein, DNA and RNA can be considered as an index of high cellular activity (Lemmens, 1995) and the variation observed in the above biochemical components among the male morphotypes may be helpful in identifying intrinsic factors underlying heterogeneous growth among morphotypes and this finding is in full agreement with the view expressed by Kuris *et al* (1987). The sequence of DNA increase or decrease followed by an increase or decrease in RNA is presumed to be an evidence of RNA synthesis and consequently protein anabolism (Zeitoun *et al.*, 1977). In the present study the DNA concentration in the morphotypes did not follow any particular trend but RNA concentration contemplated a trend very much similar to that of protein. In contrast, the RNA/DNA ratios did not follow any specific sequence. Buckley (1979) pointed out the importance of this ratio on the growth of fishes. Though the values for RNA and DNA in the present study are on a higher side when compared to Sureshkumar and Kurup (1999), the trend remained more or less the same and comparable with the above authors. The sudden decline of total and free amino acid from SM to t-SOC and

subsequently a gradual rise till OBC depicts the pattern of utilisation of amino acids by SM and OBC for somatic growth insignificantly and reciprocally the amino acid gets accumulated in the cytoplasm and hence a corresponding increase in their overall concentration can be justified. This was further confirmed while the values of protein and amino acid of different morphotypes were compared. Both these components followed an inverse trend. The results of the present study on the variation RNA/DNA ratios along with developmental stages were similar to that observed in fishes (Suyama, 1958).

The results emerged are RNA/DNA ratios and the concentration of amino acids in the tissue of male morphotypes of *M. rosenbrgii* could also be used as an index for giving explanation for the difference in somatic growth associated with male morphotypes of *M. rosenbergii*. The results of the amino acid profiling did not follow the same trend for all the amino acids studied, however, the major amino acids have shown higher value in SOC which would lend to support its faster growth rate. The results of profiling were complemented with the observations from electrophoresis of muscle protein through native and SDS PAGE (Ranjeet and Kurup, 2001) since more acidic bands being observed in SOC and its succeeding stages such as t-SOC, WBC, SBC and OBC. More or less similar findings were reported in 13 insect species by Yoon and Park (1997). Knowledge of the amino acid sequence of a protein is the fundamental necessity to have an understanding on protein structure – function relationships, especially in *M. rosenbergii* where a clear demarcation in total and free amino acids among the different male morphotypes could be established, in compliance with the differentiation in the protein binding pattern already exhibited (Ranjeet and

Kurup, 2001). Although serious efforts were made to analyse the amino acid contents in nutritional studies in non-peneaid prawns, however, so far no attempt has been made to use it as a tool to characterise male morphotypes biochemically. Amino acid composition in collagen and muscle tissue of *M. rosenbergii, P. japonicus* and *P. monodon* have been studied by Nip *et al.* (1981), Sarac *et al.* (1994) and Fang *et al.* (1992). Roustaian *et al.* (2000) quantified the amino acid composition of larval forms of *M. rosenbergii.* Their results suggest that highest representation among the total amino acids were for glutamic acid and phenylalanine (13.4 - 16.6% and 9.7 - 11.5%). In the present study, however, instead of phenylalanine aspartic acid was foremost dominant. Based on the results of the present study it may be inferred that amino acid composition of the muscle tissue of *M. rosenbergii* changes with development from larval form to adults. Thomas and Diwan (1990) reported that a juvenile in *P. indicus* are characterized by higher concentration of amino acids, which were taken as an index for faster growth. In the present study, however, the first formed morphotype, SM followed a similar pattern, which indicates its potential to transform to successive stages in the transformation pathway.

It can therefore be conclude that there exist, a clear distinction in the biochemical makeup of various male morphotypes of *M. rosenbergii.* The variations found in the biochemical composition of male morphotypes can well be treated as valid indices of size heterogeneity seen in these morphotypes. Difference in protein, RNA and DNA contents of the muscle tissue were found as useful and valid tools to explain the differential growth exhibited by the male morphotypes. Protein, amino acid and RNA/DNA ratio, which are the major

intrinsic factors influencing growth variation among morphotypes, which lend to support the view that each morphotype of *M. rosenbergii* is characterised by a definite physiological makeup. The results further revealed that the amino acid composition of individual each morphotype is totally different and hence the protein developed from binding of these amino acids also vary in composition and structure thus impacting a different composition to muscle tissue and this finding is in agreement with Ranjeet and Kurup (2001). Another inference of the present study is the possibility of the genetical involvement in size heterogeneity, since difference in protein synthesis is directly related to the percentage of RNA and DNA in the tissue. There exist a perceptible difference in the levels of RNA and DNA and therefore the difference in protein anabolism can be attributed to this. Based on the present findings, it can be concluded that the difference in the protein synthesis observed among various male morphotypes can be considered as one of the major intrinsic factors governing heterogeneous individual growth seen in the male morphotypes of *M. rosenbergii*.

Table 2.1 Biochemical evaluation of the muscle tissue of different morphotypes of *Macrobrachium rosenbergii*

Morphotypes	Protein (% wet weight)	Total amino acids (% mg)	Free amono acids (% mg)	RNA (mg/g)	DNA (mg/g)	RNA/ DNA ratio
SM	16.28 ± 0.26	14.56 ± 1.27	5.76 ± 0.27	0.476 ± 0.10	0.290 ± 0.05	1.641 ± 0.19
WOC	20.09 ± 0.31	8.70 ± 1.42	5.28 ± 0.12	0.507 ± 0.06	0.304 ± 0.01	1.667 ± 0.19
SOC	21.65 ± 0.25	7.55 ± 0.82	3.27 ± 0.87	0.792 ± 0.14	0.434 ± 0.11	1.824 ± 0.51
t-SOC	22.47 ± 0.13	5.76 ± 0.53	3.14 ± 0.62	0.893 ± 0.12	0.481 ± 0.15	1.855 ± 0.27
WBC	20.70 ± 0.35	7.14 ± 1.82	3.78 ± 0.80	0.639 ± 0.11	0.364 ± 0.01	1.755 ± 0.17
SBC	20.05 ± 0.16	8.21 ± 1.07	4.17 ± 1.15	0.624 ± 0.04	0.383 ± 0.04	1.629 ± 0.31
OBC	18.62 ± 0.23	10.67 ± 2.28	4.32 ± 0.91	0.490 ± 0.05	0.314 ± 0.03	1.560 ± 0.19
MSS between samples	3.44 df = 6	4.23 df = 7	1.2 df = 8	0.615 df = 9	0.241 df = 10	0.282 df = 11
MSS within samples	1.02 df = 72	0.52 df = 62	0.02 df = 62	0.01 df = 50	0.008 df = 50	0.08 df = 50
F value	10.734**	6.2817**	21.5236*	12.682**	1.6110+	17.8739*

Values are represented as AVG ± SD
+ Not significant
** Significant at 5 % level (P<0.05)
* Significant at 1 % level (P<0.01)

SM = Small males
WOC = Weak orange clawed males
SOC = Strong orange clawed males
t-SOC = Pretransforming strong orange clawed males
WBC = Weak Blue clawed males
SBC = Strong Blue clawed males
OBC = Old Blue clawed males

Table 2.2 Result of pairwise analysis using t-test on various biochemical constituents showing variation among different morphotypes of *Macrobrachiu rosenbergii*

Sl.No	Combinations	Muscle protein		Total amino acid		Free amino acid		RNA		DNA		RNA/DNA ratio	
		df	t	df	t	df	t	df	t	df	t	df	t
1	SM x WOC	20	0.32+	16	0.23+	15	1.42+	10	0.99+	10	6.34*	10	0.71+
2	SM x SOC	15	2.41**	15	1.57**	21	1.49+	12	6.63*	12	5.19*	12	2.61**
3	SM x t-SOC	15	3.73*	18	0.86*	16	3.91**	20	13.3*	20	4.73*	20	2.34**
4	SM x WBC	21	5.45**	18	1.55**	14	5.67*	14	0.50+	14	1.23+	14	1.44**
5	SM x SBC	16	6.42*	18	0.64*	18	2.17**	24	1.12+	24	3.56*	24	0.42+
6	SM x OBC	15	3.91*	16	0.51*	17	2.96**	10	4.96*	10	1.94+	10	8.98*
7	WOC x SOC	14	4.63**	19	0.25+	18	1.46**	8	2.93*	8	8.1*	8	1.77+
8	WOC x t-SOC	17	4.21**	18	1.45**	19	1.48**	16	1.30+	16	3.38*	16	0.08+
9	WOC x WBC	16	8.23**	19	1.29**	13	3.29*	10	2.53**	10	1.31**	10	1.22+
10	WOC x SBC	19	2.87*	20	1.02**	19	2.55**	20	0.62+	20	0.92+	20	3.61**
11	WOC x OBC	18	1.72*	19	2.53*	17	2.1**	6	0.30+	6	2.23+	6	2.58**
12	SOC x t-SOC	19	0.46+	14	1.35**	19	0.15+	18	7.29*	18	1.16+	18	3.81*
13	SOC x WBC	20	0.72+	15	0.75**	16	3.59*	12	4.71*	12	7.36*	12	6.89*
14	SOC x SBC	19	0.45+	21	2.82*	17	1.42**	22	8.66*	22	4.93*	22	3.28**
15	SOC x OBC	14	0.88**	16	2.89*	16	0.4+	10	7.55*	10	16.9*	10	2.76*
16	t-SOC x WBC	13	4.94*	15	2.35**	19	0.25+	20	4.35*	20	4.58**	20	1.29+
17	t-SOC x SBC	16	0.50+	18	1.42**	18	0.51**	15	4.40*	15	7.14*	15	1.02+
18	t-SOC x OBC	15	0.73+	13	4.51*	18	0.64**	16	4.84*	16	6.20*	16	2.53**
19	WBC x SBC	12	1.29**	19	2.08**	10	0.47**	16	0.76+	16	1.22+	16	2.14**
20	WBC x OBC	14	1.35**	18	0.54+	12	1.44**	20	3.06*	20	0.15+	20	5.21*
21	SBC x OBC	18	1.95**	12	1.55**	16	0.35+	14	3.59*	14	0.92+	14	1.54*

Values are represented as AVG ± SD
\+ Not significant
** Significant at 5 % level (P<0.05)
* Significant at 1 % level (P<0.01)

SM = Small males
WOC = Weak orange clawed males
SOC = Strong orange clawed males
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Chapter 3

Protein characterisation as an index of Heterogeneous Individual Growth (HIG) in male morphotypes of *Macrobrachium rosenbergii*

1. Introduction

Somatic growth in animals has been directly related to the anabolism associated with protein synthesis (Trudier, 1978). A diverse pattern of growth evinced among the males of *Macrobrachium rosenbergii,* technically termed "heterogeneous individual growth (HIG)" is one of the most limiting factors confronting the farming of this freshwater prawn and which reciprocally upsets the profit generated from its culture throughout the world. The extent of this differentiation is so pronounced that a clear demarcation both phenotypically and behaviorally distinct individuals could be seen within the male population. This in turn rendered earlier researchers to group the males of *M. rosenbergii* under three major and four intermediate morphotypic groups (Ra'anan, 1982; Ra'anan and Cohen, 1985; Karplus *et al.*, 1992b; Harikrishnan and Kurup, 1997) as described in the chapter 2 (please refer chapter 2 for more details). Furthermore, these morphotypes themselves develop a complex social organizational hierarchy in the cultured population (New, 1995). Hence both intrinsic factors and extrinsic factors such as social hierarchy and environmental conditions prevailing in the culture system have some bearing on this differential growth pattern (Kurup, 1996). Although the extent of extrinsic factors could be controlled through proper management measures, the role of genetical or biochemical (intrinsic) factors is still unknown. In spite of the fact that the male

morphotypes are well characterised and documented, no concerted attempt has so been made to unravel the mechanism involved in the morphogenisis; to establish the role of environmental and other factors intrinsically associated with morphotypic differentiation and also to bring out the mechanism underlying in their differential growth. Further more, though the morphotypes are phenotypically distinct and also show difference in reproductive capacity and somatic growth rate, no effort was also made to study the biochemical changes, which may take place in these morphotypes commensurating with morphotypic transformation. It is also important to bring out the physiological mechanism involved in the process of transformation in the developmental pathways of male morphotypes in grow outs. Apart from the initial works to characterize morphotypes biochemically (Sureshkumar and Kurup, 1998a), no further attempts have been made to understand and unravel the phenomena of HIG and factors associated with it from a molecular point of view. As the economic viability of farming of this species is profoundly influenced by the relative proportions of Orange clawed male (OC) and Blue clawed male (BC) morphotypes together with their transitional stages in the final harvested population, it is also found imperative to bring out the intrinsic factors governing HIG, which ultimately has much practical significance in resolving the differential growth of this species, whereby aquaculture production and marketable yield from harvested population of *Macrobrachium rosenbergii* can be increased substantially.

2. Materials and Methods

Single aged male morphotypes of *M. rosenbergii* were collected from three polders of North Kuttanad lying adjacent to the Vembanad lake in South India. All the prawns were transported in live conditions to the laboratory. A total of 36 SM, 18 WOC, 27 SOC, 29 t-SOC, 22 WBC, 28 SBC and 32 OBC were analysed in the present study. Prawns brought to the laboratory were initially sorted out to the seven morphotypes based on Harikrishnan and Kurup (1997). Morphometrics of all the prawns were measured and each prawn was individually weighed to the nearest gram using an electronic balance (0.01 g). Tissue samples from the muscles of the first abdominal segment were taken and biochemically evaluated for protein following Micro-Kjeldahl method (AOAC, 1990). The muscle tissue was also used for the electrophoresis of protein through native Poly Acrylamide Gel electrophoresis (PAGE) (Gordon, 1980) and Sodium-dodecyl sulphate (SDS) PAGE (Laemelli, 1970). A portion of the muscle tissue was used for protein fractionisation through a modified technique of Hashimoto *et al.*(1979).

The muscle protein was fractionated following a modified methodology of Hashimoto *et al.* (1979). All operations were performed at 3-4°C as quantitatively possible. 5 g of each muscle tissue was weighed into 50 ml of I = 0.05 phosphate buffer pH 7.5 (15.6 mM Na_2HPO_4; 3.5 mM KH_2PO_4) and homogenised fully with a glass homogeniser followed by stirring. The homogenate is then centrifuged at 10,000 rpm for 10 min. To the residue added 50 ml of the same buffer, and the mixture homogenised and centrifuged again. These two supernatants were combined and 5% tricholoro acetic acid was added

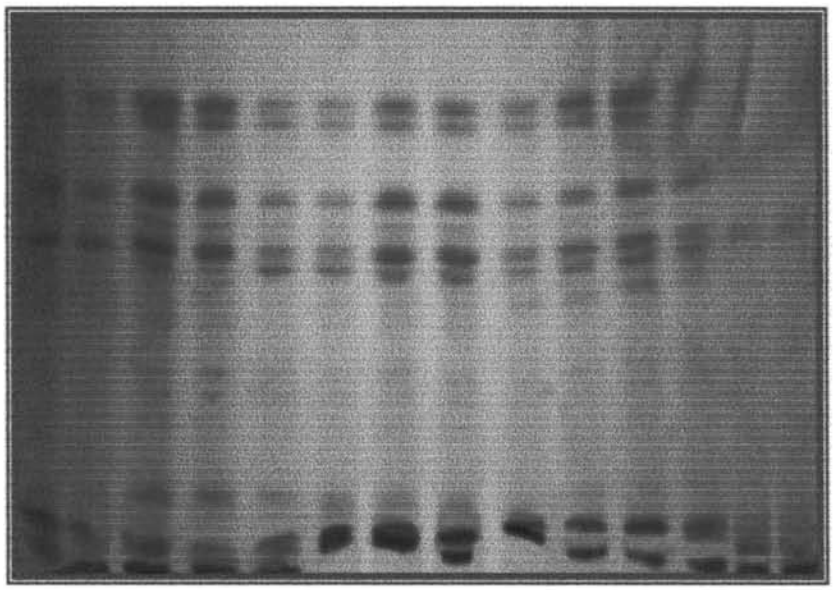
to it. The resulting precipitate was collected following filtration and used as Sarcoplasmic protein fraction. The filtrate was used as non-protein nitrogenous compound fraction. The residue obtained was subjected to an exhaustive extraction overnight with 0.1 N NaOH under stirring. The mixture was centrifuged and the supernatant was used as alkali soluble protein fraction. The final residue was used as stroma protein fraction. The protein and non-protein fractions were analysed for nitrogen by the micro Kjeldahl method (AOAC, 1965) and the protein composition of muscle calculated.

The individual fractions of sarcoplasmic and myofibrillar proteins were then run under 10% SDS-PAGE to bring out the proportion of different subfractions among the morphotypes. The photographs of gels for native, SDS-PAGE and fractionated protein PAGE were compared on the basis of the bandwidth, position and number. The results of the biochemical differentiation among the different fractions of muscle proteins were statistically evaluated by applying analysis of variance and Duncun's multiple range at $P<0.05$ using SPSS 7.5 for Windows.

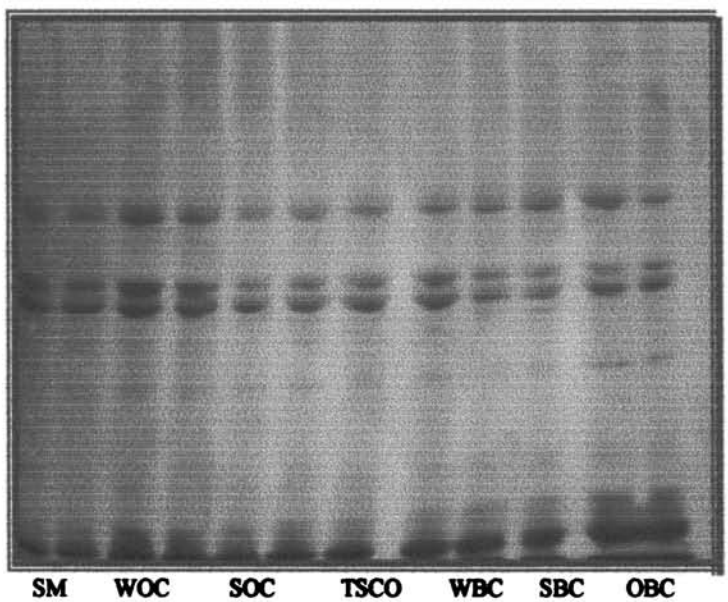
3. Results

The electrophoretic sequence of both Native and SDS PAGE are depicted in Plates 3.1 and 3.2. The results of the electrophorogram show the difference in the number and position of different protein bands among the seven major morphotypes. The difference for SDS-PAGE was more clearly represented with a clear differentiation in the number and position of protein bands that increase from SM to OBC. Also the bandwidth of lower molecular weight

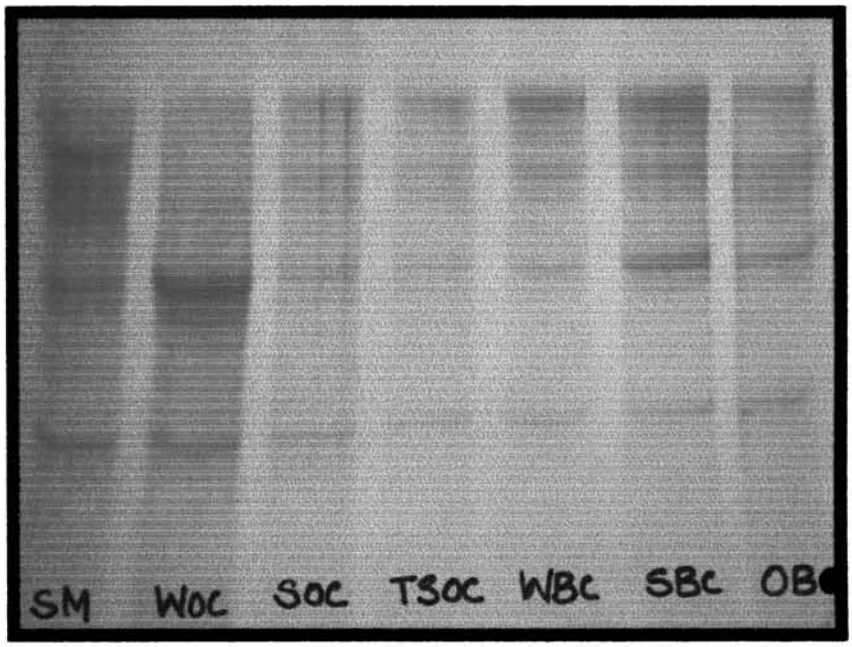
Plate 3.1



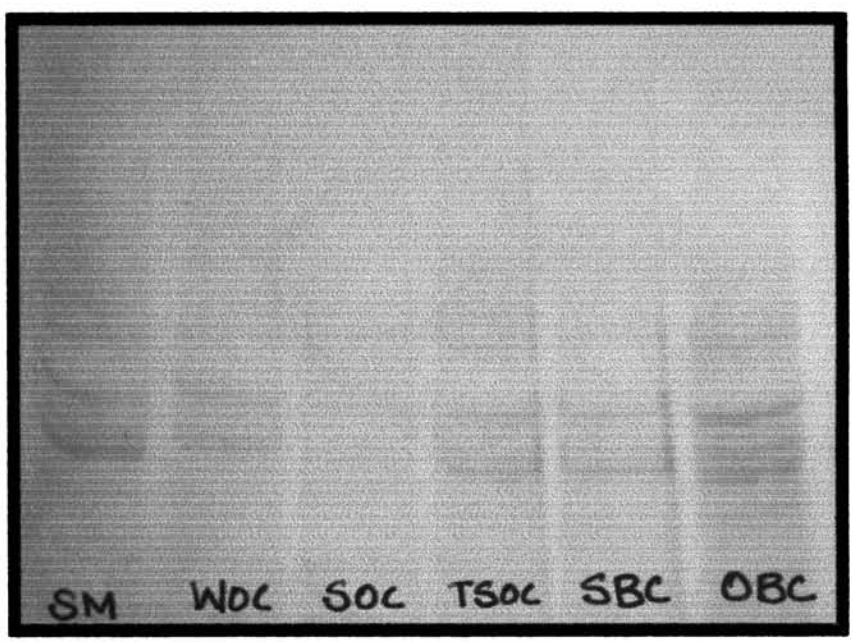
A. Native PAGE Electrophorogram of muscle protein of male morphotypes of *M.rosenbergii*



B. *SDS* PAGE Electrophorogram of muscle protein of male morphotypes of *M. rosenbergii*



A Sarcoplasmic protein fraction of muscle tissue of *M.rosenbergii* (Male morphotypes)



B Myofibrillar protein fraction of muscle tissue of *M.rosenbergii* (Male morphotypes)

proteins in terminal and sub-terminal morphotypes indicate a change in the protein nature and mass in morphotypes like WBC, SBC and OBC. When compared to SM, all the successive morphotypes possess an additional intermediate band, which increases in number for native PAGE and position for SDS-PAGE. Difference in banding pattern commensurate with developmental pathways points out the fact that there exists a significant difference in the nature (acidic or basic) or molecular weight of individual proteins differs among morphotypes. Results of SDS-PAGE clarifies the occurrence of additional low molecular weight proteins at the base of the gel for terminal stages, while initial morphotypes like SM and WOC possessed fewer low molecular weight proteins bands.

The result of protein fractionisation of muscle tissue of *M. rosenbergii* is given in Table 3.1. Maximum percentage of protein fraction for all the morphotypes is contributed by myofibrillar protein fraction followed by sarcoplasmic protein fraction. A significant difference in percentage occurrence of all the six protein fractions was noted amongst them only sarcoplasmic proteins and stroma connectin showed a definite sequence. The percentage of connectin fraction in tissue increased along with morphotypic transformation while the trend was reverse for sarcoplasmic proteins. Figs 3.3 and 3.4 show the electrophoretic evaluation of the nature of protein in sarcoplasmic and myofibrillar protein fractions respectively. The sarcoplasmic and myofibrilar protein electropherogram of the seven morphotypes differed from each other, the main difference being heavier bands at the terminal morphotypes (SBC and OBC), which were quite dense. The result of the gel electrophoresis shows that

there is a significant difference in the type and nature of protein in sarcoplasmic proteins while such a difference could not be seen for Myofibrillar protein fractions.These inturn suggest the structural differentiation in the protein structure of different male morphotypes.

4. Discussion

Phenotypic value of a quantitative character is attributable to the influence of the genotype. However, an accurate estimation of the relative effects of these factors could not be established till date. The classical approach to this problem has been to obtain correlations between phenotypic scores and genotypic relatedness. Hence an attempt to correlate phenotypic values of quantitative characters with the help of genetic indices such as biochemical and electrophoretic heterozygosity have been discussed. Growth in the animals has traditionally been expressed as a change in either length or weight or increase of dry weight body mass over a period of time (Buckley, 1979). Accompanying and underlying these changes in weight and morphology, changes in biochemistry and chemical composition of the animal might have taken place simultaneously. It has well been established that proteins are direct products of gene action (Boulton and Knott, 1984). As gene controlled proteins form the structural basis of biological diversity and therefore, protein variations can be used as excellent sources in establishing genetic basis at various levels of species organization. In the present study the protein content in the muscle tissue of different morphotypes was found to vary from 16.28 to 22.47%. The difference in the protein contents could directly be corroborated to difference in phenotypic traits

and in turn show a difference in somatic growth. The results of the present study were found comparable with that of Sureshkumar and Kurup (1998a).

Biochemical variation in protein is contemplated through the results of native and SDS –PAGE. The difference in the protein banding among the morphotypes was clearly figured for both these systems. With a progression from t-SOC onwards, the member of intermediary bands begins to rise indicating the shift of the protein nature from a relatively high molecular weight proteins of SM to low molecular weight proteins of OBC. This could basically be due to the fact that with the progression of SM → SOC → OBC, the pigmentation also increases. Since majority of the pigments fall under the low molecular weight sarcoplasmic protein fractions (Van Wormhoudt and Favrel, 1988), their presence in terminal morphotypes was highly complementary. For SDS-PAGE the presence of additional intermediate bands explains the possibility of different types of proteins (Laemelli, 1970), which indirectly points out to the presence of different sub protein fractions within each protein group (Hashimoto *et al*; 1979). A similar study on sarcoplasmic proteins was carried out in Caranagid fishes (Bindu and Jayaprakas, 1999) and the results of the present study are well comparable to them. Results of electrophoresis also revealed the nature of proteins present among the different morphotypes. In general, the trend shifted from lesser acidic protein bands in SM to a more acidic protein band of SOC. Results of the present study is useful in establishing the molecular changes in the protein nature during morphogenesis and development as envisaged by Boulton and Knott (1984) following the works in other Palaemonids using morphology and electrophoresis as yardsticks. Similar results could be established on growth

related protein variations in *Macrobrachium ohione* (Trudier, 1978). Electrophoretic analysis of muscle protein of prawns in east coast of India has been studied by Sriraman *et al.* (1995).

Results of the banding pattern from native PAGE show strong agreement with the results of amino acid profiling presented in chapter 2. More acidic bands were observed in SOC and succeeding stages such as t-SOC, WBC, SBC and OBC. Though the protein bandings patterns for all the morphotypes were visualized, the corresponding composition of each band and inter-bands could only be established through the results of protein fractionisation. Representation of different subfractions yielded a clear knowledge about the changes in protein nature and structure during morphogenesis. Moreover, with the electrophorogram of the major proteins, their relative position and contribution in the banding pattern of PAGE was well established. The major protein band seen in the native PAGE corresponds to myofibrillar fraction whereas the sarcoplasmic proteins, which differed in number and thickness, were represented as inter-bands. Hence, it can be concluded that during morphogenesis, a sizeable change in the quantity and quality of the sarcoplasmic protein may be held responsible for the changes in banding pattern and ultimately the protein structure itself. Another noteworthy finding is that, with an increase in the percentage of myofibrillar protein fraction and connectin fraction of the muscle tissue of the terminal morphotypes (SBC, OBC), the texture of the muscle also shows changes for making it more rigid. Alpha-chain sized components in the collagen of larger *M. rosenbergii* have been extracted from the muscle tissue by Kimura and Tanaka (1986). Similar findings could also be

noticed wherein in the present study the percentage of connectin was high in larger BC morphotypes. Variations in the subunit distribution of sarcoplasmic protein and myofibrillar protein during post mortem changes in *Pandalus japonica* were observed by Pyeun *et al.* (1984). The percentage of these subunits from this species was recorded to be 32% sarcoplasmic protein, 56% myofibrillar protein, 10% residual intracellular protein and 2% stroma. These results of muscle protein fractionisation in the present study also showed more or less similar values. Similarly, changes in the myofibrillar proteins and texture of freshwater prawn, *M. rosenbergii* on ice storage was reported by Kye *et al.* (1988) who reported that there was significant increase in the percentage of myofibrillar fractions during storage, which reciprocally made the texture tough. In the present study, commensurating to the morphotypic transformation a corresponding increase in the percentage of myofibrillar fractions could be discernible, which made the texture to the muscle tissue in larger morphotypes tougher.

Secondly, each morphotype by virtue of having specific biochemical composition is characterized with a biological status unique to it at which it is fitted. Thus biochemically morphotype are different from other. The nature and type of protein among these morphotypes showed distinct variations, which were further confirmed by the results of amino acid profiling. Amino acid make up of each morphotype is different and hence the protein resulting from the binding of these amino acids also vary in composition and structure giving the muscle tissue a different composition. This could be a major factor whereby a difference in texture of the prawn could be observed when SM changed into

OBC. The results of protein fractionisation further confirmed these finding. From the results of the present study it can be concluded that protein differentiation through fractionisation and electrophoretic separation can be taken as bench marks for further biochemical characterization studies related to intraspecies variations in protein synthesis. Moreover, the results of the present study manifest the possibility of genetic involvement in the size disparity among male morphotypes of *M. rosenbergii*.

Table 3.1 Protein fractionisation subunits in the muscle tissue of various male morphotypes of *Macrobrachium rosenbergii*

Morphotypes	Protein (% wet weight)	Sarcoplasmic Protein (%)	Myofibrillar Protein (%)	Alkali soluble Protein (%)	Non-protein compounds (%)	Stroma Protein (%)	Connectin (% of stroma)
SM	16.28 ± 0.26	28.87 ± 1.50	53.76 ± 2.28	1.87 ± 0.97	4.71 ± 1.18	0.98 ± 0.03	9.92 ± 1.12
WOC	20.09 ± 0.31	28.45 ± 1.09	57.42 ± 2.55	1.75 ± 0.95	3.85 ± 1.03	1.06 ± 0.04	9.81 ± 1.64
SOC	21.65 ± 0.25	21.05 ± 1.14	59.61 ± 2.73	1.06 ± 0.34	3.54 ± 0.68	1.52 ± 0.05	14.08 ± 1.68
t-SOC	22.47 ± 0.13	20.67 ± 0.73	56.82 ± 3.24	1.28 ± 0.50	3.71 ± 1.15	1.34 ± 0.02	15.97 ± 1.87
WBC	20.70 ± 0.35	17.89 ± 0.99	59.67 ± 2.12	1.87 ± 0.72	2.81 ± 0.93	1.43 ± 0.02	18.05 ± 2.12
SBC	20.05 ± 0.16	17.83 ± 0.99	60.65 ± 3.04	2.55 ± 0.16	2.65 ± 0.73	1.16 ± 0.03	23.87 ± 2.78
OBC	18.62 ± 0.23	15.42 ±1.20	63.61 ± 2.81	2.70 ± 0.41	2.08 ± 0.43	1.12 ± 0.02	28.93 ± 2.87
MSS between samples	3.44 df = 6	1.265 df = 6	0.854 df = 6	0.421 df = 6	0.625 df = 6	0.144 df = 6	0.244 df = 6
MSS within samples	1.02 df = 72	0.843 df = 62	0.124 df = 62	0.011 df = 50	0.054 df = 50	0.064 df = 50	0.013 df = 50
F value	10.734**	5.9525**	11.6543*	1.0258+	0.8546+	0.1659*	2.0361**

Values are represented as AVG ± SD
\+ Not significant
** Significant at 5 % level (P<0.05)
* Significant at 1 % level (P<0.01)

SM = Small males
WOC = Weak orange clawed males
SOC = Strong orange clawed males
t-SOC = Pretransforming strong orange clawed males
WBC = Weak Blue clawed males
SBC = Strong Blue clawed males
OBC = Old Blue clawed males

Chapter 4.

Pigment migration and related ultrastructural changes in the second cheliped of male morphotypes of *Macrobrachium rosenbergii*

1. Introduction

The colour change in crustaceans had been evinced much interest for research during the past decade. Usually changes in the colouration pattern have been attributed to changes in environment or as resultant of any stressful physiological situations (Brown *et al.*, 1952). Lately, much emphasis has been given to the role of neuro-endocrine control on the extent of colour change (Gorbman and Bern, 1974). Much of the neuro-endocrine apparatus in crustacean is located in the eyestalk in association with various medullary regions of the eyestalk nervous system, especially medulla terminalis (Wu *et al.*, 1986). The spectacular chromatic adaptations exhibited by many crustaceans are the result of complex distribution of the various pigments contained within specialized effectors known as chromatophores (McNamara, 1981a; Bauer, 1981). These effectors are situated directly beneath the epidermis or scattered in the deeper tissues of the body (McNamara, 1979). A typical chromatosome is a branched assembly of cell (chromatophores) whose overall shape does not change. But pigments contained within each chromatophore disperse or concentrate to alter the shade or tint of the chromatosome. When dark pigments are dispersed through chromatophore, an overall dark colouration of the animal will be the net effect, but when the pigment is concentrated the animal appears to be blanched (McNamara, 1981b). In *Macrobrachium rosenbergii*, the sinus gland in the eyestalk contains the hormone, which concentrates or disperses pigments in the

chromatophores (Rao and Riehm, 1989). The pigment-dispersing hormone (PDH) in crustaceans is believed to be responsible for pigment migration and corresponding colour changes. Although concerted attempts have been made to unravel the ultrastructure of various crustacean chromatophoric systems (McNamara, 1979; Bauer, 1981 and McNamara and Moreira, 1983), not much effort have been taken to bring out the mechanism underlying pigment migration and related structural changes taking place during growth and maturation in crustaceans.

Among matured population of male *M. rosenbergii* a distinct differentiation in their phenotypic trait is visible. The males not only show a diverse pattern of somatic growth but also have a perceptible difference in the colouration of their second cheliped. This enabled them to be grouped under three morphotypes. All the three morphotypes differ in their size, morphology, physiology and behaviour (Telecky, 1984; Ra'anan and Sagi, 1985; Kuris *et al.*, 1987; Harikrishnan and Kurup, 1997 and Sureshkumar and Kurup, 1998a). These morphotypes represent different phases in the male developmental pathways (Ra'anan and Sagi, 1985). Hence depending on the claw colour and size they are named as Small Males (SM), which has a small, feeble and pale second cheliped; Orange Clawed males (OC) that possess strong orange coloured second cheliped and Blue Clawed males (BC), which has the largest and strongest second cheliped with a peacock blue colouration. Each morphotype, on the basis of its claw colour, occupies a definite position in the social hierarchy either as dominant, subdominant or subordinate males (Harikrishnan and Kurup, 1997). Therefore, along with transformation to subsequent stages, the prawn also

undergoes changes in the colouration and spination of the chela apart from changes in behaviour and growth characteristics (Sagi, 1984).

Studies on the structure and development of crustacean chromatophore, especially that pertaining to *M. rosenbergii* is apparently lacking. Although it is well known that the crustacean chromatophore includes a stellate pigment-containing cells along with spatially fixed tubes through which pigment granules move either centrifugally or centripetally (McNamara, 1981a). The origin and development of these multicellular pigmentory effectors, however, remains unestablished. Although, earlier attempts were made to unravel the mechanism of colour change by studying the chromatophoric pattern in crabs (Quackenbush, 1980), other palaemonids (McNamara, 1979), post larvae of *M. rosenbergii* (Hiramatsu *et al.*, 1985) and embryos and adults of *Macrobrachium olfersii* (McNamara, 1979; McNamara and Taylor, 1987), the phenomenon of changing colour pattern of the claw commensurating with the transformation of morphotypes of *M. rosenbergii* has not yet been addressed so far. Lambert and Fingerman (1977, 1978) proposed a model for the action of red-pigment-concentrating-hormone (RPCH), and red-pigment-dispersing-hormone (RPDH) in *Palaemonetes spp.* The former was mediated through the action of cAMP while the latter acts via Ca^{2+} influx. Ultrastructural studies have revealed the presence of well-developed micro-tubular and microfilamentous systems within crustaceans (Elofsson and Kauri, 1971; Robinson and Charlton, 1973; McNamara and Taylor, 1987). Although many studies have focused on the possible relationships between microtubules, microfilament and pigment migration (Robinson and Charlton, 1973; McNamara and Sesso, 1982), the

micro-anatomy of the crustacean chromatosome is still not well known and data on structural rearrangements associated with pigment movements are lacking in particular. The role of pigment dispersing hormone on the phenomenon of pigment migration needs to be elucidated. No attempt has so far been made to investigate pigment aggregation and dispersion within chromatophores of *M. rosenbergii* during morphological transformation and thereafter the process of changing colour pattern along with morphotypic progression deserves top most attention. In view of the paucity of basic ultrastructural information essentially required for the understanding of pigment translocation and cellular function, on investigation has been undertaken to examine the microanatomy of pigmentory effectors in freshwater prawn *M. rosenbergii* with a view to bring out the structural and hormonal changes associated with pigment migration commensurating with morphogenesis.

2. Materials and Methods

Adult male specimens *Macrobrachium rosenbergii* belonging to single age were collected from grow-out of Kuttanad (S. India) and were transported in live conditions to the laboratory. The prawns were segregated into three main morphotypes (Harikrishnan and Kurup, 1997) and were maintained in oxford blue tanks for a couple of days. Live prawns were narcotised using 5% chloroform and small pieces of exoskeletal tissue from the second cheliped as well as the sinus gland from the eyestalk of the prawns were dissected out. These samples of 1 mm square size were first fixed in 2.5% glutaraldehyde solution at 4°C for 18 hrs. Preparatory techniques for chromatosomes were made following McNamara (1981a). Later the samples

were post fixed in 1% Osmium tetroxide solution for 2 hrs at 4°C. Tissues were then rinsed in 0.1 M phosphate buffer 2-3 times before dehydrating it in a series of acetone solution. Blocks of these tissues were prepared by embedding them in Araldite embedding medium. Ultra thin sections (0.25μm) on a transverse axis were cut using LKB Ultratome III and placed in thin Cu-Ni grids and stained with lead citrate before examining them under Philips CM20 transmission electron microscope at an accelerating voltage of 60KV. All measurements were taken directly by calculating the length (in mm) from the micrograph using a dial caliper and calculated with respect to the magnification of the photograph (McNamara, 1979) and is given in the text as mean values. Mean values for measurements ($P<0.05$) were tested using ANOVA for bringing out differences if any and further through Duncun's t-test in SPSS 7.5 for Windows.

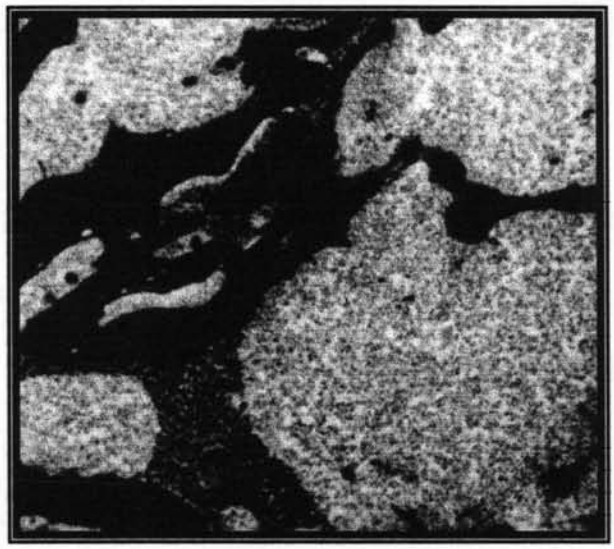
3. Results

The results on the mean values for the measurements of chromatophores and associated organelles in the three morphotypes of *M. rosenbergii* are shown in Table 4.1. ANOVA showed significant difference in the mean length of chromatophores among the three morphotypes. Mean length of chromatophores in SM was much less (51 μm) than that of OC and BC (96 and 158 μm respectively) and the difference was found highly significant (F = 16.351; $P<0.01$). Similarly, significant difference ($P<0.01$) in the number of chromatophores per chromatosome was also seen among morphotypes (F= 7.4509), which ranged between 9.8 to 18.1 in SM and BC respectively. Cell extension that implies the average number of specialized regions in

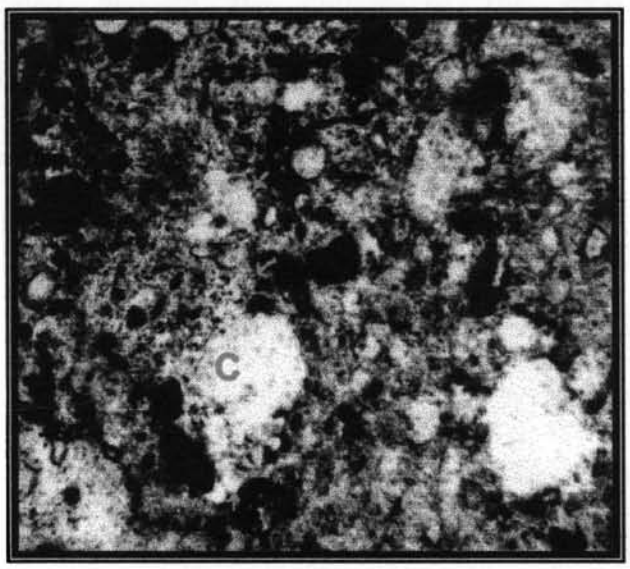
chromatophores that bulge out during the process of pigment incursion was more in BC (1.5 μm) than OC (1.1 μm) and SM (0.65 μm). Among the measurements studied, variation to the highest degree was seen in the number of filled and empty vacuoles among the morphotypes. The values were significantly higher ($P<0.01$) in OC for filled vacuoles (11 μm), on contrary, the number of empty vacuoles were found more in BC (11 μm) and SM (8 μm). Interestingly, no significant difference was seen in the total number of microtubules/ chromatophore, total length of microtubules and space between the microtubules.

The results of the present study showed that the integumentary pigmentary effectors of *M. rosenbergii* are arranged over the body in longitudinally oriented bands, each of which is composed of many juxtaposed chromatosomes and large units of tightly bound chromatophores. Plate 4.1A shows a general view of the cell body of chromatosomes in the claw of *M. rosenbergii* (1800X), while Plate 4.1B shows an enlarged view of the pigment effectors (PG) (chromatosomes) of the second cheliped (3600X). Each chromatosome comprises between 10 and 15 chromatophores and measures some 50 μm in diameter (Plate 4.1B). Plate 4.2A to 4.2C depict the shape and size of the chromatophores in SM, OC and BC respectively. Accordingly, the lengths of these chromatophores were found to be different ($P < 0.01$) from each other and were recorded in the order of 51 ± 8 nm in SM ($n = 21$); followed by 96±16 nm in OC ($n = 24$) and 158±12 nm in BC ($n = 13$). Thus, the basic size of chromatophores among the three major morphotypes was different. Plate 4.2A to 4.2C (8400X) show the general disposition of chromatophores with aggregated

Plate 4.1



A. Chromatophore in male morphotypes of *M.rosenbergii* (1800X)

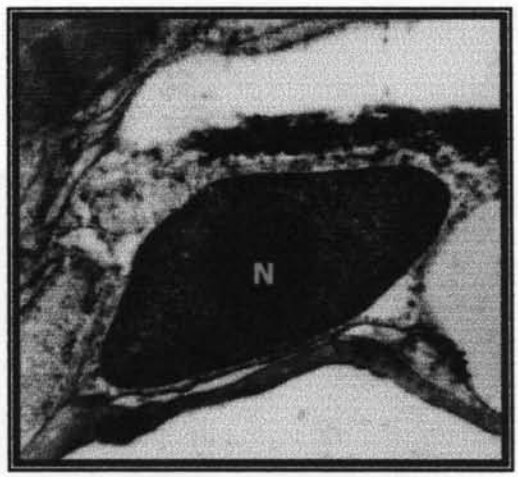


B Enlarged view of Chromatophore (3600 X)

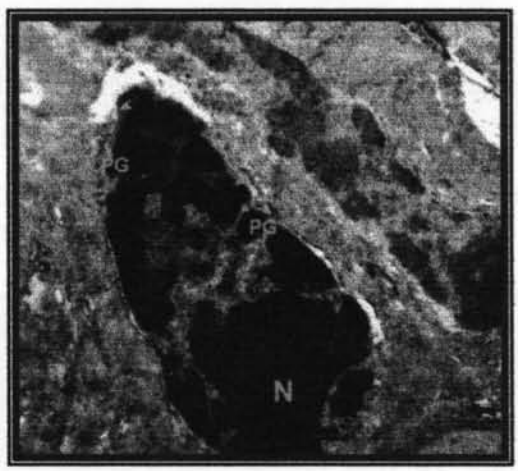
C- Chromatosome
→ Cell extension

pigments in SM, OC and BC respectively. In SM the pigments have agglomerated to form a central mass within each chromatophore while the nucleus usually lie at the base of the cell extension in OC and BC. Prominent electron dense spherical pigment granules were more in OC and BC males than SM. Chromatophores of BC with fully dispersed pigment appear as flattened, slightly elongated bodies of about 150 nm diameter (Plate 4.2C). The breadth of the cell measured between 35-40 nm. Although the cell breadth of the chromatophores of OC and SM did not vary significantly from that of BC, the shape of the cell were rather oval. This reduced the cell length for the above two morphotypes and was measured in the order of 90 and 50 nm respectively (Plate 4.2A and 4.2B). From the Plate 4.1B, it is clearly visible that colour and colour pattern of the claw of *M. rosenbergii* are formed by pigments within chromatosomes located beneath the cuticle of the second cheliped. Commensurating with transformation of SM to OC and further to BC, the shape and size of these chromatophores were found to be changing, which theoretically is a process of cell extension. In SM cell extension was recorded to be 0.65 μm, while in OC and BC it was found to be 1.1 and 1.5 μm respectively. With more and more deposition of pigments (PG) taking place within the chromatophores, the nucleus gets shifted from its initial central position in SM to the base in OC and BC. The amount of hormone present in the present study was assigned by calculating the number of filled exogenous vacuoles (FV) to that of the empty ones (Em) (Nakamura, 1978). The mechanism involved in the hormone expulsion from the active cells in the form of nascent vacuoles is shown in Plate 4.3A (12,400X), while Plate 4.3B (12,400X) shows the expelled hormonal

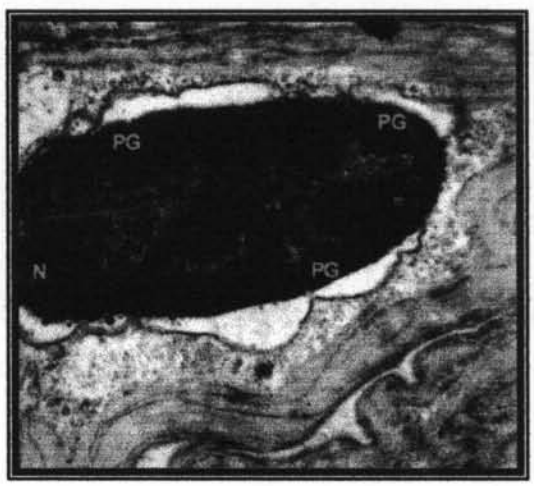
Plate 4.2



A Chromatophore in small male morphotypes of *M.rosenbergii* (8400 X)

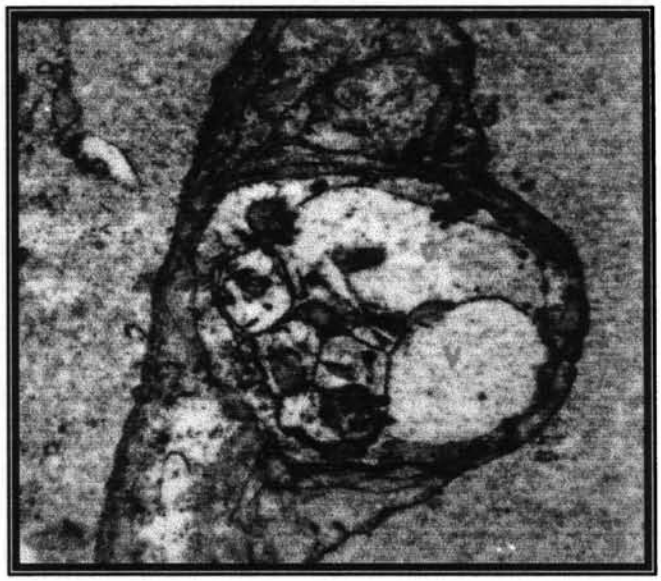


B Chromatophore in Orange clawed male morphotypes of *M.rosenbergii* (8,400 X)

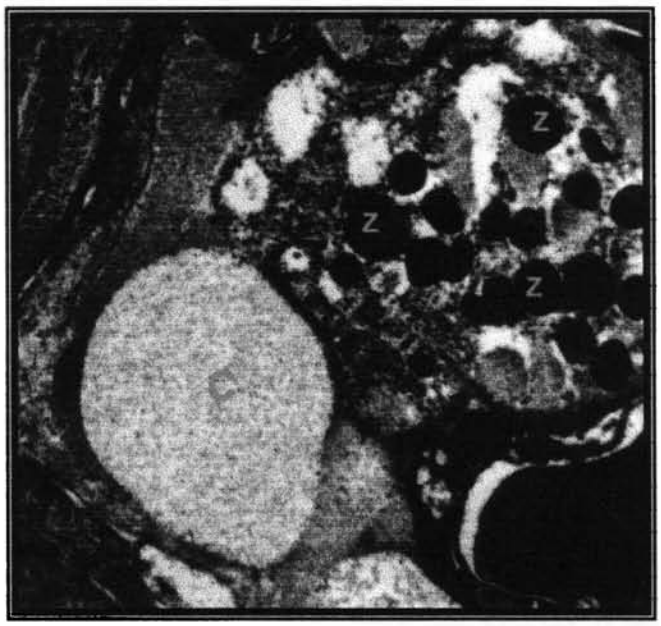


C Chromatophore in Blue clawed male morphotypes of *M.rosenbergii* (8400 X)

N -Nucleus
PG - Pigment granules



A. Formation of nascent vacuoles in orange clawed males of *M.rosenbergii* (12400 X)



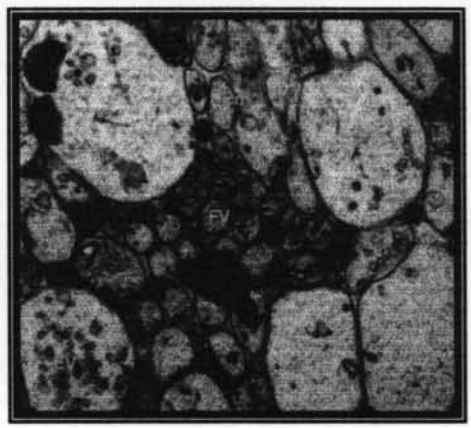
B. Excluded Zymogen granules in *M.rosenbergii* (12400 X)

V-Vacuoles
Z-Zymogen granules
Pl-Plasmalemma
C-Chromatosome

granules in the form of zymogen granules (Z). The electronmicrograph shows high hormonal activity for OC (Plate 4.4B) and SM (Plate 4.4A), while reduced numbers of filled exogenous vacuoles were encountered in BC (Plate 4.4C). The expulsion rate that was measured by counting the total number of budding vacuoles near the cell membrane was high in OC and was recorded to an average of two per cell. The enlarged view (12,400X) of the cell arrangement in sinus gland and the corresponding number of exogenous vacuoles within the intracellular space among the three morphotypes are shown in Plate 4.4A to 4.4C. On further investigation the activity of the cells in the sinus gland showed complementary results to that of the rate of hormone production through exogenous vacuoles. Plate 4.5A to 4.5C (18,200X) show detailed view of individual hormone producing cells of the sinus gland. SM (Plate 4.5A) showed a reduced cellular activity when compared with that of OC (Plate 4.5B). The cisternae and endoplasmic reticulum (ER) complex which is an index of increased protein synthesis in any cell was found high in OC. In total contrast the degenerated cells were found in BC (Plate 4.5C), which would manifest the profitability of growth reduction in this morphotype.

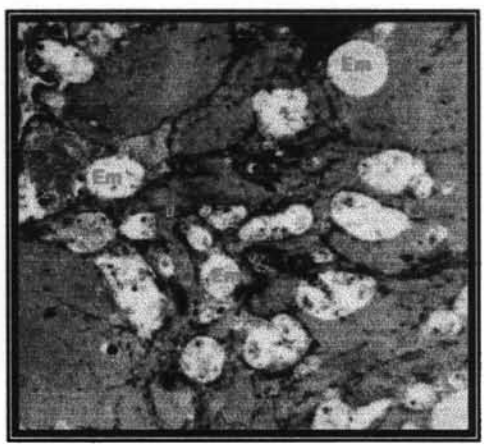
Plate 4.6A shows the enlarged view of the microtubules (10,800X) while Plate 4.6B (8400X) shows the compact arrangement of microtubules in the second cheliped. Pigment dispersion has been shown to occur with the aid of microtubules as seen in Plate 4.6A. In all the three morphotypes studied, the cell body consisted of sparsely distributed vesicles of agranular endoplasmic reticulum and a few microtubules lining the chromatophores. It is by virtue of these microtubules that the pigments enter the chromatophore and in turn cause

Plate 4.4



A Exogenous vacuoles in Small male morphotypes of *M.rosenbergii* (12400X)

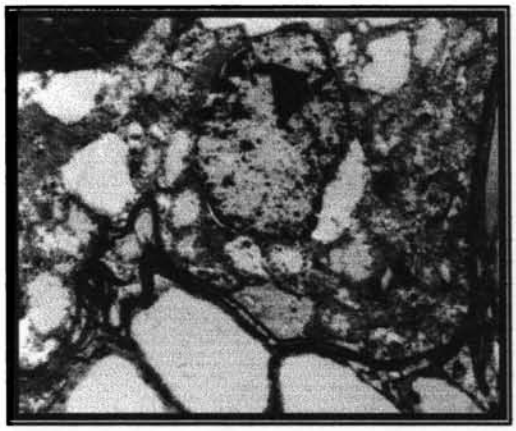
B Exogenous vacuoles in Orange clawed male morphotypes of *M.rosenbergii* (12400X)



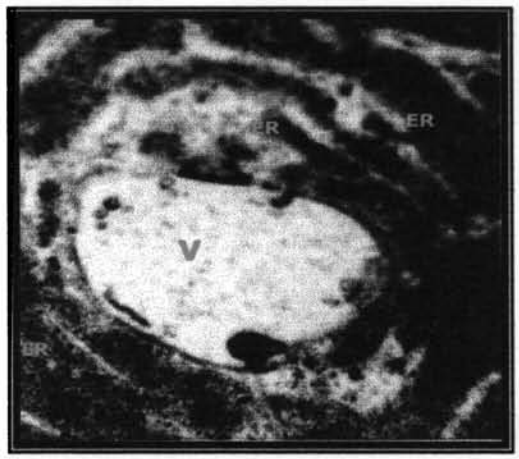
C Exogenous vacuoles in Blue clawed male morphotypes of *M.rosenbergii* (12400X)

Fv- Filled Vacuole
Em- Empty vacuole

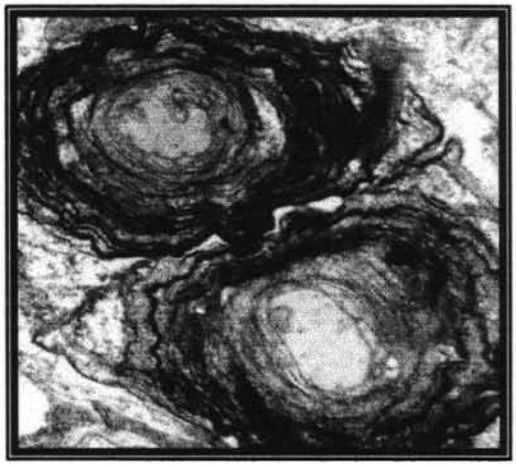
Plate 4.5



A Cells of Sinus gland of small male morphotypes of *M.rosenbergii* (18,800 X)



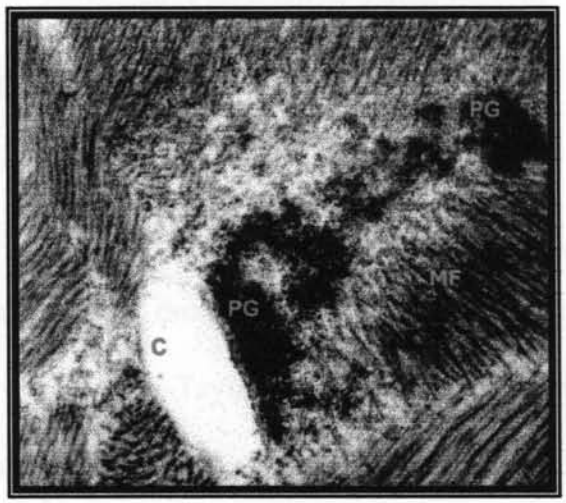
B. Cells of Sinus gland of Orange Clawed Male morphotypes of *M.rosenbergii* (18200X)



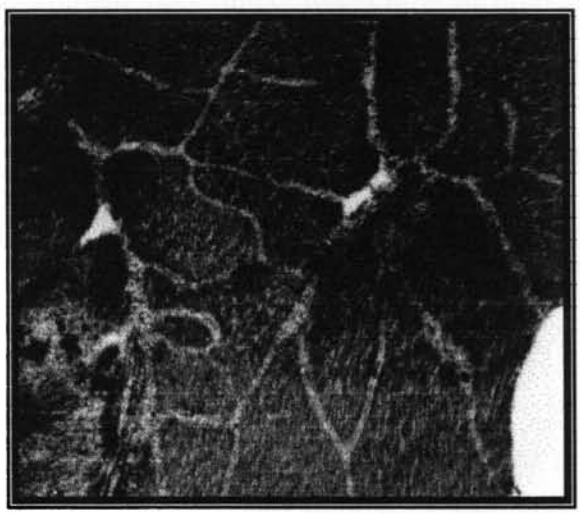
C Cells of Sinus gland of Blue clawed Male morphotypes of *M.rosenbergii* (18200X)

Er- Endoplasmic reticulam
V-Vacuole

Plate 4.6



A Myofibrillar transporting pigment granules in chromatophores of morphotypes of *M.rosenbergii* (10800 X)



B Bundles of myotubules in *M.rosenbergii* (8400X)

C-Chromatosome
PG- Pigment granules
MF- Myofibrillar Tubules

the cell extension in terminal morphotypes. The microtubule bundles do not appear to exhibit any preferential orientation within the cell body and no centres of microtubular organisation are evident. The results show that microtubules of 36-42 nm external diameter run the entire length of the cell extension and typically lie parallel to the longitudinal axis of the projection of extension. The degree of cell extension is directly dependent on the spacing between the microtubules (McNamara and Taylor, 1987). They show an even distribution throughout the cytoplasm exhibiting a centre to centre spacing of 2.7±0.14 nm and numerical density of 27±6 microtubules/ μm^2. The results of the present study show strong indication that once the pigments are fully dispersed (BC), the individuality of the chromatophores comprising the chromatosomes becomes indistinct. In each chromatophore, the pigment now surrounds the nucleus, which also appears to be displaced into the cell extension (Plate 4.2C). The microtubule bundles do not appear to exhibit any preferential orientation within the cell bodies and there is no evidence of centers of microtubular organization or nucleation.

4. Discussion

Based on the results of the present study it can reasonably be asserted that there exist marked variation in the cell shape accompanying pigment aggregation and dispersion in male morphotypes of *M. rosenbergii* and these findings show full agreement with that of McNamara (1980). The ultrastructural information presented in the current study defines the typical features of chromatophore of second cheliped of *M. rosenbergii* primarily and demonstrates certain ultrastructural modifications connected with pigment

movement. The chromatophoric pattern changes according to the growth of the prawn (Hiramatsu *et al*, 1985) i.e. prawns of the same size have almost similar chromatophore pattern but the shade of colouration may differ in individuals. In *M. rosenbergii*, the phenomenon of differential growth is very conspicuous among the single aged male population (Kuris *et al*, 1987). The difference in somatic growth is corroborated by a change in the colour pattern of the second cheliped also, which shifts from pale white in SM to dark orange in OC to ultimately peacock blue in BC. Along with the changes in other body characters, the transformations of claw colour equally qualify the prawn to attain subsequent morphotypic status in its developmental pathway. It could be seen that during the process of transformation from SM to OC and further to BC, the muscle fibre type pattern remains the same, though there are marked changes in the size and colouration of the claws (Govind and Pearce, 1993). It would thus appear that, in *M. rosenbergii*, like any other decapod crustacean, the pigment cells, chromatophores are responsible for imparting colour shades to the second cheliped. Moreover, the intensity of pigment granules differs from morphotypes to morphotypes. The translocation of pigment granules are mediated by microtubules and the quantity of their transfer to the cell may be modulated by chromatophoric or pigment dispersing hormones (PDH). Secondly, a process of cell extension was also seen among morphotypes, which would manifest the possibility that the chromatophore tends to bulge at specialised sites with the aid of microtubules to accommodate more and more pigment granules in it. Indiscriminate deposition of pigment granules and subsequent increase in the cell volume impart shades of colours, which imparts the specific colouration to a

particular morphotype. Hence, the intensity of colour specific to individual morphotype is decided by the quantity of pigment granules within the chromatophore than the type of pigment granules being accumulated. The structural basis of pigment granule translocation within chromatophores has been the topic of a series of investigations. While studying on the neurosecretion of the prawns and various hormonal changes associated with it, Nakamura (1978) reported that the rough endoplasmic reticulum shows the presence of increased protein synthesis. In the present study also, a similar observation could be noticed in hormone producing cells of OC. Also the results of pigment dispersion within the chromatophores corroborated to the earlier reports in crustaceans (Klove, 1995; Miyawaki and Taketomi, 1984; McNamara,1981a; Nowel *et al.*,1998). During pigment aggregation, the entire volumes of the pigment and the cytoplasm appear to be transferred directly to the cell body. In general, the available literature suggests that microtubules provide the motive force for pigment granule translocation within chromatophores (Murphy and Tilney, 1974). McNamara and Taylor (1987) reported that in *Palaemon affinis* microtubules form a central core that is surrounded by pigment granules. A similar pattern of pigment dispersion around the microtubules was noticed in the present study also, but no particular pattern in the distribution of pigments in the chromatophore could be observed. Therefore, it can be inferred that microtubules facilitate the transport of pigment granules directly into the chromatosome. It also appears that regular contraction or polymerisation of the microtubules around the chromatophores is possible, however, the chance of depolarisation is sparse, and thus the colour change in claws becomes progressive from pale white

to orange to deep blue. The ultrastructural features of chromatophores with dispersed pigments observed in the present study (Plate 4.2A to 4.2C) were similar to of McNamara and Taylor (1987) in *P. affinis* and *M. olfersii*. Once the pigments are fully dispersed the individuality of the chromatophore comprising of the chromatosomes become indistinct. Qualitatively, the cytoplasmic contents of cell extension filled with pigments in OC and BC are similar to those of extension without pigments as observed in SM.

Since the claw colour plays a vital important role in determining whether the individual occupies a dominant, sub-dominant or subordinate position in the social hierarchy, the knowledge of pigment migration and chromatophoric colour pattern in different male morphotypes of *M. rosenbergii* is highly essential in explaining the curious phenomenon of colour variation commensurating with morphotypic transformation. Ultrastructural evidences provided in the present study revealed the complex phenomenon involved in pigment migration, dispersion and translocation mechanism within the chromatophore of three male morphotypes of *M. rosenbergii*. Hiramatsu *et al.* (1985) are of the view that the chromatophoric pattern changes in accordance with the growth pattern of *M. rosenbergii* i.e., prawns of the same size have an almost similar chromatophore pattern but the shade of colour may differ in individual prawns. A complex structural relationship exists between the microtubular and chromatosome organelle systems. Ultrastructural changes in the myofibrils of *M. rosenbergii* have been studied by Papadopoulos *et al.* (1989). They explained the role of microtubules in pigment dispersion and brought out the view that, the granules, which usually excluded from the

microtubule bundles, may squeeze between the microtubules and the slightly elastic cell membrane, resulting in the compression of the microtubules into a single axis core. After, or during pigment aggregation, the bundle of compressed microtubules would tend to expand radially, thus because more evenly distributed throughout the cytoplasm (McNamara and Taylor, 1987). Based on the results if the present study, it can be inferred that microtubules provide the motive force and direct means for pigment granule translocation within chromatophores. Physiological experiments conducted based on microtubule-disrupting effects of colchicine (Borisy and Taylor, 1967); vinblastine (Wilson, 1970) and cytochalasin B (Wessels *et al.*, 1971) which used techniques to disrupt the contraction of microtubules through the above chemicals and subsequently monitor the decrease in pigment migration, also confirmed the involvement of microtubules in pigment migration. Although the production and external stimuli controlling the extent of dispersion of these pigments could be established in the present study, quantitative estimation of the chromatophoric and pigment-dispersing hormones through Radio Immuno Assay (RIA) would give a clear evidence of the mechanism involved in pigment aggregation and dispersion. Hence, it is found highly imperative to bring out the role of various hormones on development of colour patterns characteristics of each morphotype of *M. rosenbergii* in the population structure and the consequent social hierarchy. It can reasonably be concluded that there exist a clear differentiation in the structural and cellular activity of chromatophores in different male morphotypes of *M. rosenbergii*. Commensurating with the morphotypic transformation from SM to OC to BC, a change in cellular activity could also be observed. The pigment

migration in *M. rosenbergii* is a unidirectional process, whereby only accumulation and dispersion of pigment granules occur within the chromatophore and the pigments do not move out of the chromatophore. The claw colouration distinctive to a particular morphotype can well be attributed to the difference in the pattern of pigment migration seen among the three morphotypes. With progressive transformation of morphotype to successive stages, more and more pigments enter the chromatophore and in accordance with this, a change in the shade as well as tint of the cell could be seen. The uniqueness in the intensity of claw coloration in each morphotype is in turn the result of pigment aggregation and corresponding cellular extension. A perceptible involvement of microtubules in the transport of pigment granules to the chromatophore could be discernible. Furthermore, the influence of neuro-secretory centres of the sinus gland in the production of pigment dispersing hormone could also be located in the present study.

Table 4.1 Mean values in the size of chromatophores and associated organelle in three morphotypes of *Macrobrachium rosenbergii*

Morphotypes	Number of chromatophores per chromatosome	Cell length of individual chromatophore (microns)	Cell breadth of individual chromatophore (microns)	Cell extension in individual chromatophore (microns)	Number of filled vacuoles	Number of empty vacuoles	No. of total microtubules/ chromatophore	Total length of microtubules (microns)	Spacing between microtubules (microns)
SM	9.8 ± 2.5	51 ± 8	29.4 ± 2	0.65 ± 0.07	3 ± 1	8 ± 2	36 ± 3	22 ± 4	2.2 ± 0.18
OC	14.8 ± 1.8	96 ± 16	30.2 ± 4	1.1 ± 0.12	11 ± 3	4 ± 1	42 ± 2	26 ± 7	2.5 ± 0.34
BC	18.1 ± 3.2	158 ± 12	31.2 ± 4	1.5 ± 0.23	5 ± 1	11 ± 3	38 ± 4	27 ± 6	2.7 ± 0.14
MSS between samples	76.22 *df* = 2	19543.6 *df* = 2	6.047 *df* = 2	0.4228 *df* = 2	137.33 *df* = 2	73.55 *df* = 2	25.76 *df* = 2	54.61 *df* = 2	0.58 *df* = 2
MSS within samples	12.75 *df* = 15	469.92 *df* = 21	15.25 *df* = 18	0.0637 *df* = 18	4.1269 *df* = 21	4.477 *df* = 21	32.74 *df* = 18	24.65 *df* = 18	0.22 *df* = 21
F value	7.4509*	16.351*	0.3964	6.6355*	23.2706*	16.426*	0.7867	2.2157	2.5721

Values are represented as AVG ± SD
** Significant at 5 % level (P<0.05)
* Significant at 1 % level (P<0.01)

SM = Small males
OC = Orange clawed males
BC = Blue clawed males

Section IV

Genetical characterization of male morphotypes of *M. rosenbergii*

Chapter 5

Allozyme variations and genetic diversity among the male morphotypes of *Macrobrachium rosenbergii*

1. Introduction

Genetic resource assessment is considered as the starting point for population management in both fisheries and aquaculture. As a potential candidate species for freshwater aquaculture, *Macrobrachium rosenbergii* has attracted much attention of farmers across the world. However, the uneven growth seen in the adult prawns of a single aged population and the consequent wide size disparity of the cultured stock and skewness in their weight distribution remain a major bottleneck in generating profit from its culture worldwide. This disparity in somatic growth has been ascribed to environmental impacts (Wong and McAndrews, 1990); ontogenic causes (Mashiko, 1983); social hierarchy (Cohen and Ra'anan, 1983) and biogenetic (Malecha *et al*, 1980). The resulting population shows a wide difference in their mean length, weight and phenotypic traits such as claw colouration, enabling them to be grouped under three major morphotypes viz., Small Male (SM), Orange Clawed males (OC) and Blue Clawed males (BC). Unlike in shrimps, the economic yield of *M. rosenbergii* in grow-outs may not be a linear function of total biomass produced due to the chances of predominance of small males in the harvested population. Earlier reports on the characterization of male morphotypes have basically concentrated in defining their biochemical, structural and behavioral differentiations (Sureshkumar and Kurup, 1998c, McNamara and Taylor, 1987; Smith and

Sandifer, 1979). No efforts were made till date to characterize the morphotypes on the basis of their biochemical genetics. Moreover, the distinctive characteristics of individual morphotypes on growth and corresponding morphotypic differentiation warranted the necessity in bringing out the factors underlying differential growth of male morphotypes.

Genetic variability data have proved to be useful in identifying genetic compatibility among populations of a species or between species in hybridization programme (Ricker, 1975). Although sufficient data on the population structure of penaeid prawns are available (Benzie *et al.*, 1992; Benzie, 2000) there are only very few reports in Palaemonids especially *M. rosenbergii*. The limited works on allozymes pertaining to the geographical variations among populations in *Macrobrachium spp.* are that of Hedgecock *et al.* (1979) and Wong and Mc Andrews (1994). Many hypotheses concerning the relationship between geographic niche dimension on one hand and heterogeneity or degree of polymorphism on the other hand have been formulated (Powell, 1971). Assessment of genetic differences can be approached by estimation of genetic variability levels within the studied population. Quantification of allozymal variations has been a common successful practice to achieve information on genetic variation (Park and Moran, 1994; Velez *et al*, 1999; Shaklee *et al.*, 1990; Heath *et al.*, 2002). This method implies the tissue homogenates of individual samples from a population are subjected to electrophoretic separation, and variations in enzymatic activity is revealed by means of specific histochemical stains (May, 1992). In the present study, an attempt was made to evaluate the hypothesis that could explain the positive correlation between heterozygosity and

growth rate. If heterozygosity at the enzyme loci is responsible for the positive correlation then a correlation between electrophoretic heterozygosity and growth should be seen, which in turn may manifest the difference in phenotypic variation of male morphotypes of *M.rosenbergii.*

2. Materials and Methods

A) ***Sampling and Measurement***: Male morphotypes of *M. rosenbergii* were randomly drawn from a single aged population from the grow-out of Kuttanad (S. India) during 1999-2000. They were transported in live conditions to the laboratory and sorted out into different morphotypes (Harikrishnan and Kurup, 1997). Total body length, carapace length, length of second cheliped (ischium, merus, carpus, propodus, dactylus), length of telson, second pleura and total body weight of the specimen were measured by a vernier calipers to ±1 mm accuracy and the live weight was measured by an electronic balance nearest to 0.1 g.

B) ***Electrophoresis***: Electrophoretic analysis was made of abdomen muscle from 72 (24 prawns X 3 morphotypes) specimens (Smith *et al.*, 1978a). Just prior to electrophoresis, 0.5 g subsample of tissue was homogenised in 3 times its volume of 0.03% nicotinamide-adenide dinucleotide (NAD, in water) (Wong and McAndrews, 1990). Homogenate of muscle was centrifuged at 15,000 rpm at 4°C for 25 min. The supernatant was kept at 4°C, and used for electrophoresis within a day. Electrophoresis was conducted in a mini dual vertical gel electrophoretic apparatus (Bangalore Genei) using a running gel concentration of 7.4% (pH of stacking gel was 8.8) for 2 hr at a steady electric current of 30 amp and 100 (Benzie *et al.*, 1992. Eleven enzymes and

15 loci were studied (Nelson and Hedgecock, 1980; Li *et al.*, 1993a and Boulton and Knott, 1984) (Table 5.1).

C) Analysis of Data:

(i) **Interpretation of Electrophoretic pattern:** The enzyme banding pattern of the phenotypes were compared between individuals at a particular gel area (Nei, 1975). Phenotype variants observed between the individuals in term of differences in the distance traveled by particular band (S) at that particular gel area are designated as slow moving (S) in one individual, fast moving (F) in another individual and slow-fast moving (SF) when a combination of these two occurred in yet another individual. As a standard practice, these protein phenotypes are presumed as genotypes produced by co-dominant alleles at a particular genetic locus. Thus, individuals having S, F and SF genotypes were scored as slow moving and fast moving homozygotes and slow-fast moving heterozygote respectively. Depending on the enzyme structure, the heterozygote patterns are again scored as monomeric, dimeric and tetrameric viz. 2,3 and 5 banded patterns respectively (Velez *et al.*, 1999).

(ii) **Allelic frequencies** (Table 5.2): Allelic frequencies were calculated directly from genotype frequencies (Nei, 1975). Genotype frequencies are proportion of each genotype in total number of individuals tested for each locus.

Thus frequency of S allele = Frequency of SS genotype plus half the frequency of SF genotype.

Frequency of F allele = Frequency of FF genotype plus half the frequency of SF genotype.

Allelic frequency was calculated using the formula = $\frac{Ho \times 2 + He}{2N}$

' Ho' is the observed number of particular homozygote, 'He' is observed number of a particular heterozygote and 'N' is the total number of individuals tested.

(iii) Heterozygosity (Table 5.3): Heterozygosity was directly estimated from the number of heterozygotes present in the total number of individuals tested (Abdullah and Shukor, 1993). Average heterozygosity in the species was calculated by estimating heterozygosity for each locus in each morphotype followed by their averages for the number of locus tested. Both polymorphic and non-polymorphic loci tested were considered for calculation.

(iv) Genetic Identity and Genetic Distance: Genetic identity and genetic distance within morphotypes were tested following Utter (1987) and Nei (1975) using the formulae

Genetic distance D = -1 (I) for a single locus

Genetic Identity $I = \frac{\Sigma X_i Y_i}{\sqrt{\Sigma X_i^2 Y_i^2}}$

where values of 'Xi' and 'Yi' are the frequencies of specific alleles in the population 'X' and 'Y' respectively. When more than one locus is involved in a protein system tested, average of (I) value for all loci considered is taken for calculating the (D) value for that protein system between X and Y population. The average (I) and (D) values for all loci tested for two populations thus compared are found out.

Weir and Cockerham's (1984) *F*-statistic analysis was used to partition genetic variation into that occurring within populations (F_{IS}) and that

occurring between populations (F_{ST}). The significance of F_{IS} and F_{ST} values were tested by using chi-square. For tests of F_{IS}, chi-square equals $N(F_{IS})^2(k-1)$, with degree of freedom (*d.f*) equal to (k(k-1))/2, where N is the total number of individuals sampled and k is the number of alleles at the locus. For test of FST, chi-square equals 2NFST(k-1), with d.f equal to (k-1)(s-1), where N and K are as defined above and s is the number of population sampled (Waples, 1987).

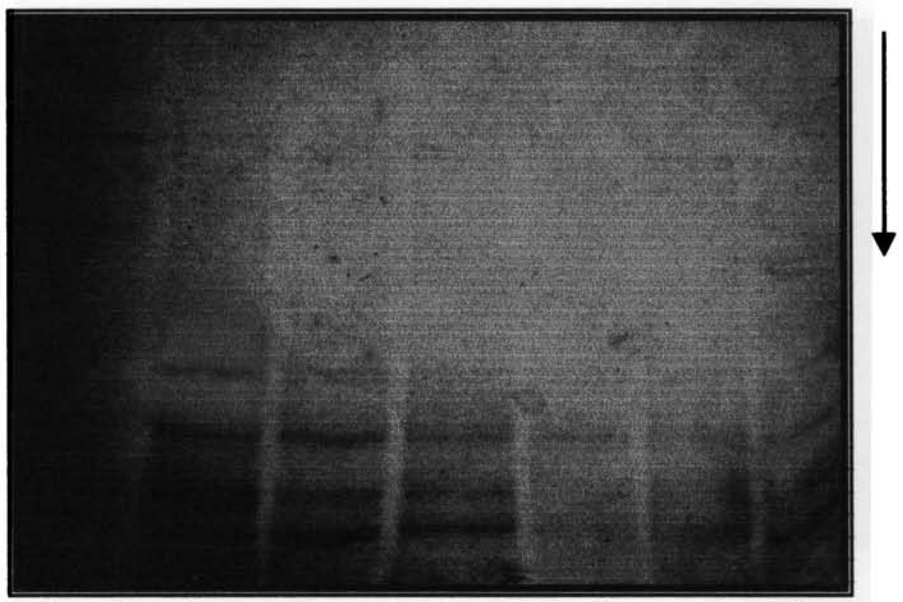
3. Results

Of the eleven enzyme system analysed difference in banding pattern was evident in 5 allozymes *viz*; *MDH**, *G6PDH**, *AAT**, *EST** and *PGM**(Plate 5.1 to 5.3). The results showed that out of the 15 loci examined 6 were monomorphic and 9 were polymorphic (Table 5.1).

(A) Polymorphic enzyme systems: The zymogram pattern of malate dehyrogenase (*MDH**) (EC 1.1.1.37) in male morphotypes of *M. rosenbergii* is shown in Plate 5.1A. The zymogram showed a single zone of enzyme activity consisting of either a single or double banded phenotype in SM, OC and BC. One polymorphic (single slow band) phenotype was seen in the present study. The expression of this band in the three morphotypes was different.

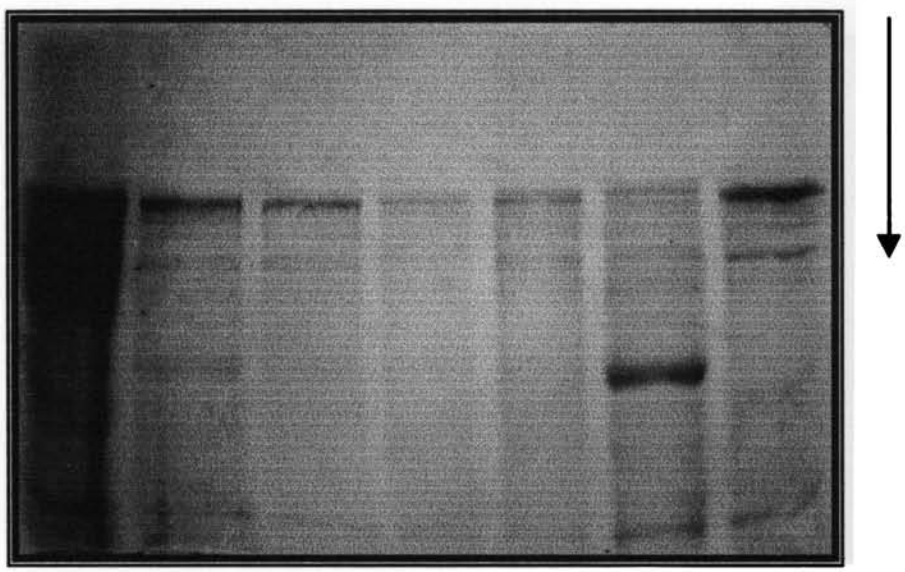
The enzyme banding pattern of Glucose 6-phosphate dehydrogenase (*G6PDH**) (EC 1.1.1.44), aspartate amino transferase (*AAT**) (EC 2.6.1.1) and esterase (*EST**) (EC 3.1.1.*) is shown in Plate 5.1B, 5.2A and 5.2B respectively. In all these three enzyme systems, a polymorphic pattern of bands was observed. A close comparison of these three phenotype variants

Plate 5.1



1 2 3 4 5 6 7

A. Allozyme banding pattern of *MDH* in male morphotypes of *M.rosenbergii*

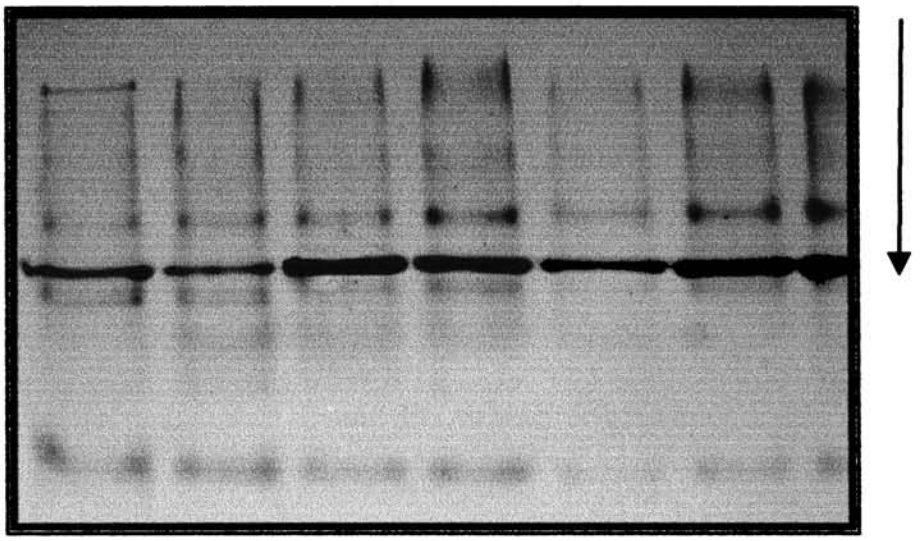


1 2 3 4 5 6 7

B Allozyme banding pattern of *G6PDH* in male morphotypes of *M.rosenbergii*

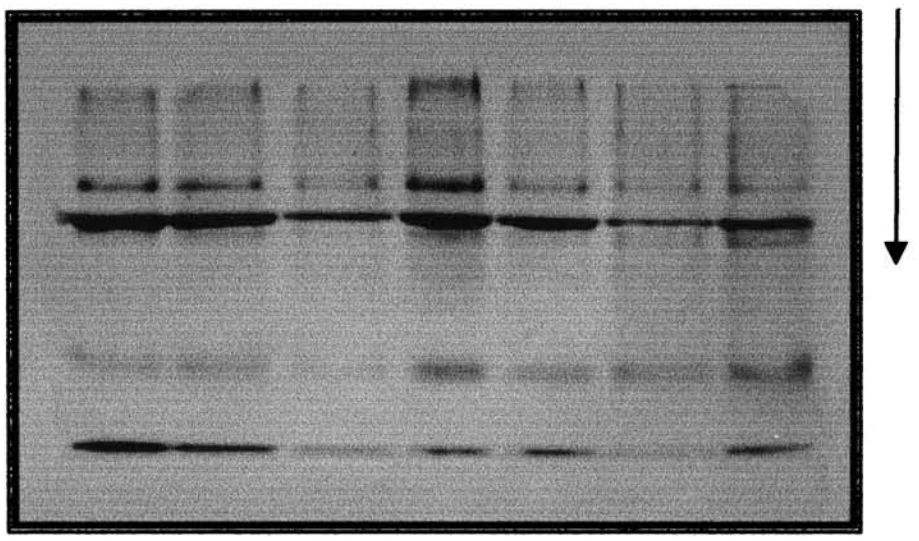
Column 1 Belongs to Small male(SM), Column 2-4 belongs to Orange clawed males(OC) and Column 5-7 belongs to Blue clawed males (BC)

Plate 5.2



1 2 3 4 5 6 7

A . Allozyme banding pattern of *EST* in male morphotypes of *M.rosenbergii*



1 2 3 4 5 6 7

B. Allozyme banding pattern of *AAT* in male morphotypes of *M.rosenbergii*

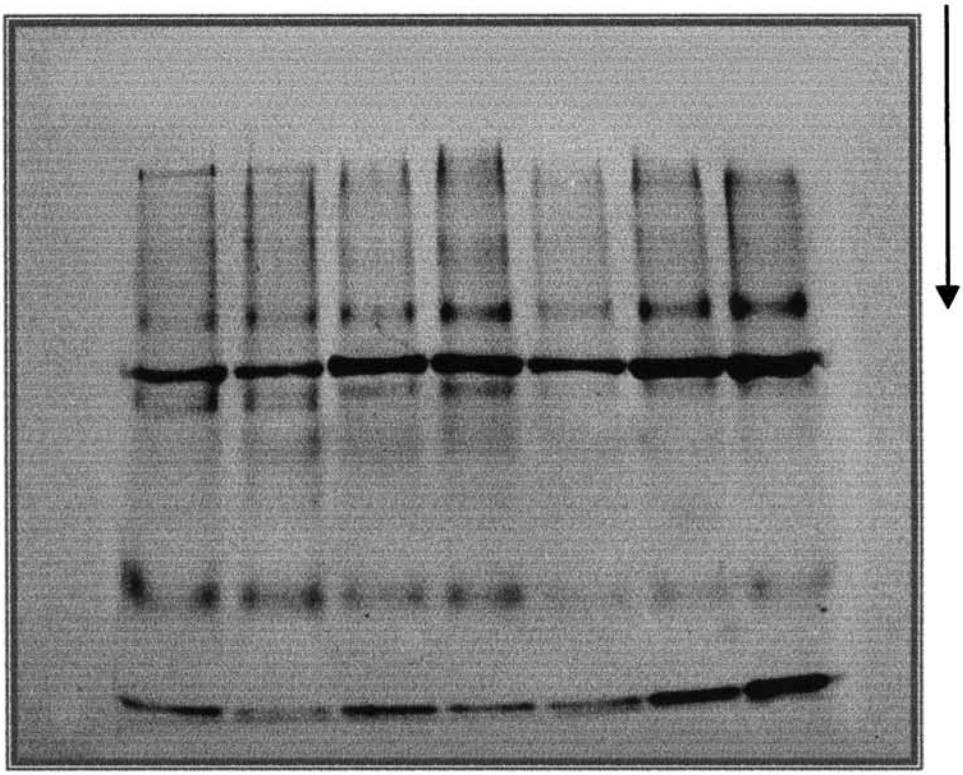
Column 1 Belongs to Small male(SM), Column 2-4 belongs to Orange clawed males(OC) and Column 5-7 belongs to Blue clawed males (BC)

suggested that they might be co-dominant di-allelic products at a single locus. The presumed genotypes are slow and fast single banded homozygote.

The zymogram pattern of phospho glucomutase (*PGM**) (EC 5.4.2.2) in male morphotypes of *M. rosenbergii* is shown in Plate 5.3. The zymogram showed multiple zone of enzyme activity consisting of either double-banded phenotype in SM, OC and BC. Three polymorphic (Two slow fast heterozygote and a single slow homozygote band) phenotype was seen in the present study. The expressions of *PGM** loci in all the three morphotypes were different.

Summary of statistics describing genetic variability (Table 5.2) showed variable degrees of polymorphism among the populations of the three morphotypes. With the exception of lower polymorphism observed in OC (16%), the proportion of polymorphic loci increased in BC (19%) and SM (28%). Allelic frequencies, observed heterozygosities and indices of heterozygote deficiency for 9 polymorphic loci are shown in Table 5.2 and 5.3 respectively. Sample size and level of polymorphism permitted goodness-of-fit tests to Hardy-Weinberg genotype proportions only in 5 cases (Table 5.2). The average number of allele per locus per morphotype ranged from 0.64 (SM) to 1.24 (BC). Average observed heterozygosities ranged from 2.6% in OC to 3.5% in BC and 4.2% in SM (Table 5.2). There was no significant difference between the observed and expected average heterozygosities (Table 5.3) in any of the morphotypes ($P>0.05$). Five out of 9 polymorphic loci were found to have significant heterozygosity of allelic frequencies among the morphotypes. The bulk of the significance was contributed by three loci *PGM**, *AAT** and *G6PDH**. Table 5.4

Plate 5.3



Allozyme banding pattern of *PGM* in male morphotypes of *M.rosenbergii*

Column 1 Belongs to Small male(SM), Column 2-4 belongs to Orange clawed males(OC) and Column 5-7 belongs to Blue clawed males (BC)

summarizes the genetic variation in the three main morphotypes of *M. rosenbergii*. Significantly higher total G values were recorded in *PGM** (27.93), *AAT** (20.06) and *G6PDH* (20.01) and these corresponded significantly to the F (ST) ($P<0.01$). Nie's (1972) unbiased genetic distance statistics for 5 enzymes showed that the genetic distance among the morphotypes was small. Distance of genetic diversity was less for SM←→OC (0.0021) followed by OC←→BC (0.0043) and SM←→BC (0.0055) (Table 5.4). The difference of banding for allozymes was found significant ($P<0.01$) showing definite loci for all the 5 enzymes were present. The mean observed heterozygosity was 0.042 ± 0.013 for SM, 0.026 ± 0.017 for OC and 0.035 ± 0.022 for BC. Table 5.5 shows the *F*-statistics within and between population and also the results of chi-square tests for gene frequencies among the three morphotypes of *M. rosenbergii.* Except for *PGM** the values for F_{IS} were significantly low in other enzyme groups showing very low genetic diversity within each morphotypes ($P>0.05$). On the contrary a distinctive difference for the five isozymal groups were evident during FST analyses. Although the mean value for F_{ST} and Nei's unbiased F_{ST} values (0.0267 ± 0.0044) showed only slight difference ($P<0.05$) among the morphotypes, the result could be used as an index in genetic diversity between the morphotypes. Comaprison of gene frequencies between populations, using chi-square, confirmed the general pattern of variation revealed by F- statistics analysis (Table 5.5).

The allozyme patterns obtained from the three morphotypes of *M. rosenbergii* for these five enzyme systems are shown in Plate 5.1 to 5.3. The muscle protein zymograms obtained for *AAT** and *G6PDH** among all the

morphotypes were clearly distinct. Specific allelic variations in the banding pattern for *PGM** was seen in OC and BC morphotypes. An overall comparison of the number, the width and staining intensity of each zymogram group indicated significant variations between the three major morphotypes studied. A close comparison of these allozyme band variations between zymogram groups at a particular gel area indicated that they might be allelic products of a particular locus. Thus general comparison of varying and non-varying bands at a particular gel area for all groups indicated the existence of one definite polymorphic loci for *MDH** and *G6PDH**, two polymorphic loci for *AAT** and *EST** and three polymorphic loci for *PGM** responsible for expression of all the muscle protein phenotype observed in the harvested population of *M. rosenbergii*.

4. Discussion

Phenotypic differences among populations may be due to both genetic and environmental differences (Malecha, 1986). Although preliminary attempts to unravel the phenomenon of HIG hidden within each morphotype have been addressed through proximate composition analysis (Sureshkumar and Kurup, 1998a) and subsequently by Ranjeet and Kurup (2001a) through electrophoresis of protein and Amino acid profiling, no concerted attempts have been made to use species-specific allozyme markers to understand the genetic diversity associated with each morphotype. The phenotypic value of a quantitative character has been attributed to the influence of the genotype and environment (Li *et al*, 1993). The classical approach to this problem has been to obtain correlation between phenotypic scores and genotypic relatedness, but there have been several attempts to correlate phenotype values of quantitative

characters in palaemonids with other genetic indices, such as enzyme heterozygosity (Boulton and Knott, 1984). The results from the present study indicate that the single aged adult males of *M. rosenbergii* from the grow-outs of Kuttanad (S. India) are different within morphotypes, both in morphology and biochemical genetical marks. The combined results of the morphology and biochemical genetic markers analysis demonstrated that SM is closely related to OC, while the population of BC was more distinct from SM and OC. In population genetics studies based on interpretation of electrophoretically detectable banding patterns, the results and their logical conclusion are as robust as the accuracy with which the observed banding patterns are interpreted. Since direct inheritance test to verify the genetic interpretation of the banding pattern for nine polymorphic loci analysed in the present study were not feasible, verification had to be done through indirect evidence of enzyme systems. The subunit structure of homologous enzymes and genetic basis of such electrophoretic variations in invertebrates has been demonstrated in *Homarus americanus* (Hedgecock *et al.*, 1979) and *Metapenaeus bennettae* (Salini, 1987). Moreover, detailed species inventorisation through the techniques of genetic markers enabled to differentiate *Palaemonetes australis* with *Macrobrachium intermedium* (Boulton and Knott, 1984). Many fish species are differentiated into subspecies, populations and semi-isolated subpopulation (Li *et al.*, 1993). If these subpopulations are more or less isolated genetically from one another, they may differ in their allelic frequencies in one or several loci. Similarly in *M. rosenbergii* with a distinct morphotypic status evident among the single aged adult male population showing a diverse phenotypic trait characteristic of each

morphotype, the distribution of allele in the gene loci were envisaged to differ. Since allozymes are the different molecular associations of the same enzyme their relative molecular weight and electrical charge are different. The differences in electrical charges are brought about by difference in the content of acidic and basic amino acid composition. Though the molecular association is different, the chemical function is almost the same. Hence allozyme pattern of the morphotypes may possibly not seem alike but their expression will be the same. Depending on the loci where they are present their expression in the form of phenotypic traits may also differ. According to available information, a significant positive correlation was found between enzyme heterozygosity and numerous phenotypic characters (Stobart and Benzie, 1994). The present study was setup under this background.

The results of these standardisation procedure in the present investigation suggest that all known protein systems may be present in all the morphotypes of *M. rosenbergii* and their presence are made visible only when suitable standardised electrophoretic method is formulated. The other important information generated by the present standardised methods are that the same identified enzyme expressed specific electrophoretic patterns in almost all the morphotypes either in terms of total number of bands or relative mobility of different bands or staining intensity of different bands or in a combination of any of these factors. This indicates suitability of muscle tissue for allozyme studies as observed in fishes (Salini and Shaklee, 1988). Earlier investigation in *M. rosenbergii* by Ranjeet and Kurup (2001a) showed that Protein, amino acid and RNA/DNA ratio, which are the major intrinsic factors associated with growth,

vary with morphotype and in turn could be inferred that each morphotype of *M. rosenbergii* is characterised by a definite physiological makeup. The present study also suggests that the visible degree of enzyme variability may be due to fundamental differences in the physiological function of each of these morphotypes. The results obtained in the present study revealed the existence of significant variation in the biochemical genetic characterisation of the male morphotypes of *M. rosenbergii*. The results were complementary to the earlier reports on the population structure difference seen in other penaeid prawn species observed by Duda and Palumbi (1999) in *P. monodon* and Velez *et al.* (1999) in *Penaeus brevirostris* and *P. vannamei*.

In the present study, nine polymorphic loci represent nine allelic loci that expresses differently in the three major morphotypes. Genetic differences induced by the allozyme-banding pattern for *MDH** and *PGM** loci seen in the present study have been reported to form polymorphic loci in *Homarus gammarus* (Jorstad and Farestveit, 1999). The allelic differentiation for *AAT**, *G6PDH** and EST have been brought out in *Sparus aurata* by Alarcon and Alvarez (1999). The analyses of population differentiation demonstrated low level genetic variations among the three morphotypes studied. Statistical significance was found for all these 5 enzyme loci, with the samples from BC showing maximum heterogeneity followed by OC. The average heterozygosity for the three morphotypes of *M. rosenbergii* ranged from 2.6 (OC) to 4.2% (SM) and were on a higher side when compared to that observed by Li *et al* (1993a) and Nelson and Hedgecock (1980) in two species of mitten crab, *Eriocheir* Similarly, the genetic identity and genetic distance observed with the three

morphotypes followed an increasing trend from SM to BC. The values recorded for genetic identity during the present study between each morphotypes was higher than those observed in other crustacean by Hedgecock *et al.* (1982). Similarly, the index of genetic diversity was more between SM←→BC (0.0021) followed by OC←→BC (0.0043) and SM←→OC (0.0055). This could be directly related to the transformation pathway envisaged by Kurup and Harikrishnan (1999). The transformation between SM and OC was relatively faster when compared to that between OC and BC. Hence, results of present study would provide invaluable support ting morphotypic pathway of Kurup *et al.*, 1998. The average number of allele per locus per morphotype ranged from 0.64 (SM) to 1.24 (BC). Proportion of polymorphic loci ($P< 0.95$) per morphotype ranged from 5.5% (SM) to 11.3% (BC). Both these results conformed to the disparity of individuals seen with in each morphotype, within each morphotype. Thus, in BC the polymorphic allelic expression is contributed to a large extent by the heterogeneous nature of their individual occupying the three morphotypic stages viz. WBC, SBC and OBC.

Twenty naturally occurring amino acids, in random combination can produce an indefinite order of protein type to create the biological diversity detectable and visible at cellular, tissue and individual level of organization (Yoon and Park, 1997). Thus protein present in any organism is an excellent source for biochemical genetic investigations. In the present study, the differences attributed to the variation in the banding pattern for specific enzyme systems may be due to the difference in the protein product of each morphotype. Concordantly, the earlier reports on the difference in the protein-banding pattern

(Ranjeet and Kurup, 2001a) could well be correlated to the present findings. In a population, all the expected genotypes need not be present. Besides, the frequency of each genotype can also vary within a population (Ayala and Kiger, 1984) and finally genotype frequencies depend on the present population genetic structure and sample population size (Smith *et al.*, 1978). Therefore, the polymorphic nature of specific allele system seen during the observation could be due to the difference in the genotypic scores of each morphotype. In biochemical genetic studies, it is also important to report whether the distribution of observed genotypes at each locus in a population is according to the expected genotypic distribution estimated as per the Hardy-Weinberg law of genotype distribution (Utter, 1987). Although in the present study the data was compiled from a single aged population of adult prawn from a definite locality of the 11 enzyme system tested for 15 gene loci, 5 loci showed significant deviation among morphotypes with P value between just less than $P<0.05$ and more than $P>0.01$. Benzie (2000) and Davis and Hetzel (2000) have collected results showing similar trend in other penaeid prawns such as *P. japonicus*, *P.monodon* and *P. vannamei*. Allozyme variation between two closely related species of *Crangon* have been studied by Abdullah and Shukor (1993).

The high value of genetic similarity between the morphotypes indicate that the degree of genetic differentiation is less, but the results of genetic distance showed a close relatedness between SM and OC morphotypes, while the BC showed maximum heterozygosity with its counterparts. This would otherwise mean that the transformation pathway of SM to OC to BC could genetically be proven. It could therefore be concluded that the results of the

present study on enzyme electrophoresis showed that the three morphotypes of freshwater prawn *M. rosenbergii* differ both in their morphology and pattern of gene expression. In all the three morphotypes 5 separate polymorphic loci were seen. These polymorphic loci occupied specific allelic loci in each morphotype and only the position of these allele differed between morphotypes. The polymorphic loci found during the present study were *MDH**, *G6PDH**, *EST**, *AAT** and *PGM** enzyme systems. The high genetic similarity observed in all the three morphotypes rules out the possibility of a separate species status for each morphotype. But a noteworthy observation was that although it was at low level, there occurs a perceptible variation in the enzyme banding pattern with in three morphotypes, which in turn manifest the possibility of genetic involvement also for the size heterogeneity seen among the single aged population of adult male morphotypes of *M. rosenbergii*.

Table 5.1: List of loci estimated from the corresponding allozyme of *Macrobrachium rosenbergii*

Enzyme	E.C. Number	Organ	Buffer	Loci	Number	Monomorphic (M)	Polymorphic (P)
Alcohol dehydrogenase (ADH)	1.1.1.1	Muscle	TME	*ADH**	1	1M	
Aldehyde oxidase		Muscle	TG	*AO**	2	1M	
Aspartate amino transferase (AAT)	2.6.1.1	Muscle	Tris HCL	*AAT-1**	3		2P
				*AAT-2**	4		
Esterase (EST)	3.1.1.*	Muscle	Tris HCL	*EST-1**	5	1M	1P
				*EST-2**	6		
Malate dehydrogenase (MDH)	1.1.1.37	Muscle	Tris HCL	*MDH**	7		1P
Isocitrate Dehydrogenase (IDH)	1.1.1.42	Muscle and HP	TCE, TBE	*IDH**	8	1M	
Glucose 6 phosphate dehydrogenase (G6PDH)	1.1.1.44	Muscle	Tris HCL	*G6PDH-1**	9		2P
				*G6PDH-2**	10		
Lactate dehydrogenase (LDH)	1.1.1.27	Muscle	Tris HCL	*LDH**	11	1M	
Phosphoglucomutase (PGM)	5.4.2.2	Muscle and HP	TCE	*PGM-1**	12		3P
				*PGM-2**	13		
				*PGM-3**	14		
Octanol dehydrogenase (ODH)	1.1.1.73	Muscle	Tris HCL	*ODH**	15	1M	

****TCE= Tris citric acid EDTA, TBE= Tris boric acid EDTA, TG= Tris glycine, HP= Hepatopancreas**

*** = Allelic loci, E.C= Enzyme classification number (IUBNC, 1984)**

Table 5.2: Allelic frequencies of isozymes of three major male morphotypes of *Macrobrachium rosenbergii* in Kuttanad

Locus	Allele	SM	OC	BC
		(20)	(22)	(21)
*ADH**	100	0.12	0.12	0.15
	111	0.88	0.88	0.85
		(16)	(18)	(22)
*AO**	100	0.04	0.05	0.05
	110	0.96	0.95	0.95
		(13)	(18)	(20)
*AAT-1**	100	0.05	0.10	0.12
	110	0.95	0.90	0.88
*AAT-2**	50	0.10	0.13	0.13
	100	0.90	0.87	0.87
		(22)	(22)	(16)
*EST-1**	100	0.02	0.05	0.05
	108	0.98	0.95	0.95
*EST-2**	81	0.95	0.90	0.95
	91	0.05	0.10	0.05
		(12)	(15)	(15)
*MDH**	100	0.82	0.84	0.80
	110	0.18	0.16	0.20
		(10)	(20)	(10)
*LDH**	100	0.93	0.90	0.90
	110	0.07	0.10	0.10
		(10)	(12)	(12)
*G6PDH-1**	100	0.90	0.86	0.85
	110	0.10	0.14	0.15
*G6PDH-2**	100	0.87	0.90	0.90
	166	0.13	0.10	0.10
		(10)	(17)	(20)
*IDH**	100	0.95	0.95	0.95
	108	0.05	0.05	0.05
		(22)	(12)	(16)
*PGM-1**	100	0.99	0.87	0.85
	91	0.01	0.13	0.15
*PGM-2**	81	0.95	0.85	0.85
	109	0.05	0.15	0.15
PGM-3	100	0.83	0.90	0.95
	110	0.17	0.10	0.05
		(10)	10)	(12)
*ODH**	100	0.90	0.90	0.91
	110	0.10	0.10	0.09
Polymorphic loci (%)		28	16	19
Heterozygosity				
Direct count (H_o)		0.042 ± 0.013	0.026 ± 0.017	0.035 ± 0.022
Expected (H_e)		0.058 ± 0.022	0.054 (0.032)	0.055 ± 0.029

SM= Small Male, OC= Orange clawed male, BC= Blue clawed male

* =Allelic loci, number of specimens analyzed given in brackets

Table 5.3: Genotype and Heterozygosities in male morphotypes of *Macrobrachium rosenbergii*

Locus	Genotype	SM	OC	BC	F (st)	G value
*ADH**	100/100	8	8	7	0.002	0
	100/111	2	2	3		
*AO**	100/100					
	100/110	10	10	10	0.001	9.38
*AAT-1**	100/100	16	16	16	0.032	20.06**
	100/110	4	4	4		
*AAT-2**	50/100					
	100/100	20	20	20		
*EST-1**	100/100	18	16	16	0.021	12.06**
	100/108	1	2	1		
*EST-2**	81/100	1	2	2		
	91/100			1		
*MDH**	100/100	14	14	14	0.021	14.37**
	100/110	5	5	5		
*LDH**	100/100				0.017	9.20
	100/110	12	12	12		
*G6PDH-1**	100/100	2	1	2		
	100/110	18	19	18	0.030	20.01**
*G6PDH-2**	100/100	16	15	16		
	166/166	4	5	4		
*IDH**	100/100					
	100/108	3	3	3	0.004	0
*PGM-1**	100/100	17	17	16		
	91/100	3	3	4		
*PGM-2**	81/100	17	17	17	0.095	27.93*
	100/109	3	3	3		
*PGM-3**	100/100	5	15	15		
	100/110	15	5	5		
*ODH**	100/100	5	5	5	0.0013	0
	100/110					

SM= Small Male, OC= Orange clawed male, BC= Blue clawed male

* = Significant at 1% level, ** = Significant at 5% level

Table 5.4: Genetic Similarity (above diagonal) and Genetic Distance (below diagonal) and Nei's unbiased genetic distance among the three major populations of male morphotypes of *M. rosenbergii*

Morphotypes	SM	OC	BC
SM	_	0. 9979	0.9945
OC	0.0021	_	0. 9957
BC	0.0055	0. 0043	_

SM= Small Male, OC= Orange clawed male, BC= Blue clawed male

Table 5.5. Weir and Cockerham's (1984) *F*- statistics describing within population (F_{IS}), between population structuring (F_{ST}), and heterogeneity chi-square tests for differences in gene frequencies among the three morphotypes of *M. rosenbergii*

Morphotypes	All populations		
	F_{IS}	F_{ST}	Chi-square
AAT-1* ***AAT-2****	-0.024 $^{n.s}$	0.032*	20.6**
EST-1* ***EST-2****	0.013 $^{n.s}$	0.021*	11.4 $^{n.s}$
MDH*	-0.001 $^{n.s}$	0.021*	12.1 $^{n.s}$
G6PDH-1* ***G6PDH-2****	0.028 $^{n.s}$	0.030*	23.9 **
PGM-1* ***PGM-2**** ***PGM-3****	0.106 **	0.95*	47.2 **
Mean	0.0244 $^{n.s}$	0.21*	23.04 **
Unbaised F_{ST}		0.0267 ± 0.0044	

** P<0.05; * P<0.01; n.s- not significant

SM= Small Male, OC= Orange clawed male, BC= Blue clawed male

Chapter 6.

Population genetic structure and difference in the freshwater prawn, *Macrobrachium rosenbergii* by RAPD analysis

1. Introduction

Macrobrachium rosenbergii is an important species for culture in freshwater, both in the tropics and subtropics (Sandifer and Smith, 1985). Although the culture of this species has been carried out in an intensive level in most part of the world, the predominance of undersized non-marketable prawns (small males) during the final harvest has reduced the economic profitability from its culture. Stunting in males of *M. rosenbergii* expressed by a high frequency of small male morphotype in the population is a major obstacle to the viability of prawn culture due to the fact that prawn prices are size dependant. Reduction or elimination of growth suppression is therefore of great importance to prawn culture. Stunting is of particular concern in monoculture and in high-density polyculture since the frequency of small males in the population is positively related to density (Karplus *et al.*, 1986a). Thus understanding the mechanism of growth suppression in male morphotypes would provide essential information in reducing the heterogeneous individual growth exhibited by them. Every animal has a distinct set of genetic materials, DNA that encodes all the details of its phenotypic characteristics. Since *M. rosenbergii* has expressed a wide variation in its phenotypic characters among the morphotypes, it was found of utmost interest to find out whether the differences in growth and appearance are directly related to any variations in the sequencing pattern of DNA. The

results of RNA, DNA, Protein and Allozymal variations between the morphotypes from previous chapters proved the possibility of the involvement of specific genes in the suppression of somatic growth in small males (SM).

Earlier reports of Dai (1988) and Li *et al.* (1993) in decapods *Eriocheir sinensis* and *E. japonicus* that have similar morphological characteristics showed distinctive biochemical genetic characteristics and allowed the taxonomists to group them under two separate species. Since a clear distinction in the morphological features for the three major morphotypes of *M. rosenbergii* viz., Small male (SM), Orange clawed males (OC) and Blue-clawed males (BC) was discernible, the present study was carried out to investigate whether they really belong to a different subspecies or variety. Secondly, are these phenotypic variations being expressed genotypically. Hence it was found highly imperative to quantify the extent of genetic diversity, if any, within the male morphotypes of *M. rosenbergii* of a single age. Heritability and genetic correlations are the parameters that define the extent of genetic variations within the species of a population (Knibb, 2000). Estimation of these parameters is crucial in improving the production from any aquaculture system (Gjerde and Gjedrem, 1984; Eknath *et al.*, 1998). Genetic improvement of farmed aquaculture species has the capacity to deliver cumulative and sustained improvements in production efficiency, product quality and ultimately, financial profitability from aquaculture. In this respect knowledge on the genomic structure of the *M. rosenbergii* shall be useful in unraveling the cause for the differential growth envisaged among its male morphotypes. This in turn would manifest the possibility of improved breeding techniques for this species that

could reduce HIG and corresponding loss due to the fragmentation in the harvested population. In order to investigate the genetic variation and relationship among the morphotypes, a novel genetic technology involving the use of DNA-based tool namely Random Amplified Polymorphic DNA (RAPD) technique (Welsh and McClelland, 1990; Williams *et al.*, 1990; Tassanakajon *et al.*, 1997) was used which is considered to be superior because of its sensitivity, ability to detect multiple loci and the fact that it is not necessary to have any DNA information available beforehand.

2. Materials and Methods

A) **Sample collection and Morphometrics**: Samples of male morphotypes of *M. rosenbergii* were collected during final harvest of a single aged population from the grow-out of Kuttanad (S. India) during 2000-2001. They were transported in live condition to the laboratory and sorted out into different morphotypes (Harikrishnan and Kurup, 1997). Detailed morphometrics on the total body length, carapace length, length of second cheliped (ischium, merus, carpus, propudus, dactylus), length of telson, second pleura and total body weight were measured (± 0.1 g, ± 1 mm). Twenty four individuals of each morphotypes were used for the analyses.

B) **DNA Extraction**: Genomic-DNA was phenol-isolated from fresh muscle tissue of each experimental individual, following the procedures described by Sambrook *et al.* (1989).

C) **RAPD-PCR**: The reaction, consisting of 20 ng of genomic DNA, 15 mM Assay buffer with $MgCl_2$, 10 mM each of dNTP (dATP, dTTP, dGTP, dCTP) mix and 1 unit of Taq DNA Polymerase (Bangalore Genei), was

carried out in a PTC-200 minicycler (MJ Research) as follows: cycle 1 to 2, 3 min. at 94°C for denaturation; 1 min. at 35°C for annealing, and 2 min. at 72°C for extension; cycles 3 to 35 and 10 min. at 72°C for final extension. This sequence was modified from Amanto and Corach (1996). Twenty primers, OPA 01-10 of Kit A and OPAD 01-10 (Operon Technologies) were used for the present study.

D) Electrophoresis: PCR Amplification products were electrophoresed on 1.2% agarose gel using TBE buffer, and stained with ethidium bromide to visualize amplified DNA fragment.

E) Data Analysis: The number of amplified bands from each individual morphotype and that shared between the morphotypes were utilized for similarity calculation based on the similarity formula (Nei, 1972)

$$S = \frac{2Nxy}{(Nx + Ny)}$$

where Nxy is the total number of bands in both present in individual x and y, and Nx or Ny is the number of bands in x or y. Since here the value of S represents the genetically homologous condition, i.e., genetic similarity, the value of I-S means genetic distance. The average similarity is the mean value of similarities between individuals of a morphotype or between two morphotypes. The Duncun's multiple range test for the average similarities among the morphotypes and within the population was carried out.

Dissimilarity Index of Gilbert *et al.* (1990) was employed and Average Percent Difference (APD) was calculated as

$$APD = \frac{1}{C} \sum_{i=1}^{c} Di \times 100$$

where C = total number of pair wise comparison; D = difference value

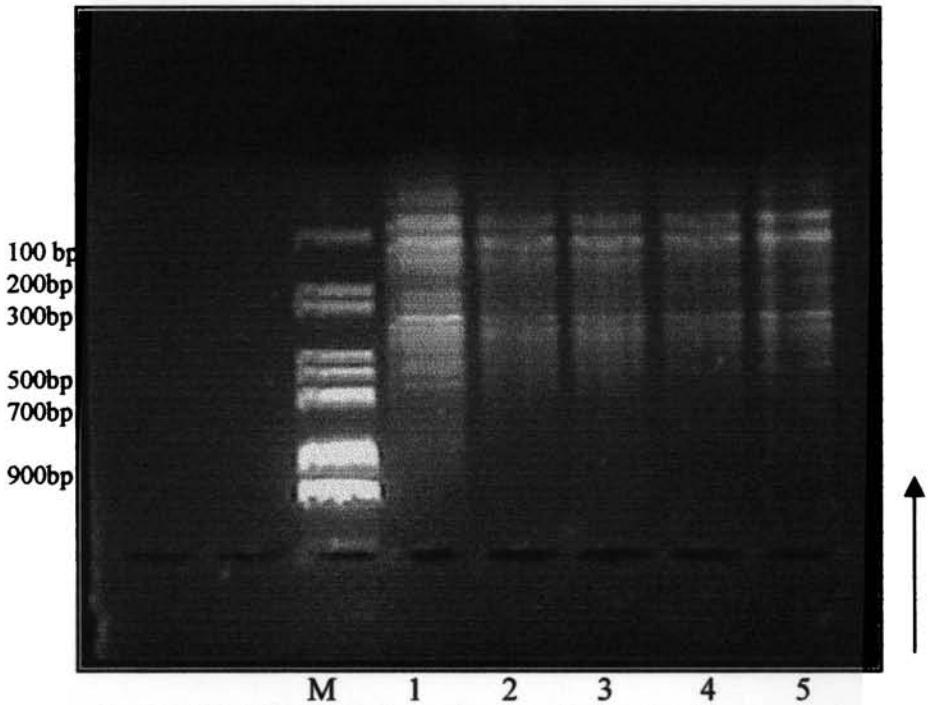
$$D = \frac{Vab}{Na + Nb}$$

where Vab = number of bands that differ between individuals a and b; Na = total number of bands in profile a; Nb number of b bands in profile b .

3. Results

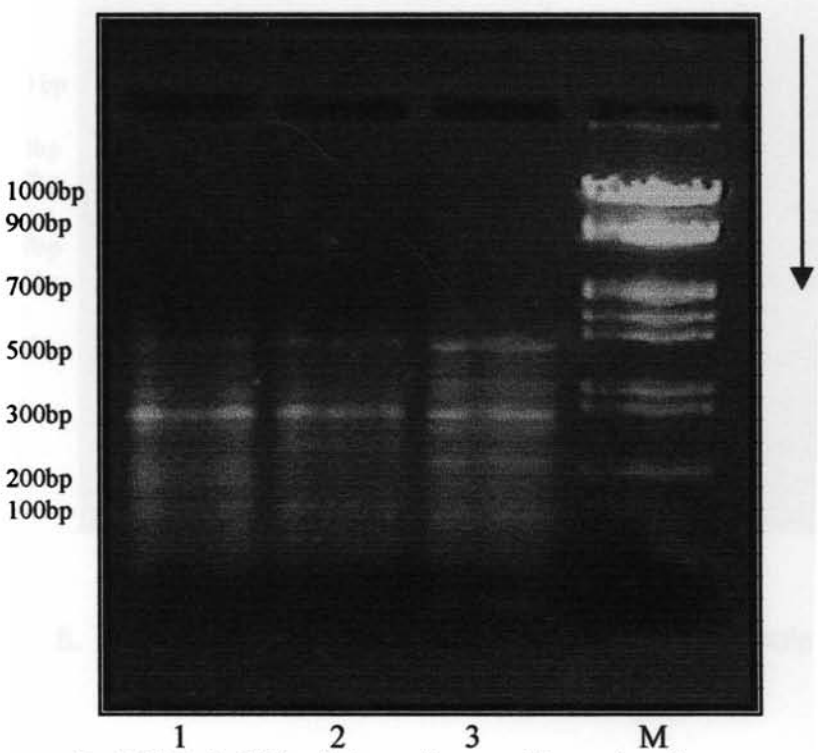
Among the 20 primers used for amplification perceptible difference in the banding pattern of the amplified DNA products were discernible only from 6 primers (OPA-02, OPA-04, OPA-06, OPA-07, OPA-08 and OPA-09) as shown in Plate 6.1 to 6.3. The difference in the banding pattern was primer specific and template specific. Characteristics of the amplification products are shown in Table 6.1. The results clearly indicate that a total of 36 amplification products were obtained with six primers, fourteen of them being constant among the three morphotypes. Another noteworthy observation of the present study was the heterozygous nature of banding pattern among the individual of SM. Of a total of 22 bands that showed difference among morphotypes 10 were common for both OC and BC, which means the homozygosity in banding pattern for these two morphotypes. Largest number of bands (12) was obtained from primers OPA-2 and OPA-9 for all the three morphotypes. Although polymorphism was established among the RAPD electrophorogram from the three morphotypes, no specific band, as a diagnostic DNA marker, was found among the male

Plate 6.1



A. RAPD (OPA-2) Banding patterns in three male morphotypes of *M.rosenbergii*

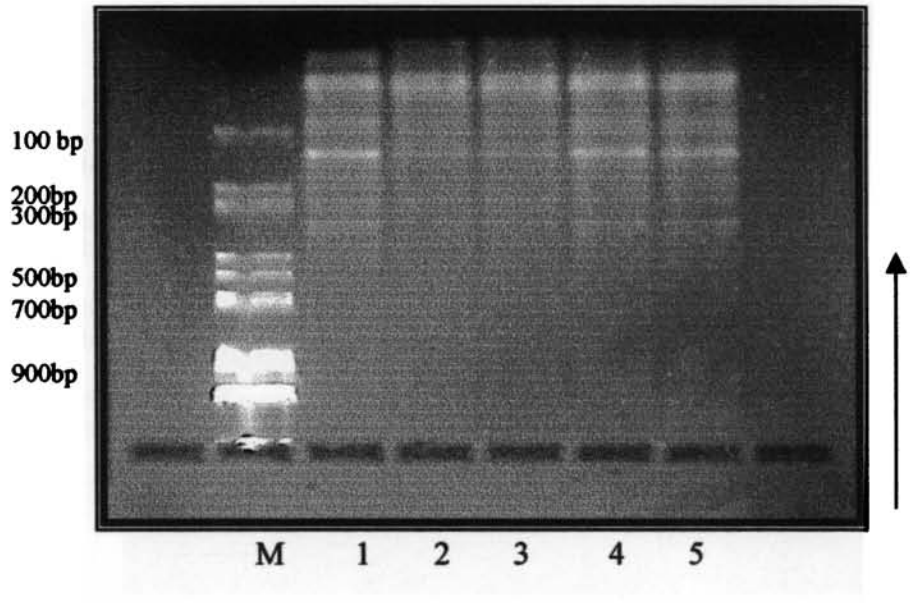
Column 1 Belongs to Small male(SM), Column 2-&3 belongs to Orange clawed males(OC) and Column 4&5 belongs to Blue clawed males (BC)



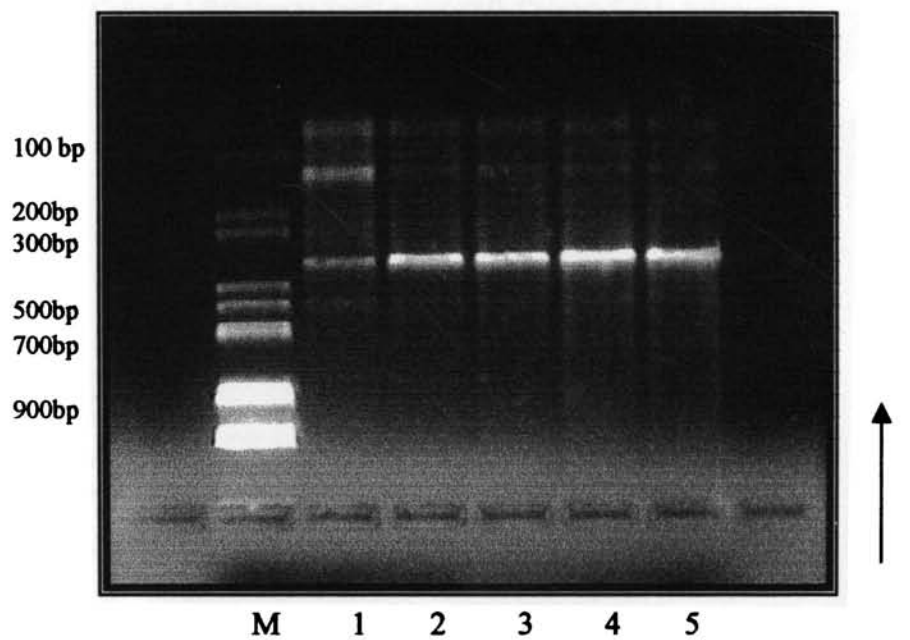
B .RAPD (OPA-4) Banding patterns in three male morphotypes of *M.rosenbergii*

Column 1 Belongs to Small male(SM), Column 2 belongs to Orange clawed males(OC) and Column 3 belongs to Blue clawed males (BC), Column M is the pattern of the molecular weight marker (100 bp)

Plate 6.2



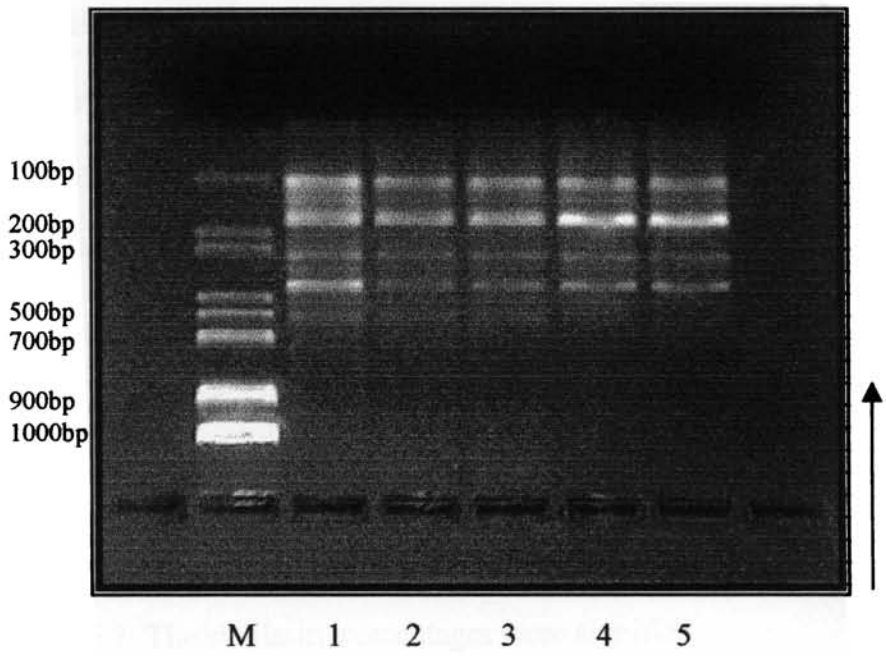
A. RAPD (OPA-6) Banding patterns in three male morphotypes of *M.rosenbergii*



B. RAPD (OPA-7) Banding patterns in three male morphotypes of *M.rosenbergii*

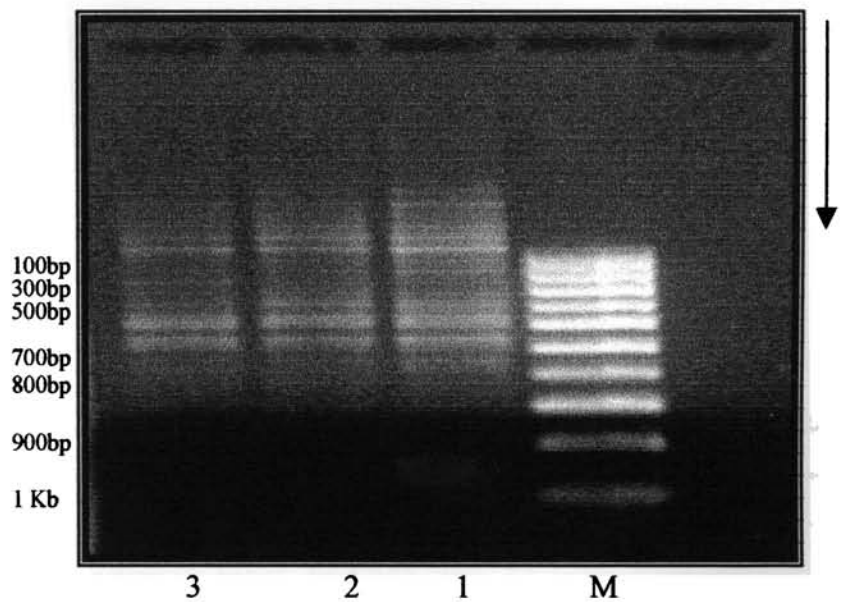
Column 1 Belongs to Small male(SM), Column 2-&3 belongs to Orange clawed males(OC) and Column 4&5 belongs to Blue clawed males (BC)

Plate 6.3



A. RAPD (OPA-8) Banding patterns in three male morphotypes of *M.rosenbergii*

Column 1 Belongs to Small male(SM), Column 2-&3 belongs to Orange clawed males(OC) and Column 4&5 belongs to Blue clawed males (BC)



B . RAPD (OPA-9) Banding patterns in three male morphotypes of *M.rosenbergii*

Column 1 Belongs to Small male(SM), Column 2 belongs to Orange clawed males(OC) and Column 3 belongs to Blue clawed males (BC), Column M is the pattern of the molecular weight marker (100 bp)

morphotypes of *M. rosenbergii*. Table 6.2 to 6.7 shows the results on genetic similarity and distance calculated for the six primers. The similarity percentage were significantly higher between OC and BC, while SM showed relatively greater distance among the morphotypes. The results of the present study did not show any significant difference ($P>0.05$) in the DNA banding sequence between individuals of a particular morphotype. Among the six primers used for the present study highest index for genetic distance for SM were recorded from OPA-02, OPA-06 and OPA-08. Variation in the mean value for intra and intermorphotypic dissimilarity verified using Duncan multiple range tests is shown in Table 6.7. The similarity percentages were significantly higher between intra-morphotypic individuals than between inter-morphotypic ones. Average similarity and standard deviations have been calculated (Table 6.7). According to the similarity data, the genetic relationships from the nearest to the most distant among the three morphotypes are sequentially from SM to OC and then to BC, which is in accordance to the morphological similarities. The results of dissimilarity index do not show any significant difference in the genetic distance between OC and BC, while 5% significance in the genetic diversity of SM was seen. Though the significance levels were low, the difference encountered in SM may be due to the intrapopulation variations. Hence the possibility of a different subspecies status to these morphotypes may not be valid. The higher values of APD for SM confirmed these findings and showed a greater degree of heterogeneity between SM and OC and SM and BC.

4. Discussion

The population genetic structure of only a few species belonging to the genus of Caridean prawn *Macrobrachium* has been studied so far. Although sufficient data on the electrophoretic and allozymal variations among the members of this genus has been documented (Sriraman *et al.*, 1995; Chow and Fugio, 1985 a and b; Hedgecock *et al.*, 1979; Malecha *et al.*, 1980), very scant information is available on their genetic differences especially from the molecular level. RAPD markers are often utilized to distinguish morphologically similar species, subspecies, populations, strains or even individuals (Welsh *et al.*, 1991; Garcia and Benzie, 1995; Lu *et al.*, 2000). Meruane *et al.* (1997) used RAPD-PCR techniques for identification of species and associated polymorphisn in *Penaeus japonicus* and *Metapenaeus ensis*. In the present work, despite the fact that the genetic variations among the three male morphotypes of *M. rosenbergii* could not be proved statistically significant, polymorphism in the DNA banding pattern was evident. However, no morphotype specific band, that could be used as an RAPD marker could be found. The nature of low degrees of genetic variability within the male morphotypes of *M. rosenbergii* seen during the present study showed close similarity to the results observed by Wong and McAndrews (1994) in two populations of *M. nipponense* inhabiting freshwater and brackishwater. The possible reason for the low variability among the morphotypes may be due to the geographical isolation and high rate of inbreeding between the morphotypes. Vembanad lake and its associated parts in Kuttanad has been the home ground of *M. rosenbergii* in Kerala. Although several strains of this species are present elsewhere in other parts of the country,

chances for inter-strain hybridization is greatly reduced due to the geographical separation. Li *et al.* (1993) showed that many fish species are differentiated into subspecies or semi-isolated sub populations. If these subpopulations are more or less isolated genetically from one another, they may differ in the banding frequencies in one or several loci. This explains the difference in the banding pattern seen in the present study. Such results of genetic variations and subsequent population subdivision in penaeid prawns have been reported by Duda and Palumbi (1999), Salini (1987) and Moore *et al.* (1999). An observation by Sureshkumar and Kurup (1998c) on the reproductive capabilities of the male morphotypes of *M. rosenbergii* showed that SM and BC are reproductively active, while the potential for OC to breed is low. This would mean that the chances for inter-morphotypic hybridization are high, especially in grow-out ponds where the boundaries are confined.

Decapods are known to display relatively low levels of enzyme polymorphisms. Mean heterozygosities of 0.048 were reported for decapods (Hedgecock *et al.*, 1982), 0.082 for crustaceans (Nevo *et al.*, 1984) and 0.028 for *M. rosenbergii* (Hedgecock *et al.*, 1979). Although, RAPD technique is expected to scan the genome more randomly, the results from the present study did not show considerable difference when compared to the values arrived from isozymal variations of Chapter 5. The APD value is a rough estimator of genetic diversity in and between populations. It indicated the levels of similarity between the individuals being compared. Thus, if APD is low, the individuals share a high proportion of bands. This was clearly visible between the band-sharing pattern of OC and BC in the present study. The APD values were significantly low for OC

(5.325) and BC (5.643) when compared to that of SM (10.723). The lower APD indicates that these morphotypes (OC and BC) have more or less similar number of bands when compared to those of SM. The measure of genetic distance indicates a high genetic similarity between populations. In the present study the genetic distance between SM and OC were more than that of OC and BC. Genetical evaluation through RAPD also confirmed the distinct position occupied by the SM in the transformation pathway, much lower to OC and BC morphotypes. Hence results emerging from the study corroborate the earlier results on the sequential morphotypic transformation of SM→OC→BC. Higher polymorphism scored with RAPD markers is probably due to preferential amplification of non-coding repetitive regions in the genome (Amanto and Corach, 1997). Which means in SM, the DNA sequence might contain more number of exons that do not take part in transcription or code during protein synthesis. This may be the reason for reduced somatic growth seen in SM. Recent works of Garcia *et al.* (1994) revealed RAPD markers associated with differential gene expression throughout development in *P. monodon.*

Malecha *et al.* (1980) established through allozymal studies that the overall intrapopulation variability in *M. rosenbergii* is low, about 28% of the loci in an individual is heterozygous. However, the genetic variability studied through DNA amplification techniques in the present study showed significant variations only in the banding pattern of SM. Hence it could be rightly concluded that SM occupies a unique place in the genetic status among the three male morphotypes of *M. rosenbergii*. The genetic diversity within the morphotypes was low and hence the possibility of a separate species status could not be

discernible. The pattern of banding for SM although was diverse, showed significance variation ($P<0.05$). The results emerging from the present study also suggest a genetic involvement in determining the phenotypic traits. The distinctive growth variations and associated changes in the morphology of the freshwater prawn were found related to the differences in the sequenced product of DNA.

ble 6.1. Amplification products obtained with 6 primers for the three morphotypes of *M. rosenbergii* fSM, fOC and fBC = frequency of bands in SM, OC and BC respectively

.No.	Primer	Sequence 5'-3'	Bands	MW (bp)	SM	OC	BC
1	OPA-02	TGCCGAGCTG	OPA 02-1	100	0.615	0.378	0.312
			OPA 02-2	200	0.115	1	1
			OPA 02-3	300	0.226	1	1
			OPA 02-4	400	0.829	1	1
			OPA 02-5	500	0.348	0.256	0.213
			OPA 02-6	600	0.523	1	1
2	OPA-04	AATCGGGCTG	OPA 04-1	100	0.218	1	1
			OPA 04-2	200	1	1	1
			OPA 04-3	300	1	1	1
			OPA 04-4	400	0.725	0.653	0.597
			OPA 04-5	500	1	1	1
			OPA 04-6	600	0.412	1	1
3	OPA-06	AGGGGTGTTG	OPA 06-1	100	0.635	0.521	0.437
			OPA 06-2	200	1	1	1
			OPA 06-3	300	0.737	1	1
			OPA 06-4	400	1	1	1
			OPA 06-5	500	0.858	1	1
			OPA 06-6	600	1	1	1
4	OPA-07	GAAACGGGTG	OPA 07-1	100	1	1	0.726
			OPA 07-2	200	0.215	0.826	0.943
			OPA 07-3	300	1	1	1
			OPA 07-4	400	1	1	1
			OPA 07-5	500	1	1	1
			OPA 07-6	600	0.638	1	1
5	OPA-08	GTGACGTAGG	OPA 08-1	100	0.234	0.421	0.554
			OPA 08-2	200	1	1	1
			OPA 08-3	300	0.472	1	1
			OPA 08-4	400	1	1	1
			OPA 08-5	500	1	1	1
			OPA 08-6	600	0.284	1	1
6	OPA-09	GGGTAACGCC	OPA 09-1	100	0.284	0.287	0.541
			OPA 09-2	200	0.242	1	1
			OPA 09-3	300	1	1	0.458
			OPA 09-4	400	1	1	1
			OPA 09-5	500	1	1	1
			OPA 09-6	600	0.327	1	1

OPA = Operon primer Kit 'A'
SM = Small Male
OC = Orange clawed males
BC = Blue clawed males

Table 6.2: OPA-02 primer results on Genetic Similarity (above diagonal) and Genetic distance (below diagonal) in the three male morphotypes of *M. rosenbergii*

	1	2	3	4	5
1	-	**0.94**	**0.94**	**0.86**	**0.86**
2	0.07	-	**1**	**0.96**	**0.96**
3	0.07	0.00	-	**0.85**	**0.85**
4	0.14	0.04	0.15	-	**1**
5	0.14	0.04	0.15	0.00	-

1 = Small Males
2 & 3 = Orange clawed males
4 & 5 = Blue clawed males

Table 6.3: OPA-04 primer results on Genetic Similarity (above diagonal) and Genetic distance (below diagonal) in the three male morphotypes of *M. rosenbergii*

	1	2	3
1	-	**0.92**	**0.95**
2	0.08	-	**0.93**
3	0.05	0.07	-

1 = Small Males
2 = Orange clawed males
3 = Blue clawed males

Table 6.4: OPA-02 primer results on Genetic Similarity (above diagonal) and Genetic distance (below diagonal) in the three male morphotypes of *M. rosenbergii*

	1	2	3	4	5
1	-	**0.85**	**0.85**	**0.85**	**0.85**
2	0.15	-	**1**	**0.97**	**0.97**
3	0.15	0.00	-	**0.97**	**0.93**
4	0.15	0.03	0.03	-	**1**
5	0.15	0.03	0.07	0.00	-

1 = Small Males
2 & 3 = Orange clawed males
4 & 5 = Blue clawed males

Table 6.5: OPA-07 primer results on Genetic Similarity (above diagonal) and Genetic distance (below diagonal) in the three male morphotypes of *M. rosenbergii*

	1	2	3	4	5
1	-	**0.93**	**0.93**	**0.93**	**0.86**
2	0.07	-	**1**	**0.97**	**0.97**
3	0.07	0.00	-	**0.97**	**0.97**
4	0.07	0.03	0.03	-	**1**
5	0.07	0.03	0.03	0.00	-

1 = Small Males
2 & 3 = Orange clawed males
4 & 5 = Blue clawed males

Table 6.6: OPA-08 primer results on Genetic Similarity (above diagonal) and Genetic distance (below diagonal) in the three male morphotypes of *M. rosenbergii*

	1	2	3	4	5
1	-	**0.87**	**0.87**	**0.87**	**0.87**
2	0.13	-	**1**	**0.98**	**0.98**
3	0.13	0.00	-	**0.98**	**0.98**
4	0.13	0.02	0.02	-	**1**
5	0.13	0.02	0.02	0.00	-

1 = Small Males
2 & 3 = Orange clawed males
4 & 5 = Blue clawed males

Table 6.7: OPA-09 primer results on Genetic Similarity (above diagonal) and Genetic distance (below diagonal) in the three male morphotypes of *M. rosenbergii*

	1	2	3
1	-	**0.92**	**0.95**
2	0.08	-	**0.93**
3	0.05	0.07	-

1 = Small Males
2 = Orange clawed males
3 = Blue clawed males

Table 6.8. Summary statistics of intra and intermorphotypic dissimilarity in males of *M. rosenbergii*

Morphotypes	***N***	**APD**	**SD**	**Number of bands (n)**
SM	22	10.723** (0.0 - 12.57)	2.554	8.13 (6-10)
OC	18	5.325 (0.0 - 8.46)	3.321	6.0 (4 - 8)
BC	16	5.643 (0.0 - 9.25)	2.869	5.25 (5 - 8)

** Probability = ($P<0.05$), The range of observed values are given in parenthesis
SM = Small Male
OC = Orange clawed males
BC = Blue clawed males

CHAPTER 7

Density dependant variations on the production and population structure of *Macrobrachium rosenbergii* in the grow- outs of Kuttanad

1. Introduction

Macrobrachium rosenbergii (de Man), the giant freshwater prawn with the trade name 'Scampi', is known to be the most commercially important freshwater prawn of Indian waters. In view of the faster growth and big size, excellent demand in export and internal markets and high price it commands, it is emerging as a prime candidate species for freshwater aquaculture. However, the wide disparity in size structure of cultured stock and the skewness in their weight distribution, which is profoundly influenced by male morphotypes, apparently appears to be the most commercial disadvantages of this species. Unlike in shrimps, the economic yield of *M. rosenbergii* in grow-outs is not a linear function of total biomass produced owing to the predominance of non-marketable small males in the harvested population. But this differential growth and consequent negative effect on economic yield can be minimized by a proper understanding of factors involved in the process of morphological transformation in the developmental pathway of male morphotypes (Kuris *et al.*, 1987). Information of this kind would be invaluable in evolving appropriate pre and post stocking management strategies for minimising heterogeneous individual growth and reciprocally improving the marketable yield (Malecha *et al.*, 1981b). The differential growth pattern of various male and female morphotypes of *M.*

rosenbergii has been well characterised in grow-out population under different levels of stocking density and management strategies (Smith *et al.*, 1978; Brody *et al.*, 1980; Cohen *et al.*, 1981; Karplus *et al.*, 1987). Information on the interaction of male morphotypes and their growth strategy will help in improving the marketable yield of *M. rosenbergii*, under captive conditions. Economic success of prawn culture in any locality is governed by the proper selection of stocking density and stocking size (D'Abramo *et al.*, 1989). As stocking density increases, an increase in yield can also normally be expected, however, a corresponding increase of non-marketable prawns in the harvested population due to the decrease in mean weight is the most commercial disadvantage of this species (Smith *et al.*, 1978; Cohen *et al.*, 1981; Karplus *et al.*, 1986a; Montanez *et al.*, 1992; Kurup *et al.*, 2000). An understanding of the factors influencing the rate of increase in size variation and skewness in *M. rosenbergii* juvenile populations deserves top most interest due to the promising potential candidature of this species for freshwater aquaculture (Ling and Costello, 1976; Hanson and Godwin, 1977).

Kuttanad, the rice bowl of Kerala, is also known to be the home ground of *M. rosenbergii.* A very lucrative natural fishery of this species is being reported in Vembanad lake and confluent rivers of Kuttanad (Raman, 1967; Kurup *et al.*, 1992; Harikrishnan and Kurup, 1997). As the seed availability of *M. rosenbergii* became a reality, a renewed interest had been noticed among the farmers of Kuttanad in the farming of this species in various natural grow-outs (Padmakumar *et al.*, 1992; Harikrishnan and Kurup, 1998). Though the farming of this species has been carried out either as monoculture or polyculture at

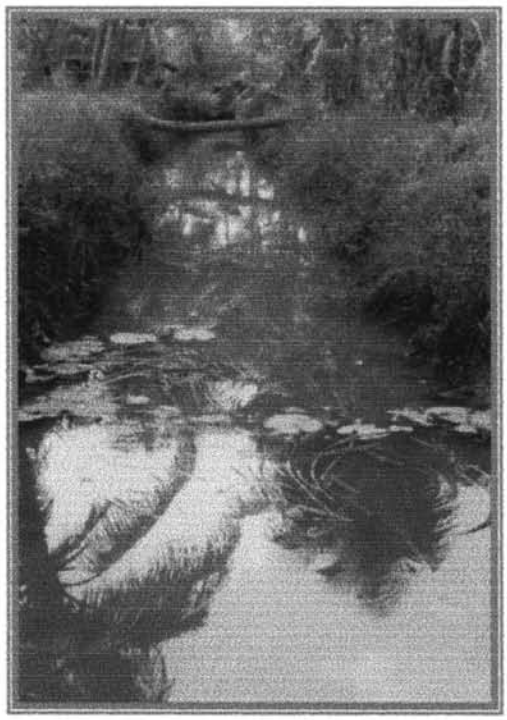
various levels in polders, coconut garden channels and homestead ponds in Kuttanad, no information is available with regard to its survival, growth performance, marketable yield structure and economic feasibility. The present study is undertaken with the objective to standardize the stocking density in two types of natural grow-out of *M. rosenbergii* in Kuttanad such as polders and coconut garden channels.

2. Materials and Methods

The data for the present study were compiled during the period 1999-2001 from twenty polders and sixteen coconut garden channels in Kuttanad where *M. rosenbergii* was cultured. The polders are traditional paddy fields lying 1m below the sea level, which were reclaimed from Vembanad lake long ago and are now separated from the lake with strong peripheral bunds. Some of these polders are used successfully as grow-outs of *M. rosenbergii*. The water-spread area of the polders used in the present study ranged from 0.5 to 2 ha. All the polders were stocked with hatchery reared post larvae and the farming operation extended for a period of 8 months. The performance of post larvae stocked under five separate stocking densities (treatments) in the polders was assessed. Stocking density was kept as the principal variable in all the treatments studied. Each set of treatment represents data collected from four separate polders (quadruplicates). In the first set of treatments (TP-1) the initial stocking density was kept @ 15,000/ha. Similarly, the stocking densities in subsequent treatments were maintained @ 25,000/ha (TP-2), 35,000/ha (TP-3), 45,000/ha (TP-4) and 60,000/ha (TP-5). Modified extensive monoculture system was followed in all

Plate 7.1

A. Polders in Kuttanad, used for the farming of *M. rosenbergii*



B. Coconut garden channels of Kuttanad used for the farming of *M.rosenbergii*

treatments after harvesting paddy in February. Prior to stocking these polders were dried and scientifically prepared (Kurup *et al.*, 1997). Liming was done @1000Kg/ha and cow dung was applied as phased manuring @5000Kg/ha. The prawns were fed initially with high protein feed @ 20% of the prawn biomass for two months and subsequently they were fed with a diet mixture of groundnut oil cake, rice bran and boiled butchery waste in equal proportions @ 10% of the prawn biomass.

All the twelve coconut garden channels selected had an equal water spread area of 1 ha each. These channels were cleared of all predatory fishes by netting, dried and deoiled cake of mahua was applied @ 5 Kg/ha. Lime @ 50 Kg/ha was added to bring pH to neutrality. Having considerable natural deposits of lime in these area, liming @ 50 Kg/ha was found enough to maintain the soil and water pH under control throughout the culture period. Farmyard manure @ 1000 Kg/ha was applied in equal monthly installments during the culture period. The channels were stocked with post larvae procured from a local hatchery. The performance of post larvae stocked under four separate stocking densities (treatments) in these channels was assessed. Each set of treatment represents data collected from four separate channels (quadruplicates). In the first set of treatments (TC-1) the initial stocking density was kept @ 5,000/ha and in subsequent treatments the initial stocking density was @ of 10,000/ha (TC-2), 15,000/ha (TC-3) and 25,000/ha (TC-4) respectively. The prawns were fed initially with a commercial feed (CP starter feed) @ 20% of the prawn biomass for three months and later they were fed with a diet mixture of groundnut oil cake, rice bran and boiled meat of edible clam in equal proportion @ 10% of the

prawn biomass. Water in the polders and coconut garden channels were exchanged periodically. Water quality parameters such as temperature, dissolved oxygen, transparency, water and soil pH were monitored on a monthly basis following AOAC (1985), while levels of total ammonia-nitrogen (TAN), nitrite-nitrogen and hydrogen sulphide in water samples collected during morning hours from each treatment were determined at fortnightly intervals using the Aquakit from MERCK. At the end of 8 months of culture periods, the polders and channels were dewatered by pumping out, and the prawns were harvested by handpicking. Random samples of 500-1000 prawns from each grow out were examined on the day of harvest. All the prawns were sorted according to their sex and morphotypes. The males were then classified into 3 morphotypes such as SM, SOC and SBC and transitional stages viz. WOC, t-SOC, WBC and OBC (Harikrishnan *et al.*, 1997). All the prawns were measured up to nearest millimeter and weighed up to nearest gram. The weight of individual morphotypes from polders and channels within each treatment were compared by applying ANOVA. In order to assess the effect of stocking density on population characteristics and yield structure the cumulative mean values of each treatment among polders (TP-1 to 5) and channels (TC-1 to 4) were compared. Statistical analysis was done using SPSS 7.5 and Excel 98 package for Windows and the results were statistically evaluated following Snedecor and Cochran (1961). Average weight gain, final mean weight and total biomass were analysed by ANOVA followed by pair-wise *t*-test.

3. Results

3.1 Polders

3.1.1 Population Characteristics

Table 7.1 consolidates the results on stocking details and corresponding yield characteristics of *M. rosenbergii* grown in four polders under a low stocking density of 15,000/ha (TP-1). Among the major observations made, survival rate, mean weight of prawns and net production among the polders in this treatment (T1) did not vary significantly and were found to range between 252.4 to 291.54 Kg/ha, 60.9 to 68.7% and 68.45 to 74.25g respectively. Dominance of males in the final harvested population was quite discernible. On further comparison of the mean weight of individual morphotypes among the four polders (TP-1) with the help of analysis of variance (ANOVA) (Table 7.2), the mean values for wet weight within the treatment (TP-1) did not vary significantly ($P>0.05$).

Details on initial stocking and results of final harvest from the four polders in which *M. rosenbergii* were cultured under a stocking density of 25,000/ha (TP-2) are given in Table 7.3. Variation in survival rate, mean weight and net production between the four polders did not show any significant difference ($P>0.05$). While survival rate ranged between 49.9 to 61.8%, mean weight and correspondingly net production were recorded to fluctuate between 60.2 to 66.6g and 300.5 to 324.56 Kg/ha respectively. As in TP-1, males dominated in the harvested population in all the polders. Results of statistical comparison of mean weight of individual morphotypes among the four polders

(TP-2) is shown in Table 7.4 and it appeared that the mean weight of morphotypes within the treatment (TP-2) did not vary significantly (P>0.05).

The stocking details and yield characteristics of *M. rosenbergii* reared in four polders under a stocking density of 35,000/ha (TP-3) are shown in Table 7.5. Net production did not vary significantly between the polders and ranged between 518.26 to 586.38 Kg/ha. Survival rate and mean weight, though diminished considerably when compared to earlier treatments, did not differ significantly within the treatment (TP-3) and ranged between 46.73 to 57.56% and 60.31 to 70.28g respectively. In all the polders, males dominated in the harvested population. Comparison of mean weight of individual morphotypes through ANOVA among the four polders (TP-3) did not show any significant difference (P>0.05) within the treatments.

Details of final retrieval rate, mean weight of prawns, sex ratio and net production from four polders under a stocking density of 45,000/ha (TP-4) is shown in Table 7.7. Net production showed glaring increase (TP-4) when compared to T3 and but did not vary significantly between the polders and ranged between 726.25 to 786.58 Kg/ha. Similarly, the survival rate and mean weight also did not differ considerably which varied between 43.43 to 52.25% and 52.47 to 58.68g respectively. The sex ratio did not follow any definite pattern among the polders. Mean weight of individual morphotypes among the four polders (Table 7.8) within the treatment (TP-4) did not vary significantly (P>0.05).

Farming details from four sets of polders following an initial stocking density of 60,000/ha (TP-5) are given in Table 7.9. Although significantly higher values for net production were encountered in this treatment (794.08 and 876.95 Kg/ha), mean weight of prawns and retrieval rate were comparatively less when compared to other treatments (41.14 to 47.61% and 46.57 to 56.24g respectively). Mean weights of individual morphotypes did not show any significant difference within the four polders ($P>0.05$) (Table 7.10).

Water quality parameters among the five treatments did not show any significant difference ($P>0.05$). Mean values for surface water temperature ranged between 26.6–33.4°C in TP-1, 27.1–33.2°C in TP-2, 26.5-34.2°C in TP-3, 25.8-34.5°C in TP-4 and 25.6-33.7°C in TP-5 (Table 7.11). Dissolved oxygen among the five treatments ranged between 4.38 mg/l in TP-1 to 6.25 mg/l in TP-4, whereas total alkalinity was in the range 48.61 mg/l in TP-3 to 75.58 mg/l in TP-4. pH for water and soil were also at the optimal levels. Water pH ranged between 6-8.5 among the treatments, soil pH varied between 5-6.5. Low levels of total ammonia-nitrogen (below 0.1 mg/l) and nitrite-nitrogen (0.02 mg/l) were recorded and thus all water quality parameters were well within the optimum ranges.

Percentage contribution by weight of various male and female morphotypes, their mean weight, percentage survival and net production observed in the final harvested population for all the five sets of treatments (TP-1 to 5) are summarised in Table 7.12. Final densities of *M. rosenbergii* at the time of harvest in treatments TP-1 to 5 were in order of 0.93,1.41,1.88, 2.15 and 2.71

no/m^2 respectively. On the contrary, the retrieval rate from the polders followed an inverse relationship with the respective stocking density. Highest survival rate was recorded from TP-1 (62 %), in total contrast it was the least in TP-5 (45%). Mean wet weight of males and females in the final harvested population also followed similar trends similar to that of survival rate. Highest mean weight for male and female were registered in TP-1 (83.02 g and 62.08 g respectively), while least values in respect of males were recorded from TP-5 (54.06 g) and for females TP-4 (44.69 g) respectively. Male-female ratio was dissimilar at the five densities, being 1:0.72 in TP-1, 1:0.91 in TP-2, 1:0.85 in TP-3, 1:1.14 in TP-4 and 1:1.33 in TP-5.

The structure of male and female population at the five levels of final density and the percentage contribution by weight of various male and female morphotypes to their respective harvested yield from these five sets of polders are given in Table 7.15. The percentage contribution by weight of individual morphotypes within the five treatments did not follow any particular trend, however, the percentage by weight of undersized SM and SF increased at higher densities and were recorded in the range of 21.36 and 13.56% respectively in TP-5, in contrast to 5.4 and 5.27% in TP-1. Percentage contribution of OC and BC by weight in the final density among the five sets of polders showed an inverse pattern with increase an in final density when compared to that of SM and were recorded as 33.45 and 26.27% respectively in TP-1 and 29.24 and 15.36% respectively in TP-5. Variation in percentage of orange-clawed females and blue-clawed females did not follow any particular pattern. The mean weight of individual morphotypes in all the five sets of treatments differed significantly

($P<0.05$). The most noteworthy finding was in respect of individual weight of SM and OBC morphotypes, which lowered from 18.41 g and 147.91 g respectively in TP-1 to 11.35 g and 91.54 g respectively in TP-5. Although there were slight variations in the mean weight of individual female morphotypes within the treatments, it was not statistically significant ($P>0.05$). The variation in mean weight of individual morphotypes has been tested by applying ANOVA (Table 7.16). The results clearly indicate that a significantly higher degree of differentiation was discernible in the terminal male morphotypes. While slight variations ($P<0.05$) was also seen among the terminal female morphotypes. Results of the pair-wise analysis of total mean weight of prawns between the treatments are shown in Table 7.17 which clearly show that there exist difference in the mean weight of individual morphotypes with respect to the initial stocking density. The differentiation was clearly visible among the BC morphotypes between treatments with low and high stocking densities. Another noteworthy observation in the present study was the non-significance of average weight of prawns between TP-4 and TP-5 (Table 7.12). From the present trend it can be inferred that, a further increase of density from 4.5/m^2 to 6/m^2 would not be helpful in the reciprocal increase in the mean weight of prawns.

The percentage composition by number of the male and female morphotypes at different levels of initial density in the five sets of treatments (polders) is shown in Fig 7.1 and Fig 7.2 respectively. In TP-1, which was characterized by lowest density at harvest (0.93/m2), SBC and its transitional stage WBC and OBC constituted 53.6% of the male morphotypes while the contribution from SM was highly insignificant (8%). On the contrary, in TP-5

with a final density of 2.71/ m^2, the percentage contribution of blue clawed males showed a perceptible decline to 39.4% in contrast to remarkable increase in the percentage contribution of SM to 28.05%. It would thus appear that while a direct relationship could be noticed in the proportion of SM with respect to the increase of density, the percentage of BC morphotypes showed an inverse relationship (Fig 7.1). Almost identical to their male counterparts, variations in the morphotypic composition of harvested female population were also discernible in relation to stocking density. It could be seen that in higher densities (TP-4 and TP-5), the cumulative percentage of SOF and its advanced stages viz. TOF, WBF and SBF accounted for 69.7 and 67.3% respectively, on the contrary, in TP-1 and TP-2, the percentage contribution of these groups were found high (78.4 and 70.6% respectively).In contrast, composition of SF followed an inverse trend, which increased from 11.9% in TP-1 to 18.3% in TP-4.

3.1.2. Yield Characteristics:

Density dependant variations in the mean weight of harvested population could be observed in the five treatments, showing highest mean weight of 72.15 g in TP-1 against 52.58 g registered in TP-3 (Table 7.1). Mean weight, standard deviation and coefficient of variation in respect of various male and female morphotypes are given in Table 7.15. Invariably, the mean weight of male and female morphotypes was highest in TP-1. Contrary to this, coefficient of variation suggests a more heterogeneous nature of population in TP-4 and TP-5, which had high initial stocking density. The net production also varied considerably within the treatments. The least among the mean net production from treatments were recorded from those stocked @1.5/m^2 (257.3 kg/ha), while

the maximum production was recorded in treatments stocked @6/m^2 (835.5 kg/ha). The results of the ANOVA on the net production (Table 7.13) among the five sets of treatments show a significant difference at 1% level (F= 49.2381). On further analysis of production within the treatments by t- test (Table 7.14), it could be seen that production in TP-3 and TP-4 showed maximum variation ($P<0.01$). Interestingly, net production between treatments 1 and 2 and treatments 4 and 5 were not significant ($P<0.05$). This directly indicates that an increase in the stocking density beyond 45,000/ha would not make any difference in production. Likewise, there was no difference in the production between stocking densities @ 15,000 and 25,000/ha and therefore stocking @ 15,000/ha would be better option from the economic point of view.

The weight distribution patterns of prawns in the five sets of treatments at different levels of stocking density are depicted in Fig 7.3 to 7.7. In TP-1 it appeared that the males profoundly influenced the weight distribution pattern, as the preponderance of >120g-weight group was quite discernible (40%), whereas, in treatments 4 and 5 this weight class constituted only 20% of the final harvested population. Weight distribution of total population in these treatments were also found to be influenced much by the female morphotypes which was evident from the high percentage occurrence of weight group 50- 80g (36%) (Fig 7.6). Percentage of undersized male and female morphotypes (<50 g) in the final population was glaringly high in treatments with high stocking densities (26 and 32% in treatments 4 and 5 respectively), in contrast, to a mere 12% in TP-1 with the least stocking density. It also appeared that, out of the total biomass produced from the five treatments, 88% of the yield from TP-1 belonged

to >50g group and therefore, was marketable whereas in TP-5 only 68% was marketable. The price packages offered by the seafood processing plants located at Cochin for *M. rosenbergii* per Kg were as follows: <50 g = Rs. 80/-; 50- 60 g = Rs. 120/-; 60-80 g = Rs. 160/-; 80-120 g = Rs. 200/-; 120-230 g = Rs. 260/-; > 230 g = Rs. 320/-. Based on the above mean tariff, the total revenue generated from the five treatments can be worked out as Rs. 56,606/- in TP-1, Rs. 68,156/- in TP-2, Rs. 1,21,924/- in TP-3, Rs. 1,63,966/- in TP-4 and Rs.1, 85,240/- in TP-5.It may, therefore be seen that though the yield from TP-1 formed only 30.8% of TP-5, income wise it fetches 40.3% of the latter because of large size of prawns as well as reduction in the percentage of undersized prawns. But as the stocking density increased in subsequent treatments, their percentage in yield and income with reference to TP-5 also differed significantly. Yield from TP-2 formed 37% of that of TP-5, while income increased to 44%. Similarly in TP-3, yield formed 66.3% of TP-5 but the income drawn was slightly high (70.7%). On the contrary, the yield registered from TP-4 formed 89.2% of that of TP- 5 but the income percentage reduced to 88.85%. A detailed micro-level economic analysis is given in Chapter 12. Based on the findings of the present study it can be asserted that a stocking density of 45,000/ha would be ideal for monoculture of *M. rosenbergii,* which would be more economically viable and technically feasible in the polders of Kuttanad.

3.2 Coconut Garden Channels

3.2.1 Population Characteristics

Stocking and corresponding yield characteristics of *M. rosenbergii* from four channels under stocking density of 5000/ha (TC-1) are shown in Table

7.18. Variations in the mean weight of prawns, survival rate and net production at the end of the culture period did not show any significant variations among the coconut garden channels and the values were in the range 95.4 to 112.5g, 62.5 to 76.4% and 82.7 to 99.4 Kg/ha respectively. Mean weight of individual morphotypes among the four channels (TC-1) when analysed revealed that there was no significant difference (P>0.05) within the treatment (TC-1) (Table 7.19).

Details of initial stocking and corresponding yield characteristics of *M. rosenbergii* at the final harvest in four channels stocked @ 10,000/ ha are shown in Table 7.20 (TC-2). There was no significant variation in net production, which ranged between 88.77 to 117.06 Kg/ha among the channels. Similarly, the variation in the survival rate and mean weight were also found insignificant and was in the range of 50.14 to 58.31% and 78.14 to 96.87g respectively. Mean weight of individual morphotypes among the four channels (TC-2) (Table 7.21) did not vary significantly (P>0.05).

Farming details of four sets of channels stocked under a density of 15,000/ha (TC-3) are given in Table 7.22. The results of mean weight of prawns, retrieval rate and net production were in the range between 143.8 to 200.1 Kg/ha, 34.32 to 41.4% and 61.35 to 78.65 g respectively. Mean weights of individual morphotypes (Table 7.23) showed significant difference (P>0.05) within the four polders.

Stocking details and yield characteristics of *M. rosenbergii* reared in four channels under a stocking density of 25,000/ha (TC-4) are shown in Table 7.24. Net production increased considerably which ranged between 227.5 to

265.1 Kg/ha in these treatments, but did not vary significantly between the channels (TC-4). Similarly, the survival rate and mean weight also did not differ considerably and were in the range of 25.6 to 30.1% and 52.4 to 61.2g respectively. Sex ratio in this treatment did not follow any particular pattern. Comparison of mean weight of individual morphotypes among the four channels (TC-4) (Table 7.25) did not vary significantly ($P>0.05$).

No significant difference ($P>0.05$) in water quality parameters could be noticed among the treatments. Table 7.26 shows the mean values calculated for different water quality parameters and their ranges registered within each treatment. Among the treatments, surface water temperature among the treatments ranged between 24.7 to 32.8°C in TC-1, 26.1 to 32.2°C in TC-2, 28.6-33.1°C in TC-3 and 24.4-34.2°C in TC-4. Mean values for dissolved oxygen ranged between 3.62-mg/l inTC-1 to 5.5 mg/l in TC-2. Total alkalinity was found to vary between 52.14 mg/l in TC-2 to 86.74 mg/l in TC-3. Water pH in all the treatments fell within the range of 5.5-8.0 while the soil pH ranged between 5-7.8. In all the treatments the total ammonia-nitrogen recorded were below 0.1 mg/l whereas, the nitrite-nitrogen was below 0.01 mg/l. All the water quality parameters determined were well within the optimum ranges.

The details of population density at harvesting on mean weight of prawns; percentage survival and contribution of male and female in the harvested population of the four treatments (TC-1 to TC-4) are given in Table 7.27. The final densities of *M. rosenbergii* at the time of harvest in treatments 1 to 4 were in the order of 0.35, 0.53, 0.57 and 0.7no/m^2 respectively. Survival in the various treatments showed a remarkable decline from 71.2 in TC-1 to 28.08% in TC-4. A

similar declining trend in the mean weight also was noticed with increase in final density among the treatments. Male: Female ratio was dissimilar at all the four densities, which were 1:2.05, 1:1.15, 1:1.11 and 1:0.77 respectively in TC-1 to TC-4. Gross production from the various treatments was also given in Table 7.27. Highest production was encountered in TC-4 (259Kg/ha), while the least was seen in TC-1 (92 kg/ha). The structure of male and female population at the four levels of final density, the percentage contribution by weight of various male and female morphotypes to their respective harvested yield is given in Table 7.30. The results showed very much agreement with that of polders. The percentage by weight of undersized non-marketable prawns (SM and WOC) increased remarkably with an increase in the stocking density. Proportion of SM and WOC was least in TC-1 (10.32%), whereas it showed significant increase to 36.33% in TC-4. On the contrary, percentage of BC males in the final population followed an inverse trend, which showed a decrease at significant levels from 34.71% in TC-1 to 14.11% in TC-4. Among the female morphotypes, only SF showed a direct relationship with stocking density as its proportion increased from 5.33 in TC-1 to 12.89% in TC-4. The coefficient of variation, which expresses the extent of heterogeneity within the population, showed higher values among larger male morphotypes (SBC and OBC), especially in TC-4.

The mean weight of individual morphotypes in all the four treatments showed significant variations ($P<0.05$). The most significant among them were the individual weight of OBC, which showed reduction from 147.9 to 103.45 g in TC-1 and TC-4 respectively. Although there were slight variations in the mean weight of individual female morphotypes within the polders, their

variations were not statistically significant ($P>0.05$). The variation in the mean weight of individual morphotypes has been studied through ANOVA (Table 7.31). There was significant difference ($P<0.01$) in the mean weight of BC and BF morphotypes among the different sets of treatments (channels). Pair-wise analyses of mean weight of prawns between the treatments (Table 7.32) showed a diverse pattern among male morphotypes with regard to change in initial stocking density. The difference was clearly visible in respect of the OC and BC morphotypes between TC-1 and TC-3. Another salient finding was that there was no difference in the average weight of prawns between TC-3 and TC-4. It may, therefore, be inferred that a further increase in the stocking density from 1.5/m^2 to 2.5/m^2 will not have any effect in reducing the mean weight of prawns at significant levels.

The structure of male and female populations from four treatments is depicted in Fig. 7.8 and 7.9 respectively. It could be seen that SM and WOC showed a direct proportion with the increasing density, on the contrary, the frequency of t-SOC and OBC showed a declining trend. The proportion of SM was appreciably high in stocking density of @ 25,000/ha (14.2%) while it was least at a density of 5000/ha (7.8%) (Fig 7.8). Similarly, high proportions of t-SOC and OBC were observed at lower density of 5000/ha (26.8% and 15.1% respectively) while their percentage showed reduction considerably at stocking density 25,000/ha (16.3% and 12.8% respectively). The population structure of female morphotypes at the final harvest also showed significant variations ($P<0.05$) in the different treatments. The percentage of undersized SF increased from 10.9 to 15.3% as the stocking density increased from TC-1 to TC-4. The

percentage of orange-clawed and blue-clawed females did not follow any particular pattern among the treatments, but their adequate representation in TC-2 (39.8 and 49.7%) and TC-3 (41.2 and 45.2%), were worth noticing.

3.1.2. Yield Characteristics

Variations in the mean weight of population commensurate with final density at harvesting could be observed in the four treatments. Highest mean weight for prawns was recorded in TC-1 (101.4 g) against 56.2 g registered in TC-4 (Table 7.27). Invariably, the mean weight of male and female morphotypes was highest in TC-1 with 128.6 g and 68.2 g respectively in TC-1 and 85.3 and 42.8 g respectively in TC-4.Commensurate with the variations in mean weights, the net production also showed differences among the treatments. The lowest mean net production was recorded in treatments with stocking density @ 0.5/m^2 (89.4 kg/ha), while the highest production was registered in channels stocked @ 2.5/m^2 (250.5 kg/ha). The results of the ANOVA in four sets of treatments showed a significant difference at 1% level (F= 22.2270) (Table 7.28), while t- test (Table 7.29) showed maximum variations ($P<0.01$) in productions between TC-1 and TC-3 and TC-2 and TC-3. Net production was insignificant between channels 1 and 2 and channels 3 and 4. Based on the results of this study, it can be concluded that the net production cannot be increased beyond a stocking density 15,000/ha in the coconut garden channels of Kuttanad.

The weight distribution patterns of prawns in the four treatments at different levels of stocking density are depicted in Fig 7.10 to 7.13. Preponderance of males in the final harvest in TC-1 seems to have influenced the

marketable weight structure which is evident from the dominance of weight group >120 g (44%). Moreover, it may also be noted that the percentage of undersized non-marketable prawns (<50 g), such as SM, WOC and undersized female morphotypes were appreciably low (6%) in this treatment. In contrast, the percentage of non-marketable prawns showed an increase to 22% in TC-4, which was characterized with highest stocking density. Furthermore, the weight class >120 g was only moderately represented (29%) in TC-3 and TC-4. In these treatments, the weight distribution of the total population was found to be much influenced by the female morphotypes since the weight group 50–80g (40%) showed predominance. It would thus appear that of the total biomass produced from each of the four treatments, 94% of the yield from TC-1 was constituted by prawns of >50g size group and therefore, were marketable whereas in TC-4 only 78% were marketable. While working out the total revenue by taking into consideration the price packages offered by the seafood processing plants located at Cochin for *M. rosenbergii* per Kg, total income would work out to be Rs. 25,032/- in TC-1, Rs. 32,312/- in TC-2, Rs. 47,528/- in TC-3 and Rs. 60,120/- in TC-4. It may therefore be seen that though the yield from TC-1 formed only 35.7% of TC-4, income wise it fetches 43.3% of the latter because of large size of prawns as well as reduction in the percentage of undersized prawns. As the stocking density increased in subsequent treatments the net yield and income with respect to treatments with highest stocking density also differed significantly. Yield from TC-2 formed 42.1% of that of TC-4, while income increased to 51.2%, similarly in TC-3 yield formed 72.9% of TC-4 but the income drawn was slightly high (77.2%). Therefore a stocking density of

15,000/ha would be ideal in the coconut garden channels of Kuttanad for the farming of *M. rosenbergii* in a more economically viable level.

4. Discussion

The developmental pathway, which follows an irreversible order from Small Male (SM) to Orange Clawed (OC) to Blue Clawed (BC) (Cohen *et al.*, 1981), is a unique feature of *M.rosenbergii*. The rate of transformation is greatly influenced by a series of extrinsic factors and stocking density in grow-outs, which are also found to alter the population composition in final harvest. In the present study, an attempt was made to establish the relationship between the stocking density and population structure of *M. rosenbergii* under two extensive monoculture systems. The results revealed that there exists a tangible difference in the morphotypic composition of this species in response to the variation in stocking density. A remarkable difference in growth and the consequent weight attained by the morphotypes at the five levels of final density in polders and four levels of final density in coconut garden channels could be discernible and their variations was well reflected in the population structure. The available reports suggest that females invariably dominate in grow-outs of *M. rosenbergii* (Smith *et al.*, 1978; 1981; Sandifer and Smith, 1975). However, in the present study, a similar trend was noticed only at higher final population density levels in polders and coconut garden channels, while at low stocking densities, males showed their dominance .The growth rate of female is slow when compared to their male counterparts and therefore the chances of their vulnerability to predation are high in higher density, especially during early phase of culture (Peebles, 1979). On the contrary, a clear preponderance of males could be noticed at the lowest final

density during harvest among polders, which is at variance with earlier reports (Smith *et al.*, 1981). Adult male prawns at a greater stocking density are prone to face competition for food and space. Owing to greater struggle and due to its highly cannibalistic nature, the proportion of male population gets considerably reduced (Kurup *et al.*, 1998). Retrieval percentage registered in the polders and coconut garden channels in the present study were found to be high when compared to similar reports (Padmakumar *et al.*, 1992; Mathew *et al.*, 1993). Mean weight of dominant male morphotypes in the high stocked grow-outs increased with reduction in survival rate and this may be due to the complex social hierarchy prevailing in the pond, while the undersized SM and WOC were deprived of food and space. This in turn reduced their average weight at higher densities. The positive correlation between prawn stocking rate and yield and negative correlation between stocking rate and individual weight have already been noted by Sandifer and Smith (1975), Smith *et al.* (1976), Willis and Berrigan (1977) and Brody *et al.* (1980).

In the present study, a dynamic shift in the proportion of male morphotype with density could also be discernible. At higher density, the proportion of SM was relatively high while the percentage of OC and its transitional stages showed a reduction. Interestingly, in channels and polders with low densities, the percentage of BC was distinctly high but the weights attained by them were comparatively lesser than that of t-SOC. This may be because of the undersized male prawns, instead of passing through the transformation pathway, skipped the intermediary stages to attain the terminal growth by leapfrog transformation as reported by Harikrishnan *et al.* (1997).

Moreover, in higher densities the relative size and weight of these males were less when compared to their counterparts in lower densities and hence instead of attaining larger size and then transforming into subsequent morphotypic stages, prawns in higher densities showed a profound change in the colouration of their claws that marked their onward transformation from SOC to t-SOC and to successive BC (Karplus *et al.*, 1992a). Thus, though the prawns could attain the terminal stage of their growth, the weight gained by them was glaringly low when compared to similar morphotypes encountered at lower densities. The shift observed in the frequency of male morphotypes may be due to the complex social organisational hierarchy in *M. rosenbergii.* At high density, the percentage of SM and WOC were high and this would suggest the chances of inhibition of growth of SM by BC due to the proximity of the latter. It may, therefore, be inferred that the rate of transformation of male morphotype to its successive stages was very rapid in low density grow outs, on account of less competition and fast growth rate and this can well be attributed as the reason for the presence of OBC in low density in appreciably high proportions. One of the principal reasons why the intensive commercial production of freshwater prawn *M. rosenbergii* could not be popularized is due to the highly skewed size distribution of harvested populations. The skewed size distribution resulted from disparate growth rates or a condition termed heterogeneous individual growth or HIG (Malecha *et al.* 1981a). Consequently, the harvested population is characterized the preponderance of non-marketable prawns (<20 g whole body weight). As stocking increases, the total yield may also increase with a dominance of marketable individuals while there is a steady tendency in the shifting of mean

length towards lower weight groups (Smith *et al.* 1978, Cohen *et al.* 1981, Karplus *et al.* 1986a).

The inverse relationship between prawn density and mean size of different morphotypes observed in this study fully agrees with the earlier findings (Cohen *et al.*, 1981; Karplus *et al.*, 1986b). Karplus *et al,* (1986a) reported that there is a decrease in mean weight for male and female morphotypes as stocking rate increases. Similar findings were recorded in respect of terminal male morphotypes in polders and channels in the present study. The reduction in prawn growth with increasing density may be attributed to a variety of reasons such as competition for food, early sexual maturity, hyperactivity of subordinate individual, loss of exuvia and aggressive and social hierarchy (Karplus *et al.*, 1986a). Increased stocking density affects the size development of juvenile prawn population in the following ways (Ra'anan and Cohen, 1985): (a) decreasing average growth and size variation with increasing density; (b) decreasing the degree of skewness with increasing density; and (c) decreasing the rate of population development with increasing density. In the present study, the heterogeneous nature of larger prawns in the high stocking density may be due to these factors. Similar effects of initial stocking density on the appearance of size variation in *M. rosenbergii* juvenile population have been reported by other investigators (Willis and Berrigan, 1977; Sandifer and Smith, 1975; Malecha, 1977). Initial small differences between individuals are getting magnified during the course of competition, resulting in suppressed growth of the smaller individuals enabling in providing an comfortable position to larger ones (Ricker, 1958). A similar phenomenon termed "shooting phenomenon" was described

(Wolfarth 1977) in fishes. *M. rosenbergii*, skewness is inversely related to density. Under less competitive situations, i.e., lower densities, the positive asymmetry of mean weight of prawns is more pronounced. The result of present study showed that the mortality rate increased with increasing population density. This effect presumably was a result of more agonistic encounters among the prawns at the higher population densities. Aggressive and cannibalistic behavior is common in *M. rosenbergii* (Wickins, 1972; Forster and Beard, 1974; Segal and Roe, 1975). Although the effect of stocking density on the production and population structure of *M. rosenbergii* either in monoculture (Siddiqui *et al.*, 1997, Kurup *et al.*, 2000), polyculture farms (Sadek and Moreau, 1998, Garcia *et al.*, 2000), laboratory (Lobao *et al.*, 1994), rice field (Janssen *et al.*, 1988) and earthen ponds (D'Abramo *et al.*, 1989; Reddy *et al.*, 1996, Valenti *et al.*, 1993) have been well documented (Daniels *et al*, 1985), no attempts has so far been made to standardize the appropriate stocking density desired in the channel and polders, especially in a tropical wetland ecosystem like Kuttanad.

In Kuttanad, a scientific culture practice for *M. rosenbergii* was lacking and most of the farmers were complying with a rather traditional type of farming which always generated only less profits. Understanding the immense potential for scampi farming in this wetland ecosystem and the role of a threshold stocking density for the economic viability of prawn farming in the polders and coconut garden channels, the present study was conceived and carried out. While working out the marketable yield structure and profit incurred from the culture under varied stocking densities, it could be seen that an increase in stocking density beyond a particular level was not helpful in the reciprocal

improvement of the profit due to the dominance of undersized prawns in the final harvest. Optimisation of stocking density also holds good since the reduction of the stocking rate below a particular level, though helpful in increasing the mean weight of prawns, would result in the poor yield and thus farming becoming economically unviable. In the present study the optimal stocking density for polders were found to be $3.5/m^2$, while that for channels a stocking density of $1/m^2$ would be advisable. The relative proportion of larger OC and BC morphotypes in the final population profoundly influences the economic viability of 'scampi' farming. In order to ensure the availability of morphotypes in the harvested population in appreciable quantities, optimisation of stocking density is a prerequisite. While maintaining an initial stocking density at $3.5/m^2$ in polders and $1.5/m^2$ in coconut garden channels, a linear relationship between the economic returns and corresponding profit could be established and similar relationship could not be arrived at other stocking densities studied. Therefore, standardisation of stocking densities at any levels of culture practice for *M. rosenbergii is* a prerequisite for improving in its economic yield and thereby making the farming economically sustainable.

Table 7.1 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 15000/ha in four polders of Kuttanad (TP- 1)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	15,000	15,000	15,000	15,000	15,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	3.0	3.0	3.0	3.0	3.0
HARVEST					
Number per square meter	0.91	0.93	0.92	0.95	0.93
Number per ha.	9142	9347	9245	9555	9314
Mean weight (g)	73.14	74.25	68.45	70.43	72.15
Gross production (Kg/ha)	255	296	280	294	280
Net production (Kg/ha)	252.4	291.54	273.5	288.4	257.3
Survival (%)	60.9	62.31	61.63	68.7	64
Mean male weight (g)	85.32	80.21	80.2	85.3	83.02
Mean female weight (g)	65.34	61.29	60.23	62.48	62.08
% by number of males in the population	52.45	57.43	60.4	55.36	58.2
% by number of females in the population	47.55	42.57	39.6	44.64	41.8
Sex ratio	1:0.91	1:0.74	1: 0.65	1: 0.81	1:0.72

Table 7.2 Comparison of mean weights of individual morphotypes in four polders of Treatment 1(TP-1)

Morphotypes	F-value
SF	1.1823
WOF	1.5889
SOF	1.3222
TOF	0.9807
WBF	1.0849
SBF	2.7907
SM	0.1263
WOC	0.7239
SOC	0.6848
t-SOC	1.8266
WBC	1.6298
SBC	2.2850
OBC	1.7302

SF = Small female
WOF= Weak orange clawed female
SOF = Strong orange clawed female
TOF = Transforming strong orange clawed female
WBF= Weak blue clawed female
SBF = Strong blue clawed female
SM = Small male
WOC = Weak orange clawed male
SOC = Strong orange clawed male
t-SOC = Transforming strong orange clawed male
WBC = Weak blue clawed male
SBC = Strong blue clawed male
OBC = Old blue clawed male

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes

Table 7.3 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 25000/ha in four polders of Kuttanad (TP- 2)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	25,000	25,000	25,000	25,000	25,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	5.0	5.0	5.0	5.0	5.0
HARVEST					
Number per square meter	1.25	1.54	1.4	1.53	1.41
Number per ha.	12486	15472	14086	15358	14125
Mean weight (g)	66.59	60.58	65.24	60.28	64.35
Gross production (Kg/ha)	305	331	329	308	315
Net production (Kg/ha)	300.54	324.56	321.53	303.91	309.8
Survival (%)	49.94	61.88	56.34	61.4	56.2
Mean male weight (g)	76.98	74.25	78.54	85.3	75.14
Mean female weight (g)	53.24	55.68	60.08	62.48	58.46
% by number of males in population	54.28	52.48	51.21	52.17	52.3
% by number of females in population	45.72	47.52	48.79	47.83	47.7
Sex ratio	1:0.84	1:0.91	1:0.95	1: 0.92	1:0.91

Table 7.4 Comparison of mean weights of individual morphotypes in four polders of Treatment 2 (TP-2)

Morphotypes	F-value
SF	1.2809
WOF	1.6875
SOF	1.4208
TOF	1.0793
WBF	1.1835
SBF	2.8893
SM	0.2249
WOC	0.8225
SOC	0.7834
t-SOC	1.9252
WBC	1.7284
SBC	1.9864
OBC	1.8288

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.5 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 35000/ha in four polders of Kuttanad (TP 3)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35,000	35,000	35,000	35,000	35,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.72	1.63	2.01	1.92	1.88
Number per ha.	17258	16357	20146	19287	18890
Mean weight (g)	60.24	61.24	54.28	58.24	58.41
Gross production (Kg/ha)	524	538	592	571	568
Net production (Kg/ha)	518.26	531.43	586.38	568.24	554.2
Survival (%)	49.3	46.73	57.56	55.1	54.8
Mean male weight (g)	67.35	70.28	60.31	62.89	65.26
Mean female weight (g)	51.95	53.84	45.67	48.25	50.43
% by number of males in population	52.48	54.37	49.85	55.27	51.69
% by number of females in population	47.52	45.63	50.15	44.73	48.31
Sex ratio	1:0.91	1:0.84	1:1	1:0.81	1:0.85

Table 7.6 Comparison of mean weights of individual morphotypes in four polders of Treatment 3 (TP-3)

Morphotypes	F-value
SF	1.3933
WOF	1.7999
SOF	1.5332
TOF	1.1917
WBF	1.2959
SBF	1.8445
SM	0.3373
WOC	0.9349
SOC	1.4547
t-SOC	2.5965
WBC	2.3997
SBC	1.8977
OBC	2.5001

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.7 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 45000/ha in four polders of Kuttanad (TP-4)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	45,000	45,000	45,000	45,000	45,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	9.0	9.0	9.0	9.0	9.0
HARVEST					
Number per square meter	2.04	2.35	2.16	1.95	2.15
Number per ha.	20487	23514	21658	19547	21578
Mean weight (g)	58.68	52.47	57.54	58.24	56.63
Gross production (Kg/ha)	742	784	762	731	757
Net production (Kg/ha)	786.58	777.56	756.38	726.25	745.3
Survival (%)	45.52	52.25	48.12	43.43	48.1
Mean male weight (g)	60.27	52.14	49.35	56.27	58.94
Mean female weight (g)	45.19	39.58	36.45	41.08	42.00
% by number of males in population	44.28	54.86	42.84	51.31	46.7
% by number of females in population	55.72	45.14	57.16	48.69	53.3
Sex ratio	1:1.25	1:0.82	1:1.33	1:0.94	1:1.14

Table 7.8 Comparison of mean weight of individual morphotypes in four polders of Treatment 4(TP-4)

Morphotypes	F-value
SF	1.9776
WOF	1.5875
SOF	2.1874
TOF	1.6752
WBF	1.8315
SBF	1.8116
SM	0.3936
WOC	1.2900
SOC	1.5108
t-SOC	1.2235
WBC	1.9283
SBC	2.4353
OBC	2.0789

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.9 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 60000/ha in four polders of Kuttanad (TP- 5)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	60,000	60,000	60,000	60,000	60,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	1.2	1.2	1.2	1.2	1.2
HARVEST					
Number per square meter	2.57	2.85	2.46	2.56	2.71
Number per ha.	25789	28569	24687	25666	27100
Mean weight (g)	53.28	46.57	56.24	51.28	52.58
Gross production (Kg/ha)	798	887	805	826	842
Net production (Kg/ha)	794.08	876.95	801.5	814.93	835.5
Survival (%)	42.98	47.61	41.14	42.77	45.7
Mean male weight (g)	58.64	46.58	58.14	49.58	54.06
Mean female weight (g)	48.21	39.58	38.14	35.68	44.69
% by number of males in population	40.58	42.15	45.36	51.31	42.9
% by number of females in population	59.42	57.85	54.64	48.69	57.1
Sex ratio	1:1.46	1:1.37	1:1.20	1:0.94	1:1.33

Table 7.10 Comparison of mean weights of individual morphotypes in four polders of Treatment 5 (TP-5)

Morphotypes	F-value
SF	1.7431
WOF	1.3530
SOF	1.9529
TOF	1.4407
WBF	1.5970
SBF	1.5771
SM	1.1591
WOC	1.0555
SOC	1.2763
t-SOC	1.9890
WBC	1.6938
SBC	2.2008
OBC	1.8444

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.11 Summary of water quality parameters in treatments 1 - 5 (Polders)

Parameters	Treatment 1 TP-1	Treatment 2 TP-2	Treatment 3 TP-3	Treatment 4 TP-4	Treatment 5 TP-5
Surface Temperature	30.28	30.85	30.4	31.26	30.81
(deg C)	(26.6 - 33.4)	(27.1 - 33.2)	(26.5 - 34.2)	(25.8 - 34.5)	(25.6 - 33.7)
Surface Dissolved Oxygen	4.38	5.26	4.82	6.25	5.68
(mg/l)	(3.3 - 7.5)	(2.5 - 6.4)	(2.3 - 6.1)	(2.6 - 8.1)	(2.9 - 6.8)
Water pH range	6.1 - 8.2	6.3 - 8.4	6.0- 7.8	6.5 - 8.2	6.1 - 8.0
Soil pH range	5.0 - 6.8	4.8 - 6.5	5.2 - 6.6	5.2 - 6.2	4.7 - 6.1
Transparancy	24.5	32.5	35	28.7	32.5
(cm)	(10 - 62)	(12 - 55)	(15 - 68)	(10 - 55)	(20 - 71)
Total Alkalinity	58.45	69.24	48.61	75.58	65.48
(mg/l)	(28 - 102)	(35 - 85)	(36 - 90)	(34 - 132)	(32 - 94)
Nitrite-N	0.1	0.1	0.2	0.1	0.2
(mg/l)	(0.02- 0.4)	(0.02 - 0.2)	(0.01 - 0.5)	(0.01 - 0.2)	(0.02 - 0.4)
Total Ammonia	0.1	0.05	0.05	0.1	0.1
(mg/l)	(0 - 0.2)	(0 - 0.1)	(0 - 0.1)	(0 - 0.4)	(0 - 0.2)
Hydrogen sulphide	0.01	0.01	0.01	0.01	0.01
(mg/l)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.05)	(0 - 0.04)

Figures are means of four replicates and 18 sampling dates (N = 72). The range of observed values are given in parenthesis

Table 7.12 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared under different stocking density in five treatments (Polders)

	Treatments				
	TP-1	TP-2	TP-3	TP-4	TP-5
STOCKING					
Number per ha.	15,000	25,000	35,000	45,000	60,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	3.0	5.0	7.0	9.0	1.2
HARVEST					
Number per square meter	0.93	1.41	1.88	2.15	2.71
Number per ha.	9314	14125	18890	21578	27100
Mean weight (g)	72.15	64.35	58.41	56.63	52.58
Gross production (Kg/ha)	280	315	568	757	842
Net production (Kg/ha)	257.3	309.8	554.2	745.3	835.5
Survival (%)	64	56.2	54.8	48.1	45.7
Mean male weight (g)	83.02	75.14	65.26	58.94	54.06
Mean female weight (g)	62.08	58.46	50.43	42.00	44.69
% by number of males in popı	58.2	52.3	54.1	46.7	42.9
% by number of females in pc	41.8	47.7	45.9	53.3	57.1
Sex ratio	1:0.72	1:0.91	1:0.85	1:1.14	1:1.33

Table 7.13. Analysis of Variance in the net production from the five sets of treatments (Polders)

Source of Variation	*Sum of square*	*df*	*Mean Sum of square*	*calculated F*
Between Groups	729735.67	4	182433.92	49.2381*
Within Groups	40756.48	15	3705.1345	
Total	770492.15	19		

Table 7.14 t-Test comparison on the net production between the treatments

	Treatment 1 vs 2	Treatment 2 vs 3	Treatment 3 vs 4	Treatment 4 vs t 5	Treatment 2 vs 3	Treatment 3 vs 4	Treatment 4 vs 5	Treatment 3 vs 4	Treatment 4 vs 5	Treatment 4 vs 5
t- value	1.5648	5.2771*	14.9317*	18.499*	4.9498*	23.5957*	33.2847*	3.9168**	5.8792*	6.5062

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

Table 7.15 Percentage contribution of male and female morphotypes by weight from five treatments

Treatment		Mean weight of male morphotype							Mean weight of female morphotypes					
		SM	WOC	SOC	t-SOC	WBC	SBC	OBC	SF	WOF	SOF	TOF	WBF	SBF
	Number sampled	151	127	79	113	110	124	161	140	163	48	118	124	153
	% by weight	5.40	5.90	6.20	21.35	2.60	11.00	12.67	5.27	6.14	3.18	8.12	7.82	4.35
TP-1	Mean weight	18.41	42.53	93.33	103.62	21.22	97.78	147.91	16.04	31.50	60.63	71.99	60.95	70.00
(n=4)	Standard deviation	5.82	3.87	18.63	18.09	14.58	19.08	25.25	5.31	16.67	16.78	16.07	17.93	11.55
	Coeff. of variation	13.42	27.36	15.93	70.61	24.00	117.85	182.64	3.11	52.94	27.68	22.33	29.41	16.50
	Number sampled	115	123	16	154	154	172	137	123	21	50	95	123	124
	% by weight	9.87	2.14	10.63	23.67	1.26	1.52	15.56	7.61	3.94	1.98	12.88	3.29	5.65
TP-2	Mean weight	25.60	32.20	87.50	108.70	101.28	82.80	121.30	12.55	28.57	43.24	49.30	52.38	66.94
(n=4)	Standard deviation	7.49	16.92	23.69	43.59	45.51	23.74	12.87	2.64	7.57	1.02	11.72	13.04	13.03
	Coeff. of variation	25.81	18.73	38.70	44.28	54.57	37.92	87.81	21.56	22.44	28.34	19.15	13.16	23.76
	Number sampled	137	57	11	135	162	186	96	141	100	31	124	112	122
	% by weight	14.66	2.10	8.89	16.38	8.14	7.71	8.06	11.34	2.94	1.04	6.79	8.17	3.78
TP-3	Mean weight	18.23	36.43	68.50	76.37	106.91	78.12	93.40	12.26	27.92	38.66	48.17	51.18	65.41
(n=4)	Standard deviation	6.57	7.36	8.82	4.23	39.21	3.85	4.96	3.66	8.59	23.74	12.74	14.06	14.05
	Coeff. of variation	32.45	25.55	12.80	18.08	36.68	15.73	13.44	29.88	30.76	37.92	27.47	21.48	32.08
	Number sampled	129	129	33	110	151	133	140	53	48	18	129	117	112
	% by weight	14.59	5.40	5.28	16.21	3.82	8.26	6.75	9.82	5.91	1.54	12.44	4.69	5.29
TP-4	Mean weight	15.57	32.47	45.61	61.57	90.43	61.00	76.53	13.50	61.20	64.40	58.40	61.14	86.02
(n=4)	Standard deviation	48.52	21.83	36.82	12.82	46.14	83.56	87.32	3.31	31.32	9.98	27.11	24.51	29.68
	Coeff. of variation	68.86	61.82	21.53	19.43	51.03	39.15	53.56	36.21	62.02	21.99	56.75	49.59	43.00
	Number sampled	115	33	16	154	124	82	137	23	21	50	135	123	94
	% by weight	21.36	0.77	10.00	18.47	8.29	2.46	4.61	13.56	6.23	1.93	7.23	3.01	2.08
TP-5	Mean weight	11.35	27.45	51.17	68.32	25.02	67.15	91.54	13.55	30.86	46.26	53.24	56.57	72.30
(n=4)	Standard deviation	2.61	8.54	8.34	19.96	38.62	24.00	27.06	2.85	8.18	1.10	12.66	14.08	14.07
	Coeff. of variation	23.76	27.32	23.35	23.10	37.31	30.30	23.07	23.28	24.24	30.61	20.68	14.21	25.66

TP = Treatments in polders

For expansion of morphotypes refer Table 7.1

Table 7.16 Comparison of mean weight of individual morphotypes among the five treatments

SF	0.3147
WOF	0.7213
SOF	0.4546
TOF	2.3517*
WBF	1.2173**
SBF	4.9231*
SM	0.2587
WOC	0.8563
SOC	0.3761
t-SOC	3.5179*
WBC	5.3211*
SBC	2.9763*
OBC	6.4215*

Table 7.17 Comparison of mean weight between five treatments through t-Test

Morpotype	Treatment 1 vs 2	Treatment 1 vs 3	Treatment 1 vs 4	Treatment 1 vs 5	Treatment 2 vs 3	Treatment 2 vs 4	Treatment 2 vs 5	Treatment 3 vs 4	Treatment 3 vs 5	Treatment 4 vs 5
SF	0.1124	0.2516	0.1782	0.3147	0.1056	0.3112	0.4733	0.1573	0.7512	0.5348
WOF	0.3481	1.1412**	1.2457**	1.7330**	0.4213	0.5796	1.6434*	0.1702	0.5796	0.7456
SOF	0.5932	0.7510	0.8539	1.9634**	0.6642	0.8696	0.0243	0.7661	0.4552	0.7970
TOF	1.3429**	1.7370**	2.3557*	4.1702*	0.9895	1.1113**	2.4716*	2.4431*	0.7976	0.1086
WBF	0.5932	0.9352	1.5486**	1.2927**	0.9143	1.1964**	5.7511*	1.1250**	1.0606*	0.9529
SBF	1.7682**	1.7825**	2.7204*	3.0308*	0.9186	1.1939**	2.8578*	0.712	0.815	0.4152
SM	0.2791	0.2478	0.2750	0.2012	0.8586	0.7371	0.1324	0.1141	0.2588	0.2185
WOC	0.9613	0.8123	1.0134**	0.6107	0.5678	0.2228	0.4511	0.2283	0.4827	0.9619
SOC	0.6323	3.3743*	1.3912**	1.9356**	0.5718	0.4504	0.4717	0.9356	0.4620	0.7518
t-SOC	1.3563**	2.6691**	3.1470*	3.3743*	0.486	1.9525**	1.0031	0.3147	3.3252*	1.0952**
WBC	1.9948**	1.9876**	32616*	1.3912**	0.8526	5.6922*	1.3563**	0.3201	1.9362**	1.5290**
SBC	1.8463**	1.3480**	3.2009*	1.9356**	0.4515	6.3745*	1.9199**	1.3200*	1.3928**	3.4283*
OBC	1.3738**	1.8172**	1.7426**	1.0462**	1.1463**	5.7128*	1.3738**	1.7426**	1.0751**	1.1753**

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

For expansion of morphotypes refer Table 7.1

Table 7.18 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 5000/ha in four channels of Kuttanad (TC-1)

	CHANNELS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	5,000	5,000	5,000	5,000	5,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	1.0	1.0	1.0	1.0	1.0
HARVEST					
Number per square meter	0.32	0.37	0.38	0.31	0.35
Number per ha.	3254	3684	3821	3129	3560
Mean weight (g)	112.50	98.40	95.4	100.3	101.4
Gross production (Kg/ha)	102	85.1	89.7	95.2	92.1
Net production (Kg/ha)	99.4	82.7	85.6	92.7	89.4
Survival (%)	65.08	73.68	76.42	62.58	71.2
Mean male weight (g)	132.8	125.4	118.8	136.2	128.6
Mean female weight (g)	72.3	65.4	62.4	73.80	68.2
% by number of males in population	71.05	54.36	58.17	66.25	67.26
% by number of females in population	29.95	45.64	41.83	33.75	32.74
Sex ratio	2.37:1	1.19:1	1.39:1	1.96:1	2.05:1

Table 7.19 Comparison of mean weights of individual morphotypes in four channels of Treatment 1 (TC-1)

Morphotypes	F-value
SF	1.4962
WOF	1.9028
SOF	1.6361
TOF	1.2946
WBF	1.3988
SBF	1.9474
SM	0.4402
WOC	1.0378
SOC	1.5576
t-SOC	1.6994
WBC	1.5026
SBC	2.0006
OBC	2.1030

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.20 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 10000/ha in four channels of Kuttanad (TC- 2)

	CHANNELS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	10,000	10,000	10,000	10,000	10,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	2.0	2.0	2.0	2.0	2.0
HARVEST					
Number per square meter	0.52	0.58	0.57	0.5	0.53
Number per ha.	5247	5831	5721	5014	5380
Mean weight (g)	91.54	78.14	84.37	96.87	88.3
Gross production (Kg/ha)	95.45	121.55	118.92	92.47	110.1
Net production (Kg/ha)	91.58	117.06	115.23	88.77	105.4
Survival (%)	52.47	58.31	57.21	50.14	53.8
Mean male weight (g)	111.89	86.45	94.68	127.24	105.2
Mean female weight (g)	65.38	52.48	50.18	60.48	52.5
% by number of males in population	55.24	58.15	56.14	51.98	57.5
% by number of females in population	44.76	41.85	43.86	48.02	42.5
Sex ratio	1.23:1	1.38:1	1.27:1	1.08:1	1.15:1

Table 7.21 Comparison of mean weights of individual morphotypes in four channels of Treatment 2 (TC-2)

Morphotypes	F-value
SF	1.2395
WOF	1.6461
SOF	1.3794
TOF	1.0379
WBF	1.1421
SBF	2.8479
SM	0.1835
WOC	0.7811
SOC	0.7420
t-SOC	1.8838
WBC	1.6870
SBC	2.3422
OBC	1.7874

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.22 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 15000/ha in four channels of Kuttanad (TC- 3)

	CHANNELS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	15,000	15,000	15,000	15,000	15,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	3.0	3.0	3.0	3.0	3.0
HARVEST					
Number per square meter	0.62	0.54	0.51	0.58	0.57
Number per ha.	6210	5433	5148	5824	5690
Mean weight (g)	61.35	74.29	78.65	62.16	70.5
Gross production (Kg/ha)	198.7	149.2	158.4	204.4	188.3
Net production (Kg/ha)	195.4	143.8	154.7	200.1	182.8
Survival (%)	41.4	36.22	34.32	38.82	37.93
Mean male weight (g)	88.47	115.14	108.7	94.28	101.8
Mean female weight (g)	44.58	51.28	56.34	48.25	50.05
% by number of males in population	52.48	53.27	54.24	50.14	55.5
% by number of females in population	47.52	46.73	45.76	49.86	44.5
Sex ratio	1.04:1	1.13:1	1.18:1	1:1	1.11:1

Table 7.23 Comparison of mean weights of individual morphotypes in four channels of Treatment 3 (TC-3)

Morphotypes	F-value
SF	1.8942
WOF	1.5041
SOF	2.1040
TOF	1.5918
WBF	1.7481
SBF	1.7282
SM	0.3102
WOC	1.2066
SOC	1.4274
t-SOC	1.1401
WBC	1.8449
SBC	2.3519
OBC	1.9955

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.24 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 25000/ha in four channels of Kuttanad (TC- 4)

	CHANNELS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	25,000	25,000	25,000	25,000	25,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	5.0	5.0	5.0	5.0	5.0
HARVEST					
Number per square meter	0.75	0.68	0.74	0.64	0.7
Number per ha.	7532	6821	7425	6415	7020
Mean weight (g)	52.47	61.24	54.98	53.24	56.2
Gross production (Kg/ha)	269.4	248.4	271.8	232.5	259.1
Net production (Kg/ha)	264.35	241.84	265.14	227.54	252.41
Survival (%)	30.12	27.28	29.7	25.66	28.08
Mean male weight (g)	78.25	89.45	78.14	94.12	85.3
Mean female weight (g)	38.26	41.86	40.17	45.82	42.8
% by number of males in population	35.58	39.42	48.14	51.24	38.5
% by number of females in population	64.42	60.58	51.86	48.76	51.5
Sex ratio	0.55:1	0.65:1	0.93:1	1.05:1	0.77:1

Table 7.25 Comparison of mean weights of individual morphotypes in four channels of Treatment 4 (TC-4)

Morphotypes	F-value
SF	1.3944
WOF	1.8010
SOF	1.5343
TOF	1.1928
WBF	1.2970
SBF	2.0028
SM	0.3384
WOC	0.9360
SOC	0.8969
t-SOC	2.0387
WBC	1.8419
SBC	2.0999
OBC	1.9423

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 7.1

Table 7.26 Mean and range of water quality parameters in treatments 1-4 (Coconut garden channels)

Parameters	Treatment 1 TC-1	Treatment 2 TC-2	Treatment 3 TC-3	Treatment 4 TC-4
Surface Temperature	28.4	29.3	31.4	30.4
(deg C)	(24.7 - 32.8)	(26.1 - 32.2)	(28.6 - 33.1)	(24.4 - 34.2)
Surface Dissolved Oxygen	3.62	5.5	5.06	4.49
(mg/l)	(3.0 - 6.2)	(2.8 - 5.8)	(2.3 - 7.1)	(3.6 - 6.1)
Water pH range	5.48 - 7.8	6.2 - 7.9	6.4 - 8.1	5.9 - 7.4
Soil pH range	5.3 - 7.8	5.6 - 7.9	5.2- 6.8	5.2 - 6.9
Transparancy	12.4	18.6	21.4	28.4
(cm)	(8 - 35)	(10 - 38)	(15 - 38)	(10 - 45)
Total Alkalinity	68.4	52.14	86.74	64.29
(mg/l)	(32 - 87)	(35 - 92)	(56 - 114)	(44 - 102)
Nitrite-N	0.1	0.1	0.1	0.1
(mg/l)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)
Total Ammonia	0.1	0.05	0.05	0.1
(mg/l)	(0 - 0.2)	(0 - 0.1)	(0 - 0.1)	(0 - 0.4)
Hydrogen sulphide	0.01	0.01	0.01	0.01
(mg/l)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)

Figures are means of four replicates and 22 sampling dates (N = 88). The range of observed values is given in parenthesis

Table 7.27 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared @ 5,000 - 25,000/ha in four sets of treatments (Channels)

	Treatments			
	TC-1	TC-2	TC-3	TC-4
STOCKING				
Number per ha.	5,000	10,000	15,000	25,000
Mean weight (g)	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	1.0	2.0	3.0	5.0
HARVEST				
Number per square meter	3560	5380	5690	7020
Number per ha.	0.35	0.53	0.57	0.7
Mean weight (g)	101.4	88.3	70.5	56.2
Gross production (Kg/ha)	92.1	110.1	188.3	259.1
Net production (Kg/ha)	89.4	105.4	182.8	250.5
Survival (%)	71.2	53.8	37.93	28.08
Mean male weight (g)	128.6	105.2	101.8	85.3
Mean female weight (g)	68.2	52.5	50.05	42.8
% by number of males in population	67.26	57.5	55.5	38.5
% by number of females in population	32.74	42.5	44.5	51.5
Sex ratio	2.05:1	1.15:1	1.11:1	0.77:1

TC = Treatments in channels

Table 7.28. Analysis of Variance in net production from four sets of treatments (Channels)

Source of Variation	*Sum of square*	*df*	*Mean sum of square*	*calculated F*
Between Groups	39716.657	3	13238.88556	22.227*
Within Groups	4764.88	12	595.61	
Total	44481.537	15		

Table 7.29 t-Test comparison on net production between treatments (Channels)

	Treatment 1 vs 2	Treatment 1 vs 3	Treatment 1 vs 4	Treatment 2 vs 3	Treatment 2 vs 4	Treatment 3 vs 4
t- value	1.735	5.5459*	6.5635**	4.4937*	5.8487	2.3727

* Significant at 1% level (P<0.01)
** Significant at 5% level (P<0.05)

Table 7.30 Percentage contribution by weight in the harvested population and mean weight of male and female morphotypes from the four treatments (Coconut garden channels)

Treatment		Mean weight of male morphotype												
		SM	WOC	SOC	t-SOC	WBC	SBC	OBC	SF	WOF	SOF	TOF	WBF	SBF
	Number sampled	65	25	6	72	41	12	24	29	34	15	26	51	50
	% by weight	6.28	4.04	4.56	28.45	5.00	8.37	21.37	5.33	1.16	0.10	7.34	4.76	3.24
TC-1	Mean weight	18.41	42.53	93.33	103.62	97.78	129.40	147.91	12.05	54.64	57.50	52.14	54.59	76.80
(n=4)	Standard deviation	5.82	3.87	18.63	18.09	19.08	14.65	25.25	3.94	31.95	10.61	27.74	25.14	30.31
	Coeff. of variation	13.42	27.36	15.93	70.61	117.85	36.55	182.64	32.67	58.48	18.45	53.21	46.05	39.46
	Number sampled	24	14	47	45	48	35	52	24	48	1	15	24	24
	% by weight	15.79	11.28	3.77	20.12	6.22	6.20	15.09	7.37	0.81	0.42	3.55	4.47	4.91
TC-2	Mean weight	25.60	32.20	87.50	108.70	82.80	120.53	121.30	12.26	27.92	0.00	48.17	51.18	65.41
(n=4)	Standard deviation	7.49	16.92	23.69	43.59	23.74	18.63	12.87	3.66	8.59	0.00	12.74	14.06	14.05
	Coeff. of variation	25.81	18.73	38.70	44.28	37.92	15.93	87.81	29.88	30.76	0.00	27.47	21.48	32.08
	Number sampled	13	24	15	35	31	20	35	24	48	15	12	16	122
	% by weight	20.21	15.92	2.66	11.58	8.02	4.20	10.62	9.33	0.52	0.14	7.87	3.55	3.38
TC-3	Mean weight	18.23	36.43	68.50	76.37	78.12	102.45	111.45	16.04	31.50	60.63	71.99	60.95	70.00
(n=4)	Standard deviation	36.57	7.36	18.82	24.32	23.85	31.95	24.96	5.31	16.67	16.78	16.07	17.93	11.55
	Coeff. of variation	132.43	5.55	12.80	18.08	15.73	58.48	13.44	3.11	52.94	27.68	22.33	29.41	16.50
	Number sampled	29	29	13	10	51	33	40	53	48	18	29	17	12
	% by weight	23.66	12.67	3.73	14.29	4.42	2.00	7.69	12.89	1.72	1.56	5.88	6.34	2.15
TC-4	Mean weight	15.57	32.47	45.61	61.57	61.00	95.34	103.45	13.50	61.20	64.40	58.40	61.14	86.02
(n=4)	Standard deviation	48.52	21.83	36.82	12.82	83.56	16.92	88.32	3.31	31.32	9.98	27.11	24.51	29.68
	Coeff. of variation	168.72	61.82	21.53	19.43	39.15	18.73	53.57	36.21	62.02	21.99	56.75	49.59	43.00

TC = Treatments in Coconut garden channels
For expansion of morphotypes refer Table 7.1

Table 7.31 Comparison of mean weight of individual morphotypes among the five treatments

Morphotypes	F-value
SF	0.4167
WOF	0.9550**
SOF	0.6019
TOF	3.1136*
WBF	1.6117**
SBF	4.5127*
SM	0.3425
WOC	1.1337**
SOC	0.4980
t-SOC	4.6570*
WBC	3.2541*
SBC	3.9406*
OBC	2.1573**

Table 7.32 Comparison of mean weight between the four treatments through t-Test

Morpotype	Treatment 1 vs 2	Treatment 1 vs 3	Treatment 1 vs 4	Treatment 2 vs 3	Treatment 2 vs 4	Treatment 3 vs 4
SF	0.1198	0.3529	0.5367	0.1784	0.8519	0.6065
WOF	0.4778	0.6573	1.8636	0.1930	0.6573	0.8455
SOF	0.7532	0.9861	0.0276	0.8688	0.5162	0.9038
TOF	1.1220**	1.2602**	2.8027*	2.7704*	0.9045	0.1232
WBF	1.0368	1.3567*	6.5217	1.2757**	1.2027**	1.0806
SBF	1.0417	1.3538*	3.2407	0.8074	0.9242	0.4708
SM	0.9737	0.8359	0.1501	0.1294	0.2935	0.2478
WOC	0.6439	0.2527	0.5115	0.2589	0.5474	1.0908
SOC	0.6484	0.5108	0.5349	1.0610	0.5239	0.8525
t-SOC	0.5511	2.2141*	1.1375**	0.3569	3.7707*	1.2420
WBC	0.9668	6.4549*	1.5380**	0.3630	2.1956*	1.7338**
SBC	0.5120	7.2286*	2.1771*	1.4968**	1.5794**	3.8876*
OBC	1.2999	6.4783*	1.5578**	1.9761*	1.2191**	1.3327**

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

For expansion of morphotypes refer Table 7.1

Fig 7.1 Morphotypic composition of males of *M. rosenbergii* in the harvested population from different Treatments (Polders)

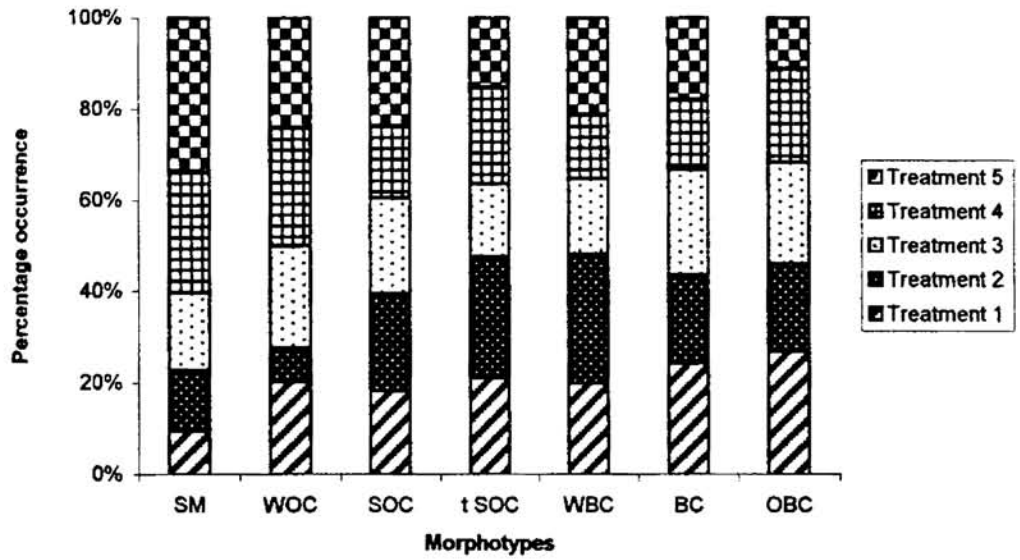


Fig 7.2 Morphotypic composition of females of *M. rosenbergii* in the harvested population from different Treatment (Polders)

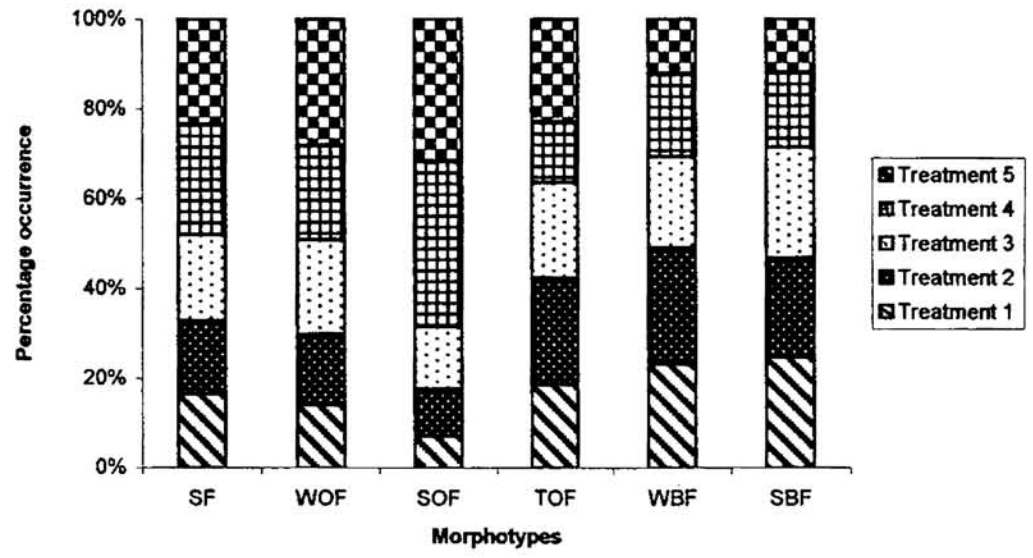


Fig. 7.3 Market structure of harvested population of *M. rosenebrgii* in Treatment 1 (Polders) (15,000/ha)

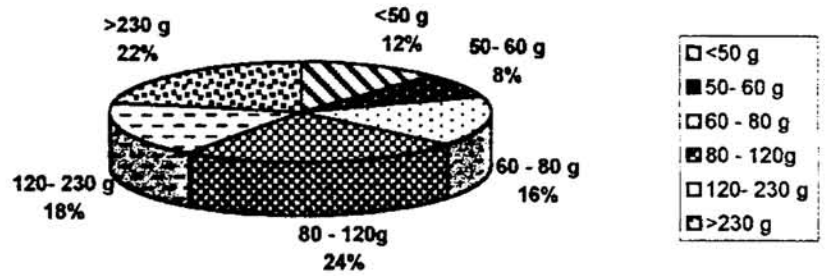


Fig. 7.4 Market structure of harvested population of *M. rosenbergii* in Treatment 2 (Poders) (25,000/ha)

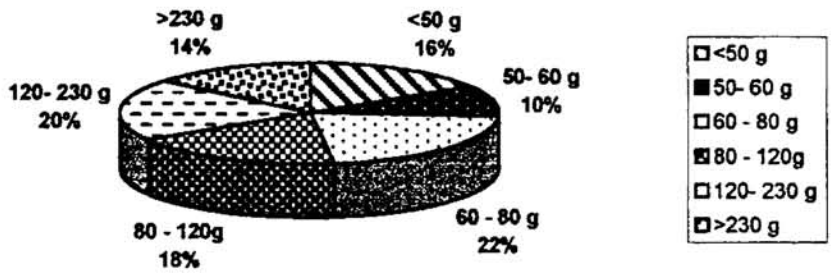


Fig 7.5 Market structure of harvested population of *M. rosenbergii* in Treatment 3 (Polders) (35,000/ha)

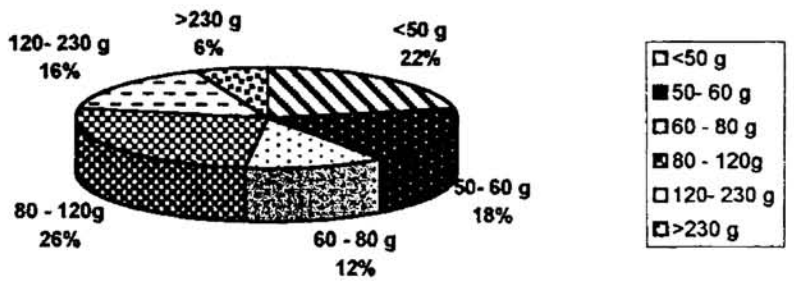


Fig. 7.6 Market structure of harvested population of *M. rosenbergii* in Treatment 4 (Polders) (45,000/ha)

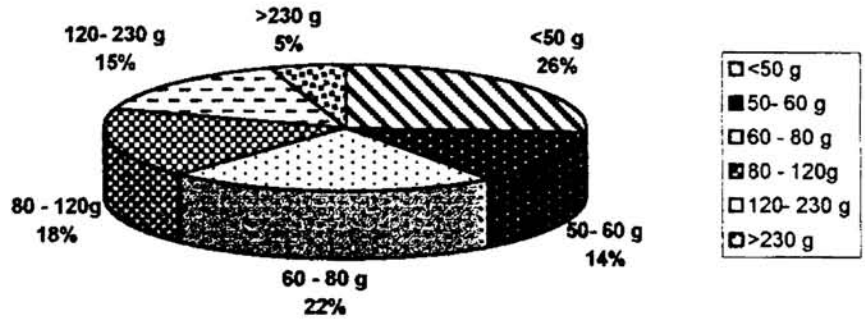


Fig. 7.7 Market structure of harvested population of *M. rosenbergii* in Treatment 5 (Polders) (60,000/ha)

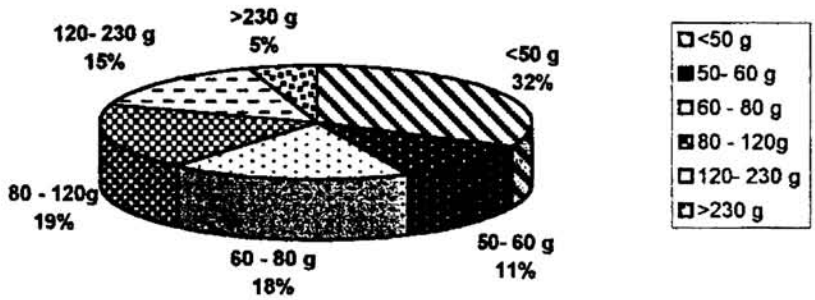


Fig 7.8 Morphotypic composition of males in final harvested population of *M. rosenbergii* in different treatments (Channels)

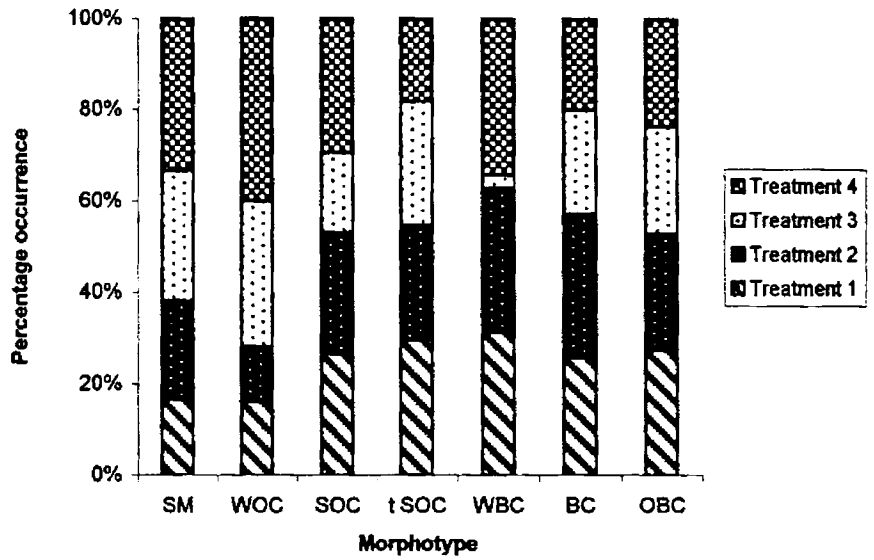


Fig 7.9 Morphotypic composition of females in harvested population of *M.rosenbergii* in different treatment (Channels)

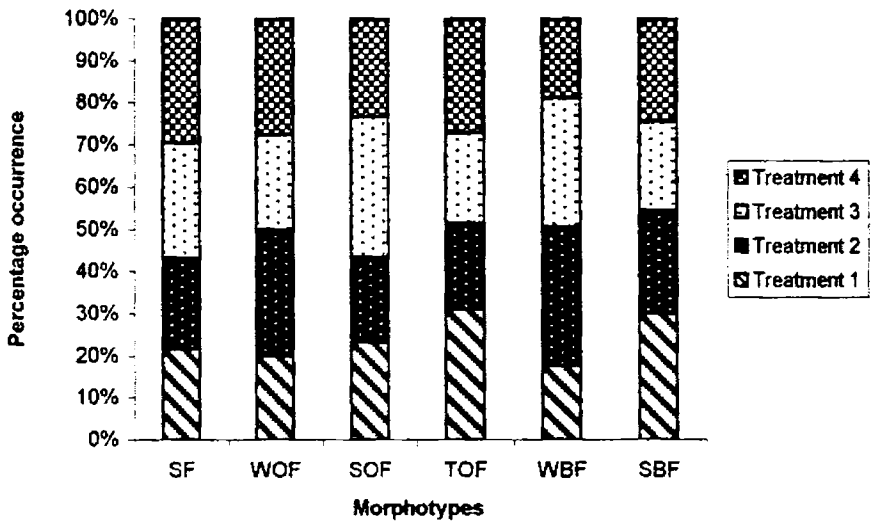


Fig 7.10 Market structure of harvested population of *M. rosenbergii* in Treatment 1 (Channels, 5000/ha)

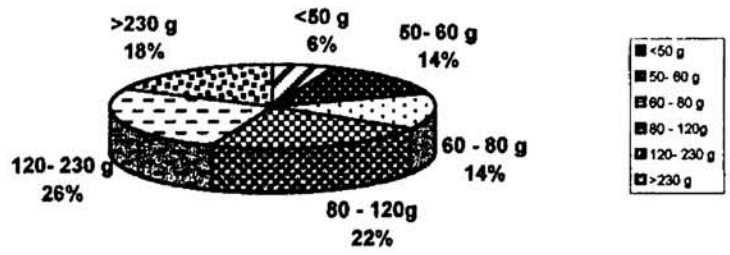


Fig 7.11 Market structure of harvested population of *M. rosenbergii* in Treatment 2 (Channels, 10,000/ha)

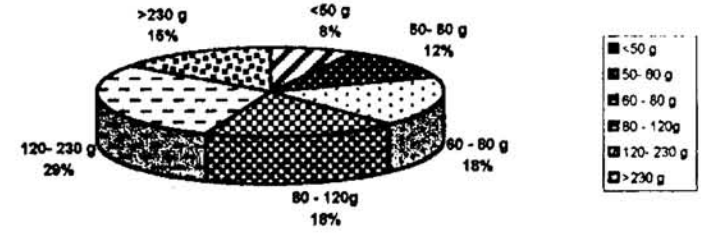


Fig 7.12 Market structure of harvested population of *M. rosenbergii* inTreatment 3 (Channels, 15,000/ha)

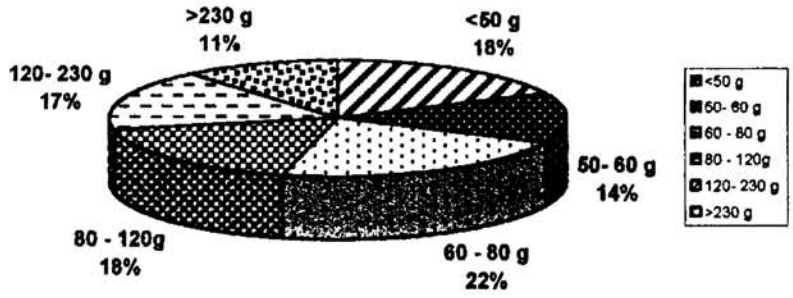
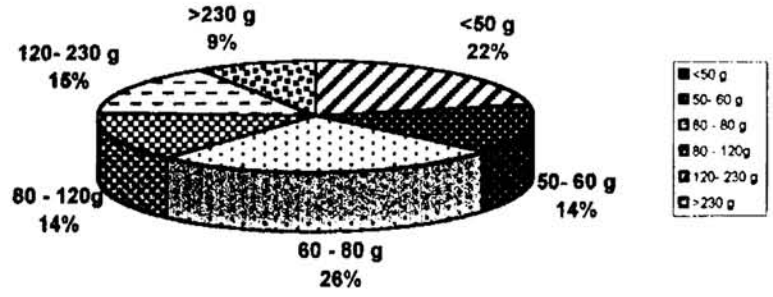


Fig 7.13 Market structure of harvested populaion of *M. rosenbergii* inTreatment 4 (Channels, 25,000/ha)



Chapter 8.

Effectiveness of Nursery phase in enhancing survival and production of *Macrobrachium rosenbergii* in the grow-outs

1. Introduction:

Macrobrachium rosenbergii is receiving remarkable attention as a candidate species for intensive culture throughout the world. Attempts to develop a threshold grow-out strategy for this species is gaining importance in recent years (Wickins, 1972; Sandifer and Smith, 1975, 1977; Malecha, 1977). However, the success of the culture is dependent on survival rates and average weight for prawns at the time of harvest. During the past two decades substantial development has taken place in the hatchery and grow-out techniques of *M. rosenbergii* ever since the accomplishment of its life cycle by Ling (1962). Stocking with batch graded and size graded post larvae and juveniles (Karplus *et al.*, 1987; Sandifer and Smith, 1975, 1977), of this species have been attempted to improve the yield by minimizing differential growth, however, limited information is available on the role of nursery rearing in improving the production. Most works on prawn nursery rearing deals with intensive indoor nursery systems in temperate regions (Kneale and Wang, 1979; Smith and Sandifer, 1979; Smith *et al.*, 1983), since temperature acts as a limiting factor on growth, survival and activity of prawns when it fluctuates outside the range of 22-33°C (Sandifer and Smith, 1985). In South India, farming of these species can be carried out almost round the year, however, in many parts, water temperature drops below 22°C during winter months, affecting the growth of these prawns adversely (Bindu *et al.*, 1999). The major economic constraints met within the

winter farming of this species are the necessity of roof cover and the maintenance of water temperature within desirable limits (Rao and Tripathi, 1993). In a tropical wetland ecosystem such as Kuttanad, however, the major impediment faced in the freshwater prawn farming is the low survival rates as well as differential growth of adult male prawns (Ranjeet and Kurup, 2001a). Hence, development of a threshold grow-out strategy for *M. rosenbergii* culture is most warranted in order to make its culture viable and profitable. Usually the absence of a nursery phase after the hatchery cycle results in low survival rates in the grow-outs (Bindu *et al.*, 1999). Regardless of grow-out approach, the incorporation of prawn nursery system seems to offer potential for increasing the yields, value and predictability of the crop (Smith *et al.*, 1976, 1978; Willis and Berrigan, 1977).

Kerala is endowed with large freshwater resources and low-lying paddy fields, which have been traditionally been utilized for rice cultivation alone. Although paddy cultivation in these polders has been practiced on a regular basis, its integration with scampi culture has given a new thrust to the economy of traditional farming in Kuttanad. Integration of paddy with prawn/fish has turned out to be a viable alternative to utilize the vast amount of fertile water available in Kuttanad effectively. Paddy has been traditionally cultivated in two seasons such as "Punja" and "Virippu". Punja season extends from October-November to February-March depending upon the locality, while Virippu extends from May-June to August-September. Since the two crops are not successfully carried out in most of the regions of Kuttanad, the prawn farming is usually rotated with one crop of paddy on a crop rotation basis. But

due to the delay in harvesting of paddy and subsequent conditioning of polders for prawn culture, the farmers generally get less than 8 months for prawn culture in these polders after each paddy crop. Moreover, being a low-lying area prone to recurrence of flood during the monsoon season culture period is getting reduced to even 6 months. In those areas where, the grow-out period available is less than 6 months, nursery systems shall be an integral requirement for successful commercial prawn farming. In such areas, a portion of the polder is getting transformed to nursery, which would operate simultaneously along with the paddy-cropping season. This would facilitate farmers to rear the post larvae procured from the hatchery in a closed environment for an additional one or two months, thus enabling them to stock juveniles originated from the nursery phase to the grow-out which would be characterized by excellent growth and high survival rate (Smith and Sandifer, 1979). In the present study, an attempt was made to investigate the effectiveness of a fixed outdoor nursery system in the farming of *M. rosenbergii*. Effort was also made to compare the results of grow-outs incorporated with different types of nurseries with a view to bring out the effectiveness of different types of nurseries which can offer high mean weight, survival and net yield *M. rosenbergii* in the polders of Kuttanad.

2. Materials and Methods

Twelve polders of Kuttanad, which followed modified extensive monoculture of *M. rosenbergii*, were monitored during 1999-2001. They were grouped under three sets of treatments (T1 to T3). Each set of treatment represents separate polders (quadruplicates). Hence Treatment 1 (T1) comprised of polders that did not follow any nursery system while Treatment 2 (T2)

incorporated polders that followed a definite nursery phase of one month prior to stocking whereas Treatment 3 (T3) include those polders that followed a 'two-phase' nursery system. After harvesting the 'Punja' paddy crop in the month of February these polders were totally dried and scientifically prepared (Kurup *et al.*, 1996). Liming was done @500 kg/ ha and cow dung was applied for phased manuring @1000 kg/ ha. The stocking density for all the treatments except T3 was maintained at 35,000/ha. In the third set of treatments (T3) the stocking density was slightly higher (40,000/ha), which was done in batches, i.e., the first set of 20,000 post larvae were introduced and reared in the nursery during December, which was released into the pond in January. Soon afterwards, in March the second set of 20,000 post larvae were introduced into the nursery and they were reared till the end of April. Prior to the release of nursery-reared juveniles to the pond, a cull harvesting of adult prawns greater than 80 gm was carried out in the polder that was initially stocked during January. The second batch of juveniles was then let out into the polder in May. Artificial substrates in the form of broken pipes were provided in each polder at a definite percentage of the total pond bottom (Tidwell *et al.*, 1998a). Prawns were fed initially with a high protein content feed comprising of groundnut oil cake, rice bran and boiled butchery waste @10% of the total body weight of prawn and later with an on-farm made feed with 35-40% protein ratio @ 5% of the body weight on a daily basis in two installments, 40% in the early morning and 60% in the evening. Water was exchanged once in a week with the help of pumps. Water quality parameters such as temperature, dissolved oxygen, transparency, water and soil pH were monitored on a daily basis following AOAC (1985), while levels of

Plate 8.1

A A typical out door nursery of *M.rosenbergii* in Kuttanad

B. A concrete nursery used for *M.rosenbergii* in Kuttanad

total ammonia-nitrogen (TAN), nitrite-nitrogen and hydrogen sulphide in water samples collected from each pond at around 1300 hr were determined on a fortnightly basis using the Aquakit from MERCK. On termination of farming after eight months, the polders were harvested by pumping it dry and the prawns were hand picked from the bottom. Random samples between 500-1000 prawns each from the fourteen polders were examined on the day of harvest. All the prawns were sorted to sex and morphotypes (Kuris *et al.* 1987). The males were then classified into three morphotypes such as Small males (SM), Strong orange clawed males (SOC) and Strong blue clawed males (SBC) and four of their transitional stages viz. Weak orange clawed males (WOC), transforming Strong orange clawed males (t-SOC), Weak blue clawed males (WBC) and Old blue clawed males (OBC). Similarly, females were also sorted into three morphotypes such as Small females (SF), Strong orange clawed females (SOF) and Strong blue clawed females (SBF) and three transitional stages viz. Weak Orange clawed female (WOF), transforming strong orange clawed females (TOF) and Weak blue clawed females (WBF). All the sampled prawns were measured up to nearest millimeter and weighed up to nearest gram. The weight of individual morphotypes from polders within each treatment was compared using ANOVA. In order to compare the effect of a nursery phase on population characteristics and yield structure, the cumulative mean values of each treatment (T1 to T3) was compared. Statistical analyses were done using SPSS 7.5 and Excel 98 package for Windows and the results were scrutinized following Snedecor and Cochran (1961). The data on average weight gain, final mean weight and total biomass

compiled from various treatments were statistically evaluated using ANOVA followed by pair-wise analysis through *t*-test.

3. RESULTS

3.1. Population structure

- Stocking and yield characteristics of *M. rosenbergii* from four polders lacking a nursery phase (Treatment 1) is shown in Table 8.1. The variations in the mean weight of prawns, survival rate and net production at the end of the culture did not show any significant variations among the polders and the values were in the range 42.56 to 58.32g; 35.96 to 42.23% and 158.4 to 182.68 Kg/ha respectively. Comparison of mean weight of individual morphotypes among the four polders (T1) by applying ANOVA is shown in Table 8.2. The results revealed that the mean weight of morphotypes within the treatment (T1) did not vary significantly ($P>0.05$).

Table 8.3 shows the farming details of four sets of polders following a single-phase nursery operation (Treatment 2). Mean weight of prawns, retrieval rate and correspondingly better net production were higher in (T2) when compared to T1. However interestingly, there was no variation in any of the polders in the net production (452.8 to 501.4 Kg/ha) survival rate (46.7 to 49.2%) and mean weight (48.96 to 55.14g). Results of ANOVA within the four polders for mean weight did not show any significant difference ($P>0.05$).

Details on the initial stocking and corresponding yield characteristics of *M. rosenbergii* at the final harvest reared in four polders following a two- phase nursery system (T3) are shown in Table 8.5. Net production ranged between 715.3 to 777.9 Kg/ha among the polders and there

was no significant variation. Similarly, the variation in the survival rate and mean weight were also found insignificant and was in the range of 60.3 to 65.71% and 58.2g to 68.2g respectively. Average values of physcio-chemical factors parameters from the three treatments polders are also represented in Table 8.5. Mean weight of individual morphotypes among the four polders (T3) analyzed applying ANOVA did not vary significantly ($P>0.05$) within the treatment (T3) (Table 8.6).

No significant difference ($P<0.05$) was found for the different water quality parameters analysed during the present study among the polders. Mean value calculated for different water quality parameters are given in Table 8.7. Surface water temperature among the treatments ranged between 25.8-34.5°C (T1), 25.6-33.7°C (T2) and 26.5-34.2°C (T3). Mean values for dissolved oxygen ranged between 4.82 mg/l in T3 to 6.25 mg/l in T1. Total alkalinity ranged between 48.61 mg/l in T3 to 75.58 mg/l in T1. Water pH in all the treatments ranged from 6-8.2 and soil pH was slightly less in T2 (4.7) but did not differ significantly with the other two treatments, which ranged between 5.2-6.6. Total ammonia-nitrogen recorded was below 0.1 mg/l while nitrite-nitrogen below 0.2 mg/l.

Details regarding the stocking and the yield characteristics of prawns during the final harvest in the three treatments are given in Table 8.8. Incorporation of a nursery phase was found helpful in increasing the growth and survival rate of prawns. Final mean weight, survival and net yield from T3 that followed a 'two-phase' nursery system were significantly higher when compared

with that of other two treatments. The results of the present study showed that with the adoption of a two-phase nursery system, an increase in the overall retrieval rate by about 40% could be recorded. Male: Female ratio at the time of final harvest were dissimilar in the three sets of trials, being 1:1.15 in T1; 1:0.85 in T2 and 1:0.76 in T3. In treatments in which a nursery phase was adopted, the predominance of males was quite noteworthy. Average weight of prawns ranged from 46.70 g in T1 to 63.32 g in T3. Result of ANOVA on the net production from the three sets of polders is given in Table 8.9 and it shows that with the incorporation of a fixed nursery phase; there was an increase in production at substantial levels within the treatments (F= 74.555, P<0.01). On further analysis of the production performance between the treatments following pair wise analysis (t-test), it could be seen that all the treatments differed significantly (P<0.01) in the final harvest (Table 8.10). The increase in the net production from 453.1 kg/ha in T2, with the incorporation of a 'single-phase' nursery system to 732.4 kg/ha in T3 with the involvement 'two-phase' nursery system deserves special attention.

The percentage contribution by weight of various male and female morphotypes to the respective harvested yield from the three treatments is given in Table 8.11. Percentage representation of various individual morphotype by weight showed that with an increase in the nursery period, there was a corresponding increase in the mean weight of male and female morphotypes. At higher stocking densities the mean weight of prawn was not adversely affected. This was clearly evident in the case of larger morphotypes such as SBC and OBC, which ranged from 89.45 and 119.24 g respectively in T1 to 171.22 and

172.07 g respectively in T3. Fig 8.1 to 8.3 represents morphotypic composition of males of *M. rosenbergii* in the three treatments. Contribution of SM and WOC by weight in the final harvested population in T1 was 18%, whereas in T2 and T3, which is characterized by a definite nursery phase, their percentage was in the order of 13 and 10% respectively. On the contrary the percentage of larger BC males was high in those treatments, which had either a mono phasic, or di-phasic nursery phases. The representation of BC males in the control pond was 53%, while in T2 and T3 its concentration was in the order of 58 and 63% respectively. Population structure of female morphotypes in harvested population of the three treatments also shared similar variation, almost identical to their male counterparts. The morphotypic composition of females in the three treatments is depicted in Fig 8.4 to 8.6. In the treatments endowed with a nursery system (T2 andT3), the percentage of terminal morphotypes WBF and SBF accounted for 53 and 63% respectively in contrast to a mere 34% in non-nursery reared treatments. The percentage representation of lower morphotypes such as SF and WOF to the final population was similar to the trend shown by SM and WOC in the three treatments. Percentage contributions of male morphotypes to total harvested population by number in the three treatments are depicted in Table 8.11. In T1 the dominant morphotypes were t-SOC (26.31%) and TOF (15.57%), while in T2 and T3 the dominant morphotypes were OBC (34.26% and 24.84%) and WBF (8.19% and 21.81%) respectively.

3.2 Yield characteristics:

Stocking densities and yield characteristics of *M. rosenbergii* reared in the three treatments are given in Table 8.8. In T1 and T2, the stocking

was done @ 3.5/ m^2, while in T3 the same was @ 4/m^2. However, a remarkable variation could be noticed in the density at harvest, especially in T3. The percentage retrieval varied from 38.92% in T1 to 54.21% in T3. Mean net production varied from 169.42 (T1) to 732.45 Kg/ha/8 months (T3). Mean weight, standard deviation, coefficient of variance and skewness in respect to various male and female morphotypes are given in Table 8.11. Invariably, the mean weights of all male and female morphotypes were relatively higher in T2 and T3, where additional substrates were provided. Mean weight of male morphotypes in the three treatments ranged between 53.45 in T1 to 62.05 g in T3, while among female morphotypes highest values for mean weight was recorded in polder 2 (39.97 g) and was least in T1 (34.37 g). In order to test the differences, if any, in the weight attained by various morphotypes of *M. rosenbergii* and their yield from the three treatments studied, the data was statistically analyzed using two-way ANOVA. The results revealed that there was no significant difference between treatments ($F= 2.3564$), however, the weight attained by various morphotypes showed significant difference ($F= 67.344$, $P< 0.01$). Table 8.12 shows the result of two-way ANOVA of various morphotypes under different levels of managements. Significant difference ($P<0.01$) in the F- values were recorded among larger morphotypes such as TOF, WBF, t-SOC, WBC, SBC and OBC showing the diverse nature of their representation by weight in the three sets of treatments studied. Nevertheless, the non-significance of SM, WOC, SOC and SBF by weight in any of the three sets of treatments was noteworthy. The result of pair wise analysis of the different morphotypes under three different sets of nursery system is shown in Table 8.13.

From the results of "t" test, it was obvious that the mean weight attained by male and female morphotypes differed significantly between each other (t = 2.93). The results also showed that there exists a visible difference in the mean weight of morphotypes in treatments without any nursery phase when compared with their counterparts in polders incorporated with a di-phasic nursery period. However, the results of pair-wise analysis by applying t-test (Table 8.13) did not show any significant difference in mean weight for morphotypes (P>0.05) within the two different nursery systems.

The weight distribution pattern and marketable yield structure of prawns in the three treatments incorporating different levels of nursery phases are depicted in Fig 8.7 to 8.9. The percentage composition of undersized prawns (< 50g) was glaringly high in polder 1 (26%) while the same showed a drastic decline in polder 3, which is preceded by a two-phase nursery rearing system (9%). Dominance of weight group >120 g was very apparent in polders 2 and 3 (29% and 32% respectively). On the contrary, the weight distribution in the total population was found profoundly influenced by the intermediary morphotypes and female population in polder 1 and consequently, 50-60 g group showed its distinct predominance (20%) in this treatment. The price packages for per kg of *M. rosenbergii* offered by the seafood processing plants located at Cochin are given in Chapter 7. The percentage of highly profitable weight group i.e., > 120 g was lowest in polder 1 (20%). Maximum biomass was reckoned in polder 3 that encompassed 91% of the prawn above 50 g in weight. At the time of final harvest while computing the mean revenue of the three treatments based on the above tariff, total income from these grow-outs would workout as Rs. 27,880/- in

T1, Rs. 81,080/- in T2 and Rs. 1,34,450/- in T3. It may, therefore, be seen that by incorporation of a two-phase nursery system was effective in augmenting the net revenue by around 380%. Thus the di-phasic nursery grow-out apparently appears to be superior and thus generating more profit than to the conventional monophasic nursery rearing system or grow-outs without a nursery system.

4. Discussion

The necessity of a nursery system as an intermediate step between hatchery and grow-out phase was first indicated by Ling (1969). The utilization of nursery systems to improve profitability of existing traditional and commercial farms of *M. rosenbergii* in tropical regions is found most promising. Nursery rearing has been found to be beneficial for several reasons including the following (Mulla and Rouse, 1985); (a) small nursery ponds can be more closely managed to ensure higher survival during a time when the young prawns are most susceptible to predation and harsh environmental condition, (b) juvenile prawns are relatively larger and can be more easily enumerated for accurate stocking of production ponds, (c) the expected higher survival of juveniles than post larvae in production ponds increases the final production and (d) higher stocking densities in nursery ponds result in more efficient utilization of food and space in the pond. Better mean weight, survival rate and dominance of male morphotype in the final harvested population for treatments T2 and T3 in the present study would suggest that mortality and size disparity could be greatly reduced when a nursery phase was incorporated. Various methods have been employed to increase per unit production of prawns including increased size at

stocking and increased stocking densities (D'Abramo *et al.*, 1989), grading animals prior to stocking (Daniels *et al.*, 1995), etc.

The results of the present study clearly shows that the net productions in T2 and T3, which incorporated nursery phases, were substantially higher when that compared to the treatment which lacked a nursery phase (T1). With the adoption of a two-phase nursery system, an increase in the net biomass production at a rate of over 330% could be attained in T3. Increase in the net production can be attributed to two reasons, most importantly, the dominance of male morphotypes especially OBC (24.84%), which was characterized with high mean weight of prawns, registered at the time of harvest and secondly, the higher retrieval rates registered in those treatments (54.21%). Another noteworthy finding was the higher percentage of terminal blue-clawed males and females in the final harvested population, which was @ 53% in T2 and 63% in T3. The ratio between SM:OC: BC in the three treatments also differed significantly. In T1 it was found to be 1: 5.7: 7.5, while in T2 and T3 it was recorded as 1: 2.2: 4.6 and 1: 2.3: 5.7 respectively. It is worthwhile to note that, in spite of the dominance of males in the population of T3, the survival rate was not affected adversely. Moreover, with the adoption of a nursery phase, especially diphasic nursery system, faster morphotypic transformation might have taken place, at a faster rate, which would in turn culminated in the presence of larger prawns at the time of harvest. Fujimura and Okamoto (1970) reported that density, food, temperature, shelter and insect predator were important factors affecting survival in shrimp nursery ponds. In the present study a slight increase in the density did not have any adverse affect on the survival rate or market structure of the

prawns. There are direct benefits associated with stocking of larger juveniles in ponds. These include: the production of larger, more valuable prawns, greater crop yield and increased crop predictability (Smith *et al.*, 1983). Higher values in net yield registered in treatment with two-phase nursery system during the present study might be due to better survival rate and larger juveniles stocked evolved from of the nursery phase in the grow-out. Marketable yield structure of the three treatments was diverse and the predominance of undersized prawns (< 50 g) was high in T1 and 2 (26% and 22% respectively), on the contrary, the more profitable weight group (>120 g) was more in T3 (32%). The presence of higher percentage of non-marketable prawns (<50 g) in the final harvested population resulted in reduction in net yield from T1, while the net income incurred from T3 was nearly 380% higher than that of T1. The decrease in yield from T1 in final population could be due to the low mean weight of individual prawns. The present findings were in full agreement with that of Marques *et al.* (2000) who documented the effect of stocking density at nursery phase on growth, biomass increase and survival of post larvae of *M. rosenbergii* in cages.

The high density nursery rearing of prawns has been studied by a number of researchers in relation to shelter (Smith and Sandifer, 1975), feed (Willis *et al.*, 1976), stocking density (Sandifer and Smith, 1975; Wickins, 1972), age and temperature (Kneale and Wang, 1979). The necessity of nursery phase for improving the net yield can well be established on the basis of the present findings for *M. rosenbergii*. Interestingly, the percentage of undersized SM and WOC were high in the nursery-stocked prawns (15% and 14% respectively for T2 ad T3). The lower percentage of undersized SM and WOC in the control pond

may be due to increased mortality in these treatments. When the stocking density got reduced to less than half the initial stock, the transformation of undersized prawns to successive stages might have increased. Malecha *et al.* (1989) stated that small-sized prawns have an inherent capacity for compensatory growth in the absence of dominant males. This was confirmed by noticing the mean weight of WBC, SBC and OBC morphotypes that were far inferior to their counterparts in T2 and T3 respectively. Percentage by weight representation of larger morphotype, BC was high in the treatments that incorporated a definite nursery phase. Similarly the percentage contribution of SBC and its transitional stages such as WBC and OBC also showed an increasing trend in these treatments with 53% in T1, 58% in T2 and 63% in T3. In total contrast to this, the representation of SOC and its intermediate morphotypes, WOC and t-SOC showed an inverse trend with higher values in T1 (40%), which gradually reduced to 29 and 26% in T2 and T3 respectively. This indicates that the prawns that emerged out of the nursery phase had a faster growth rate and transformed to their successive morphotypic stages at a faster rate when compared to those without a nursery phase. These results are complimentary to the findings of Arieli and Rappaport (1982) who reported an increase in the mean weight of 40 g in one season when compared with prawns reared without a nursery phase. Janssen *et al.* (1988) showed that rice fields could be used as nursery grounds for prawn PL-1 to PL 60 concurrent with rice cultivation

Kuttanad waters had been traditionally known to be the home ground of *M. rosenbergii*. Farming of this species in an unscientific method has been employed in the fallow polders without resorting to any scientific norms.

Hence the net unit production of *M. rosenbergii* in Kuttanad is far below and this is mostly due to low survival rates and size disparity in the harvested population. In light of the complex population structure and resulting size disparity among adult population of *M. rosenbergii*, incorporation of a nursery phase in enhancing the net yield and income from the culture of *M. rosenbergii* in a tropical wetland ecosystem such as Kuttanad was found highly imperative. The results emerged from the present study showed that nurseries are integral part for farming of *M. rosenbergii* that facilitates the production of larger juveniles for stocking in ponds or other grow-out systems and thus ensure high production and marketable yield structure, high revenue of the harvested population, and improved predictability of yields. Secondly, homestead ponds, which are available in plenty in Kuttanad, can effectively be utilized as nursery ponds in places where separation of polders as nurseries are not viable. Present findings provides more impetus to a di-phasic mode of nursery operation, where a multiple stocking and periodic release of juveniles from the nursery to grow-out is envisaged.

Table 8.1 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared without a nursery phase in four polders of Kuttanad (Treatment 1)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35000	35000	35000	35000	35000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.38	1.25	1.47	1.32	1.36
Number per ha.	13876	12589	14782	13258	13620
Mean weight (g)	44.58	58.32	42.56	48.27	46.70
Gross production (Kg/ha)	197	165	201	174	181
Net production (Kg/ha)	182.68	161.4	175.1	158.45	169.42
Survival (%)	39.64	35.96	42.23	37.88	38.91
Mean male weight (g)	52.14	49.35	56.27	50.17	53.45
Mean female weight (g)	39.58	36.45	31.07	32.05	34.37
% by number of males in population	42.85	47.33	45.51	55.36	46.38
% by number of females in population	57.15	52.67	54.49	44.64	53.62
Sex ratio	1:1.33	1:1.11	1: 1.19	1: 0.81	1:1.15

Table 8.2 Comparison of mean weight of individual morphotypes in four polders of Treatment 1 (T1)

Morphotypes	F-value
SF	1.5422
WOF	1.6832
SOF	1.7054
TOF	1.3070
WBF	1.4285
SBF	2.5591
SM	0.3102
WOC	1.0074
SOC	1.0549
t-SOC	1.7203
WBC	1.8241
SBC	2.4299
OBC	1.9412

SF = Small female
WOF= Weak orange clawed female
SOF = Strong orange clawed female
TOF = Transforming strong orange clawed female
WBF= Weak blue clawed female
SBF = Strong blue clawed female
SM = Small male
WOC = Weak orange clawed male
SOC = Strong orange clawed male
t-SOC = Transforming strong orange clawed male
WBC = Weak blue clawed male
SBC = Strong blue clawed male
OBC = Old blue clawed male ·

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes

Table 8.3 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared with a single phasenursery in four polders of Kuttanad (Treatment 2)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35000	35000	35000	35000	35000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.63	1.72	1.68	1.67	1.69
Number per ha.	16358	17245	16850	16724	16920
Mean weight (g)	53.47	48.96	55.14	52.78	52.25
Gross production (Kg/ha)	457	506	487	462	486
Net production (Kg/ha)	452.87	501.48	482.65	458.17	453.11
Survival (%)	46.73	49.27	48.14	47.78	48.35
Mean male weight (g)	59.58	58.21	54.68	55.87	57.97
Mean female weight (g)	41.2	36.45	41.07	37.58	39.97
% by number of males in population	53.87	52.14	56.42	48.21	54.11
% by number of females in population	46.13	47.86	43.58	51.79	45.89
Sex ratio	1:0.86	1:0.91	1: 0.77	1:1.07	1:0.85

Table 8.4 Comparison of mean weight of individual morphotypes in four polders of Treatment 2 (T2)

Morphotypes	F-value
SF	1.4666
WOF	1.8732
SOF	1.6065
TOF	1.2650
WBF	1.3692
SBF	3.0750
SM	0.4106
WOC	1.0082
SOC	0.9691
t-SOC	2.1109
WBC	1.9141
SBC	2.5693
OBC	2.0145

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 8.1

Table 8.5 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared with a two-phase nursery system in four polders of Kuttanad (Treatment 3)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	40000	40000	40000	40000	40000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.72	1.86	1.95	1.91	1.89
Number per ha.	25202	24123	25704	26285	25328.5
Mean weight (g)	68.25	64.17	58.25	60.74	63.32
Gross production (Kg/ha)	721	728	782	775	757
Net production (Kg/ha)	715.8	715.3	777.9	769.2	732.45
Survival (%)	63	60.3	64.26	65.71	63.32
Mean male weight (g)	59.28	69.28	58.27	60.17	62.05
Mean female weight (g)	39.72	42.15	34.29	42.15	38.28
% by number of males in population	62.14	59.89	51.02	48.26	56.82
% by number of females in population	38.86	40.11	48.98	51.74	53.18
Sex ratio	1:0.62	1:0.66	1: 0.96	1: 1.07	1:0.76

Table 8.6 Comparison of mean weight of individual morphotypes in four polders of Treatment 3 (T3)

Morphotypes	F-value
SF	1.9776
WOF	1.5875
SOF	2.1874
TOF	1.6752
WBF	1.8315
SBF	1.8116
SM	0.3936
WOC	1.2900
SOC	1.5108
t-SOC	1.2235
WBC	1.9283
SBC	2.4353
OBC	2.0789

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 8.1

Table 8.9. Result of Analysis of Variance on the net production from the three sets of treatments

Source of Variation	*Sum of square*	*df*	*Mean sum of Square*	*Calculated F*
Between Groups	545548.8555	2	272774.4278	74.55517612
Within Groups	40245.6122	9	3658.692018	
Total	585794.4677	11		

Table 8.10 Result of t-Test comparison on the net production between the treatments

Net Production	Treatment 1 vs 2	Treatment 1 vs 3	Treatment 2 vs 3
t- value	7.8721*	19.504*	6.6762*

* Significant at 1% level (P<0.01)
** Significant at 5% level (P<0.05)

Table 8.11 Percentage contribution of male and female morphotypes by weight in the harvested population and mean weight of male and female morphotypes of the three treatments (T1 - T3)

	Pond	Mean weight of male morphotype												
		SM	WOC	SOC	t-SOC	WBC	SBC	OBC	SF	WOF	SOF	TOF	WBF	SBF
	Number sampled	134	111	50	174	84	129	88	119	42	0	110	180	144
	% by weight	0.59	0.13	1.45	26.31	18.57	11.08	16.03	0.50	0.77	0.00	15.57	7.89	1.11
	Mean weight	1.32	26.82	50.00	66.76	65.61	89.45	119.24	12.26	27.92	0.00	48.17	51.18	65.41
T1	Standard deviation	3.63	9.56	0.00	20.98	25.02	28.08	42.74	3.66	8.59	0.00	12.74	14.06	14.05
(n=4)	Coeff. of variation	32.08	35.64	0.00	31.42	38.62	31.39	35.84	29.88	30.76	0.00	27.47	21.48	32.08
	Standard error	0.62	2.88	0.00	2.44	2.73	5.21	4.56	0.84	2.48	0.00	1.21	1.05	2.12
	Skewness	0.41	0.20	0.00	1.83	0.31	-0.09	0.75	0.35	1.14	0.00	0.30	0.09	0.51
	Number sampled	95	39	26	147	129	136	158	74	24	12	49	105	65
	% by weight	1.14	0.28	0.76	23.73	16.67	7.66	34.26	0.76	0.16	1.09	3.65	8.19	1.65
	Mean weight	10.61	21.67	83.33	112.96	90.43	148.89	151.72	12.05	54.64	57.50	52.14	54.59	76.80
T2	Standard deviation	4.20	8.66	17.22	34.80	46.14	38.71	33.53	3.94	31.95	10.61	27.74	25.14	30.31
(n=4)	Coeff. of variation	39.61	39.97	19.50	30.81	51.03	26.00	22.10	32.67	58.48	18.45	53.21	46.05	39.46
	Standard error	0.49	2.89	7.03	2.87	4.06	6.45	2.67	0.59	8.54	7.50	3.96	2.45	7.83
	Skewness	1.04	1.20	2.72	0.03	0.50	1.11	0.34	0.91	0.04	0.00	0.46	-0.12	0.86
	Number sampled	129	26	30	147	110	41	59	74	30	18	137	82	110
	% by weight	0.91	0.70	0.12	11.69	13.09	6.14	24.84	0.55	0.79	0.00	12.54	21.81	6.81
	Mean weight	12.93	40.00	92.00	113.36	106.91	171.22	172.07	16.04	31.50	60.63	71.99	60.95	70.00
T3	Standard deviation	4.91	0	15.49	31.96	39.21	27.22	39.71	5.31	16.67	16.78	16.07	17.93	11.55
(n=4)	Coeff. of variation	37.99	0.00	16.84	28.19	36.68	15.90	23.08	3.11	52.94	27.68	22.33	29.41	16.50
	Standard error	0.91	0	4.9	2.64	3.74	4.25	5.17	1.08	5.27	5.93	1.37	1.98	3.65
	Skewness	0.47	0.00	1.38	0.45	0.73	0.06	0.32	0.26	0.91	0.66	0.25	0.23	0.66

T1 = No nursery, T2 = Single phase nursery, T3 = Two-phase nursery
For expansion of morphotypes refer Table 8.1

Table 8.12 Comparison of mean weight of individual morphotypes among the three treatments (T1 - T3)

Morphotypes	F-value
SF	5.2617*
WOF	7.7718*
SOF	0.1736
TOF	65.3279*
WBF	4.7771*
SBF	0.7003
SM	2.5155
WOC	2.1937
SOC	0.1575
t-SOC	62.9071*
WBC	26.4160*
SBC	58.7652*
OBC	35.6605*

Table 8.13 Comparison of mean weight between three treatments by as applying t-Test

Morpotype	Treatment 1 vs Treatment 2	Treatment 1 vs Treatment 3	Treatment 2 vs Treatment 3
SF	2.9186*	2.3691*	0.1702
WOF	3.0752*	0.0278	3.3717*
SOF	0.7081	-	-
TOF	5.2356*	12.8152*	0.6153
WBF	1.9163**	3.5108*	0.6635
SBF	0.5386	0.518	0.8781
SM	1.9907**	1.0625	1.139
WOC	6.3508*	4.5738*	1.2629
SOC	0.4278	-	-
t-SOC	0.0247	12.807*	12.072*
WBC	3.0322*	8.8778*	5.0294*
SBC	2.8900*	12.154*	7.1651*
OBC	3.4987*	7.6670*	6.0804*

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

For expansion of morphotypes refer Table 8.1

Fig 8.1 Percentage composition of various male morphotypes of *M. rosenbergii* in harvested population in Treatment 1 (No nursery)

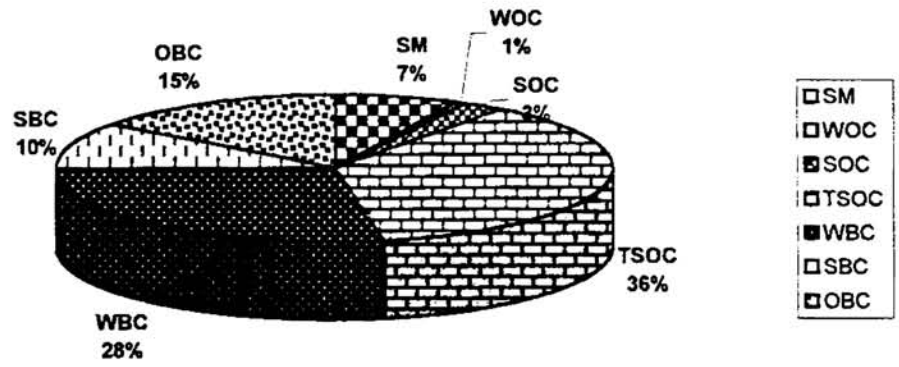


Fig 8.2 Percentage composition of various male morphotypes of *M. rosenbergii* in harvested population in Treatment 2 (Single phase nursery)

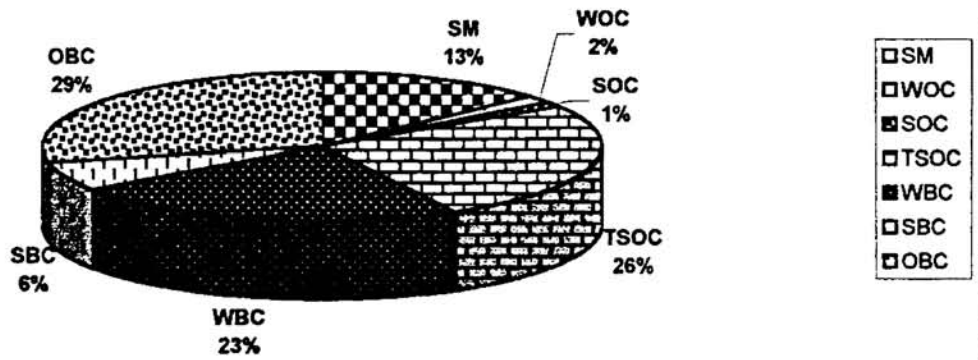


Fig 8.3 Percentage composition of various male morphotypes of *M. rosenbergii* in harvested population in Treatment 3 (Two-pase nursery)

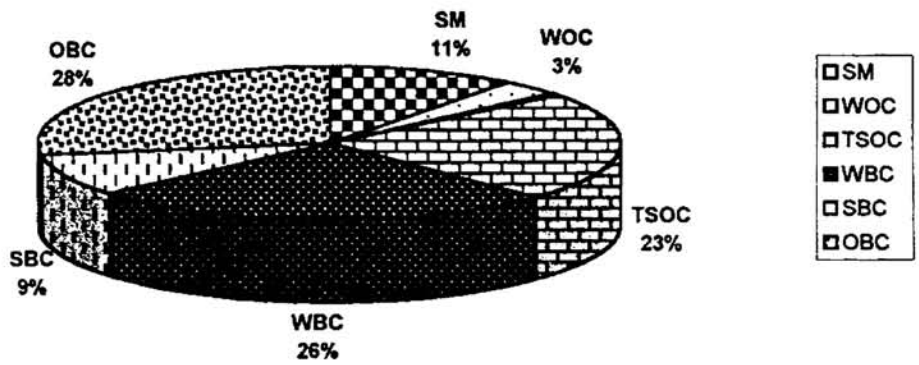


Fig 8.4 Percentage composition of various female morphotypes of *M. rosenbergii* in harvested population in Treatment 1 (No nursery)

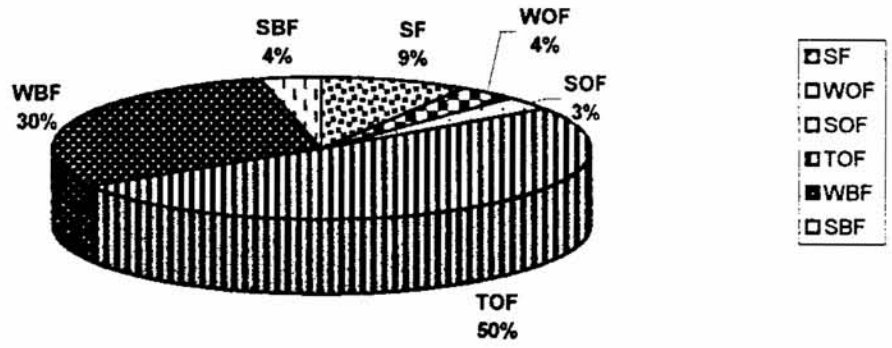


Fig 8.5 Percentage composition of various female morphotypes of *M. rosenbergii* in harvested population in Treatment 2 (Single phase nursery)

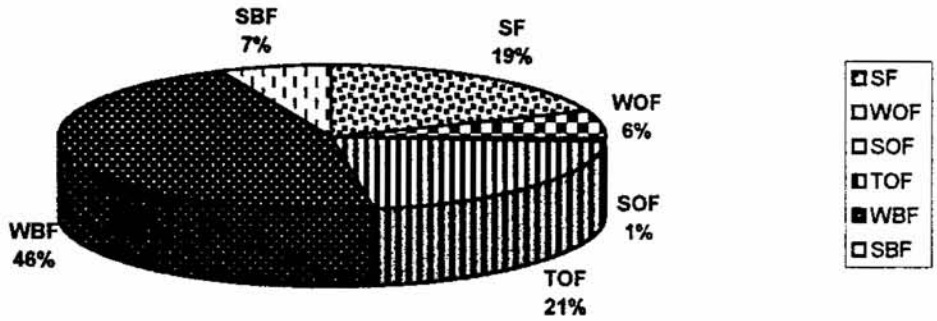


Fig 8.6 Percentage composition of various female morphotypes of *M. rosenbergii* in harvested population in Treatment 3 (Two-phase nursery)

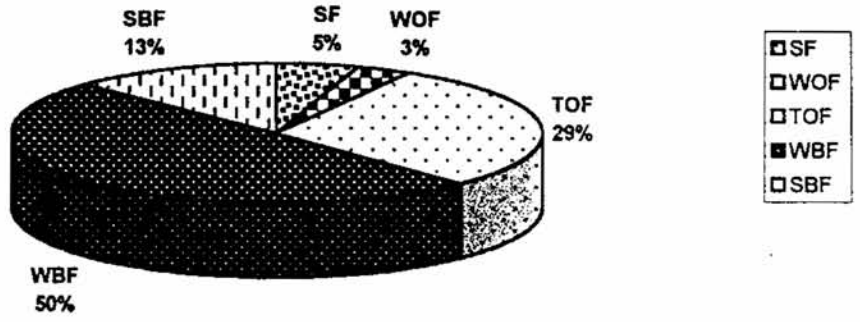


Fig 8.7 Percentage composition of various market size groups of *M. rosenbergii* in harvested population fromTreatment 1 (No nursery)

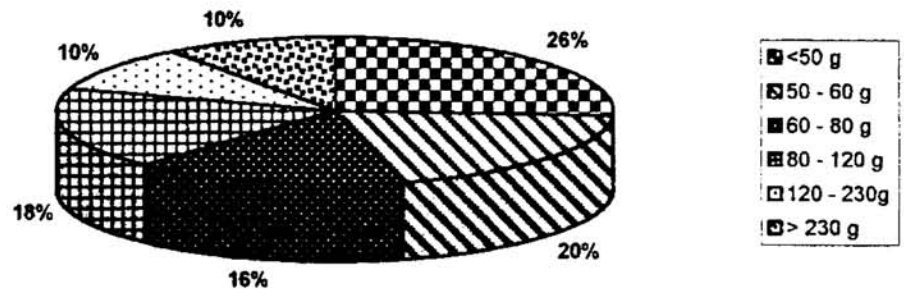


Fig 8.8 Percentage composition of various market size groups of *M. rosenbergii* in harvested population fromTreatment 2 (Single phase nursery)

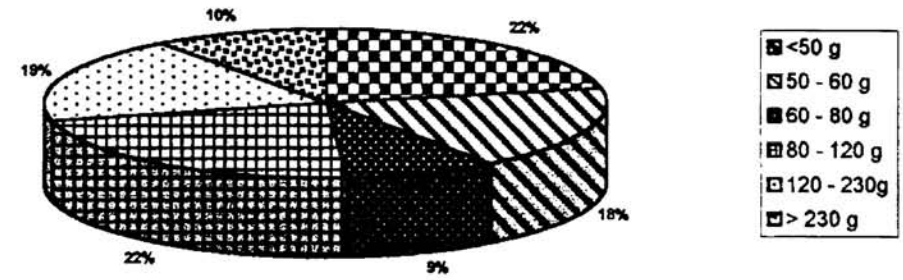
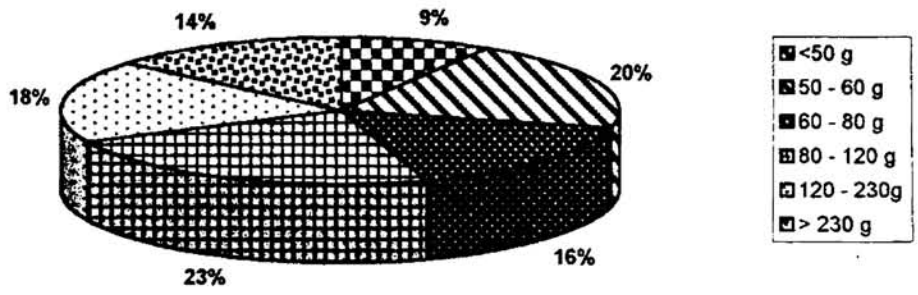


Fig 8.9 Percentage composition of various market size groups of *M. rosenbergii* in harvested population fromTreatment 3 (Two-phase nursery)



CHAPTER 9.

Adaptive trials incorporating batch graded, size graded and modified batch graded post larvae to improve net yield from the grow-outs of *Macrobrachium rosenbergii*

1. Introduction

Farming of *Macrobrachium rosenbergii* gained much attention among enterprising farmers due to its excellent demand in foreign markets, disease resistance, easy acceptance of both plant and animal-based diets, capability to withstand wide fluctuations in water quality and fast growth rate (Smith *et al.*, 1982). Despite of having all these favorable factors, its inherent social structure, acts as the principal limiting factor against popularization of culture (D'Abramo *et al.*, 1991). The differential growth associated with male morphotype of this species draws special interest. Starting with a cohort of post-larvae having a normal size distribution (Sandifer and Smith, 1975; Ra'anan and Cohen, 1985), growth of juvenile prawns is dispensatory and the variation become more perceptible with the advancement of growth (Malecha, 1980). About half of the population grows rapidly and variably, while the other half grows slowly and relatively uniformly, leading to marked, positively skewed size distribution (Wickins, 1972; Forster and Beard, 1974; Ra'anan, 1983; Ra'anan and Cohen, 1985). This complex social structure that is basically persists in males, a condition termed as "Heterogeneous individual growth" or HIG. Both sexes have similar size frequency distributions at immature stage (Ra'anan and Cohen, 1985). As the individuals mature, the size distribution becomes so contrasting between males and females. Mature females grow more slowly than

males of similar age, and the size distribution of the females regain an approximately normal pattern (Cohen *et al.*, 1981). For the males, the skewed distribution is extended, even reinforced, upon maturation (Fujimura and Okamoto, 1970; Smith *et al.*, 1978; Brody *et al.*, 1980; Malecha *et al.*, 1981b), and this is associated with the differentiation of three anatomically, physiologically and behaviourally distinctive adult male morphotypes (Ra'anan, 1982; Ra'anan and Cohen, 1985; Ra'anan and Sagi, 1985; Kuris *et al.*, 1987; Sagi and Ra'anan, 1988; Sagi *et al.*, 1986). The size differentiation so evinced among the mature males in a population happens to be the major impediment in the successful farming of this species. There are instance where almost 50% of the prawns in the final harvest is contributed by the undersized Small males (SM) characterized by small claws, slow growth, thenceforth exhibiting varying size frequency distribution. About 40% of the prawns that have relatively larger orange claws, grow rapidly and are highly variable in their size distribution, which belonged to the Orange clawed males (OC). While the rest 10% belongs to Blue clawed males (BC) that are bigger in size, larger than the OC and are behaviourally dominant over other morphotypes and shows strong territoriality (Ra'anan *et al.*, 1991). Thus dominance of undersized and non-marketable SM makes the culture of this species highly uneconomical. Various management approaches followed to minimize HIG have focused mostly on selective harvesting or size grading of pond populations of prawns in order to minimise the differential growth (Karplus *et al.*, 1986 a and b, 1987). However, more research on the most effective of size-grading approach is indispensable, as no concerted attempts have so far been made to segregate the post larvae soon after

the hatchery phase according to their growth and assess their performance in the grow-outs, Therefore an attempt made to investigate the effects of size grading, batch grading and modified batch grading of post larvae prior to stocking on production characteristics, population structure, morphotypic differentiation and net yield from the grow-outs of Kuttanad (S. India).

2. Materials and Methods:

Three sets of adaptive trials were carried out in the coconut garden channels of North Kuttanad (S. India) that encompass a total water spread area of 4.2 ha. The whole area was equally divided into channels of 0.2 ha, as a result 21 individual channel systems were demarcated which were separated using mud bunds and fine nylon mesh. Each set of experiments was carried out in duplicates in such a way that the post larvae for each trial were stocked in three consecutive channels of 0.2 ha area. Hence under each treatment a total of six channels were maintained. All the channels were maintained as separate units with the help of nylon nettings. The initial stocking density in all the channels was maintained @1.2PL/m^2. The depth in all the channels was maintained at around 1.8 m. Ponds were drained, dried and lime was applied @ 100 Kg/ha. After liming, water was filled to about 1.0 m depth and manured with cow dung and urea @ 200 and 50 Kg/ha respectively. After one week, channels were filled with water to the level of 1.5 m depth and the post larvae (PL-20) were stocked. The first 18 channels covered an equal area of 0.2 ha and were separately stocked with six different sets of post larvae in triplicates. The seventh set of channels was maintained as control that did not follow any grading of post larvae prior to stocking. An "on-farm" made feed basically comprised of clam meat, ground nut

oil cake, rice bran and wheat flour was prepared and broadcasted in the form of dough balls at specific sites on the channels @ 5-10% of the total biomass through out the culture period. Feed was given daily and the ratio was determined from time to time based on the intake shown by the prawns. Routine observation on physio-chemical parameters such as dissolved oxygen, pH, alkalinity, hardness, ammonia, nitrite, hydrogen sulphide and phosphate were made on a monthly basis (AOAC, 1985).

(A). Farming trials using batch-graded post larvae based on hatching order:

In the first set of treatment the larvae from a single clutch were separated on the basis of differential hatching order. Based on hatching order, the larvae that hatched out of the egg were divided into two groups, (a) the larvae that emerged first from the egg namely the "first hatched" (T1) and (b) the larvae which hatched later from the egg namely the "later hatched" (T2) (Mashiko, 1987). The post-larvae thus emerged from each group were reared in hatchery till PL-20 stage and were stocked @12000/ha in the two channels of each having s 0.2 ha area.

(B) Farming trials using modified batch-graded post larvae:

In the second set of treatments, the post-larvae that settled during the first one-week were collected and grown in separate tanks -first settled groups (T3). Similarly, another batch of post-larvae settled in the subsequent week were sorted as second settled group (T4) and reared in separate 1000 litre tanks till the PL-20 stage. They were then stocked in duplicates in two sets of channels of 0.2 ha area each @12,000/ha.

(C) Farming trials using size graded post larvae

In the third set of treatments, newly metamorphosed post-larvae of *M. rosenbergii* were reared at a density of 12/litre for 20 days in two 1000 litre Oxford blue FRP tanks accommodated at the Prawn hatchery complex of the School of Industrial Fisheries. At the PL-20 stage the post-larvae were size graded into two approximately equal populations on the basis of their faster growth, movement and feeding behavior. The size group, which showed faster growth, better movement and greater feeding aptitude were categorised as "Jumpers" (T5) while, the group, which showed comparatively slower growth, sluggish movement and low feeding aptitude were sorted as "Laggards"(T6) (Karplus *et al.*, 1987). They were then stocked in duplicates in two sets of channels of 0.2 ha area each @12,000/ha.

The population of prawns in each treatment was sampled every month commencing from March to Nov 99. During the final harvest the individual prawns were sexed, measured for claw and body length and weight was measured using a single pan balance. Morphotypes were segregated following Kuris *et al* (1987), their individual length and weight, length of carapace, ischium, merus, carpus propodus, dactylus, second pleura and telson were measured following Harikrishanan and Kurup (1997). More than 90% of the total harvested prawns were individually examined for morphometrics. Monthly mean growth, percentage weight gain and retrieval rate, sex ratio and gross production at the end of the culture period were calculated (Sagi *et al.*, 1986). Data obtained at the final harvest were subjected to one-way analysis of variance (ANOVA). Statistical analysis was done using SPSS 7.5 and Excel 98

package for Windows and the results were statistically evaluated following Snedecor and Cochran (1961). Data on average weight gain, final mean weight and total biomass compiled from various treatments were statistically evaluated using ANOVA followed by pair-wise analysis applying *t*-test.

3. Results

3.1 Population Characteristics of final harvested population

Table 9.1 shows the initial stocking details and yield characteristics observed at final harvest among the first hatched groups (T1) that were reared in three channels. Net production between the channels did not vary significantly which ranged between 53.14 to 66.45 Kg/ha. Similarly, survival rate and mean weights of individuals also did not differ significantly and varied between 19.89 to 21.44% and 41.85 to 44.58 g respectively. Predominance of females in the harvested population was quite discernible. Comparison of mean weight of individual morphotypes among the three channels (T1) is shown in Table 9.2. Mean weight for morphotypes within the treatment (T1) did not vary significantly ($P>0.05$).

Mean weight, sex ratio, retrieval rate and net production from three channels stocked with later hatched post larval groups (T2) are shown in Table 9.3. No significant difference in the net production, survival rate and mean weight of prawns within the three channels were observed. While the net production ranged between 81.26 to 96.58 Kg/ha, the survival rate ranged between 16.4 to 18.45% and mean weight between 61.89 and 69.25 g respectively. In all the channels, dominance of females was discernible in the

harvested population. Comparisons of mean weight of morphotypes among the three channels (T2) are given in Table 9.4. F value denotes that the mean weight for morphotypes within the treatment (T2) did not vary significantly (P>0.05).

Stocking particulars and yield characteristics of first settled groups (T3) reared in three channels are shown in Table 9.5. Net production between the channels did not vary significantly and was in the range between 91.23 to 102.5 Kg/ha. Similarly, the survival rates recorded were between 15.35 to 15.79% and mean weight from 66.38 to 71.08 g, both of them did not show significant variation. Mean weight of the morphotypes within the treatment (T3) did not vary significantly (P>0.05) (Table 9.6).

Stocking details and yield characteristics of *M. rosenbergii* from three channels stocked with second settled groups (T4) are shown in Table 9.7. The variations in the mean weight of prawns, survival rate and net production did not show any significant variations among the channels and the values were in the range 45.98 to 56.27g, 16.37 to 18.63% and 61.38 to 84.26 Kg/ha respectively. In all the channels, females dominated in the final population. Comparison of mean weight of individual morphotypes among the three channels (T4) is shown in Table 9.8. F value suggests that the mean weight of morphotypes within the treatment (T4) did not vary significantly (P>0.05).

Stocking and production characteristics from channels stocked with Jumpers (T5) are shown in Table 9.9. None of the parameters observed showed significant variation among the three channels studied. Net production ranged from 50.24 to 64.89 Kg/ha, survival rate ranged from 12.13 to 13.10%

and mean weight of prawns in T5 ranged from 80.26 to 88.58 g. Result of ANOVA showed that the mean weight within the treatment (T5) did not vary significantly (P>0.05) (Table 9.10).

Stocking details and yield characteristics of channels stocked with Laggards (T6) reared in three channels are shown in Table 9.11. No significant difference in the net production, survival rate and mean weight of prawns within the three channels could be observed. While higher values for net production and survival rate were registered (93.45 to 98.71 Kg/ha, 21.18 and 25.83% respectively), the mean weights of prawns were found to be lower than that of T5 and were in the range 51.21 to 59.52 g. Females showed a clear dominance in all the channels. Mean weight of morphotypes among the three channels (T6) did not vary significantly (P>0.05) (Table 9.12).

Table 9.13 shows the stocking details and yield characteristics of the three sets of channels maintained as control (T7). Net production was in the range 118.24 to 142.68 Kg/ha, the retrieval rate between 26.07 and 30.68% and mean weight of prawns ranged from 54.58 g to 59.52 g and there was no significant variation among the channels. Mean weight within the treatment (T5) did not vary significantly (P>0.05) (Table 9.14).

No significant differences in water quality parameters could be observed among treatments in the adaptive trials (P>0.05). Table 9.15 shows the mean values observed in respect of different water quality parameters and the range within each treatment. Surface water temperature among the treatments ranged between 26.1–32.5°C (T1), 27.2–31.8°C (T2), 24.7-32.8°C (T3), 26.1-

32.2°C (T4), 28.6-33.1°C (T5), 24.4-34.2°C (T6) and 27.4-32.6°C (T7). Mean values for dissolved oxygen ranged between 3.62 mg/l in T3 to 5.5 mg/l in T4. Total alkalinity ranged between 40.5 mg/l in T2 to 86.74 mg/l in T5. Water pH in all the treatments was in the range of 5.4-7.6 and soil pH between 5.3-7.8. In all the treatments the total ammonia-nitrogen recorded below 0.1 mg/l and nitrite-nitrogen observed below 1.07 mg/l. Repeated liming maintained the alkalinity in the pond water between 37 to 120 mg/l of $CaCo_3$.

The stocking details and population characteristics of the different sets of treatments conducted in the coconut garden channels are shown in Table 9.16. Among them, better survival rates were recorded in treatments stocked with Laggards (T6) and those maintained as control (T7). While the retrieval rate of T6 showed a peak value of 24.1% among the different treatments studied, in their counterpart Jumpers (T5), which was stocked with the same initial density of $1.2/m^2$, showed a poor survival rate (12.5%). In the rest of the treatments, the retrieval rate was more or less uniform ranging from 15.4% in the first settled groups (T3), 17.4% in second hatched post-larval group (T2), 18% in second settled groups (T4), 20% in first hatched group (T1) and 22.2% in the control group (T7). Except for T5, in most of the treatments, females were dominant in the final population and the Male: Female ratios were in a ratio of 1:1.57, 1:1.32, 1:1.08, 1:1.69, 1:1.33 and 1:2.04 for T1, T2, T3, T4, T6 and T7 respectively, in contrast, in T5 dominance of males was discernible. (1.39:1). Mean weight gained by Jumpers at the time of harvest was glaringly higher (83.11 gm). First settled and second hatched post larval groups also attained relatively high mean weight with 69.52 g in the former and 67.62 g in the latter. In contrast, in control

the average weight was only 58.72 g and this was due to the preponderance of undersized females. Moreover, the percentage of undersized morphotypes like Small Males (SM), Small Females (SF), Weak Orange Clawed males (WOC) and Weak Blue clawed Females (WBF) showed their predominance in T1, T4 and T6. As a result, the final mean weight of prawns in these treatments was in the order of 43.76, 48.74 and 56.53 gm respectively. Interestingly, the treatments, which registered better average weight at the time of harvest, the dominance of larger male morphotypic stages such as transforming Strong Orange Clawed males (t-SOC) and Blue clawed males (BC) was quite discernible in these treatments, where the average weight were comparatively high.

The percentage contribution and mean weight of individual morphotype under the three separate sets of adaptive trials are shown in Table 9.19. Among the batch graded post larvae, high mean weight and faster morphotypic transformation was registered in the later hatched groups (T2). The percentage contributions by weight of undersized male and female morphotypes in these treatments were comparatively low (4.9%) when compared to their counterparts from first hatched groups (8.6%). Similarly, lower percentage of undersized prawns among the size graded post larvae were characteristic in Jumpers (2.3%) when compared with Laggards (3%). Among the modified batch graded population, almost similar performance was observed in first settled larval groups (2.3%) when compared to the later settled groups (9%). The coefficient of variation, which expresses the extent of heterogeneity among the various morphotypes in the population, was high in T2, T3 and T4. Analysis of

mean weight of terminal male and female morphotypes in the three treatments showed better results for OBC and SBF from second hatched batch (T2) (146.4g and 75.3g respectively), first settled batch (T3) (112.8g and 71.3g respectively) and Jumpers (T5) (151.7g and 76.8g respectively). Results of analysis of variance of mean weight of morphotypes under different sets of adaptive trials is shown in Table 9.20. Significant difference ($P<0.01$) in the F value of mean weight was observed among larger morphotypes such as SOC, t-SOC, WBC, SBC and OBC showing the diverse nature in their representation by weight in the three sets of trials conducted. Further analysis on the mean weight of individual morphotypes through t-test (Table 9.21) revealed the fact that, among the various combinations T1 and T2 showed significant difference in weight ($P<0.05$) in respect of terminal male morphotypes. Similar results were also observed for the different sets of adaptive trials incorporating size graded and modified batch-graded populations. In the channels stocked with Jumpers, a significant difference ($P<0.05$) in the degree of heterogeneity was seen in the mean weight among all the morphotypes except for SM. In all the other sets of trials, maximum differentiation ($P<0.05$) was encountered in the intermediate stages t-SOC, WBC and SBC. A lesser extent of heterogeneity ($P<0.01$) was observed in the terminal stage OBC in T2 and T4. It would thus appear that with an increase in the proportion of males in the final population, the degree of heterozygosity also becomes alleviated. Results of analysis of variance of mean weight of prawns within morphotypes support the earlier finding that during the initial stages of transformation the weight differentiation was not well pronounced since in most of the experimental groups there wasn't any significant variation in the

mean weight for SM. However, in terminal stages especially in t-SOC and BC, the difference in the mean weight was significant ($P<0.05$). This indicates that the skewness of mean weight among individual morphotypes was greater among T5 followed by T3 and T2. This disproportionate growth rate seen among the treatment was mainly manifested by the presence of larger males in the final population.

Figure 9.1 shows the population characteristics of *M. rosenbergii* in channels stocked with batch graded post larvae. The second hatched group showed a better performance in growth and rate of morphotypic transformation as the predominance of t-SOC (16%) and Blue clawed (22%) morphotypes were quite discernible. Conversely, the first hatched group showed poor growth performance as evident from the final population structure, which had a high proportion of blue-clawed female (22%), transforming orange female (16%) and SM (14%). The population structure and morphotypic composition of modified batch-graded prawns at the final harvest is shown in figure 9.2. It appears that the percentage of undersized males (SM and WOC) was comparatively less in T3 (17%) than T4 (22%). On the contrary, larger morphotypes (BC) followed an inverse pattern with high values in T3 (27%) than T4 (17%). Figure. 9.3 shows population characteristics of *M. rosenbergii* in channels stocked with size graded post larvae. The results show that T5 had better growth performance and faster morphotypic transformation. The contributions of t-SOC (22%) and Blue clawed (26%) were far more than that recorded in any of the treatments with different batch graded or size graded post larvae. Another interesting observation was that in spite of the fact that most of the male morphotypes attained the terminal

stages, Strong Orange clawed (9%) still persisted in the final harvested population in appreciable number in the treatment. On the other hand, the percentage of SM (5%) and other undersized male morphotypes such as WOC (5%) and Small females (9%) were very less. However, the presence of a high proportion of undersized prawns (34%) including SM, WOC and SF was worth noticing in T6.

3.2 Market structure and Yield characteristics of different sets of Adaptive trials

The result of ANOVA of net production from three different sets of adaptive trials is given in Table 9.17. F value (F= 11.603) showed that there exits significant difference (P<0.01) in the production pattern of different channels, which highlights the importance of batch grading, size grading and modified batch grading techniques prior to stocking in the culture of *M. rosenbergii*. Results of pair wise analysis within the three sets of treatments are depicted in Table 9.18. Barring T3 and T4, all other treatments showed significant difference in net production (P<0.05). More clear differentiation was observed between treatments T1 and T2 and T5 and T6. Yield characteristics and percentage contribution of each weight class to the final yield between T1 and T2 are given in Table 9.19. The contribution of <50 gm class group of prawns were alarmingly high in T1 (25.63%). Conversely, the percentage of weight class >120g was high in T2 (61.4%) while < 50 gm weight class comprised only 16%. The trend followed more or less a similar pattern in size graded post larval groups also. The percentage contribution of non-marketable prawns (<50g) in T6 accounted for 27.78%, in contrast, their percentage showed a sizeable reduction in T5 (11.98%). Figure 9.5 shows the yield characteristics of *M. rosenbergii* in

channels stocked with modified batch graded post larvae. Net production showed significant variation among the channels. Higher values for net production were recorded from T2 (88.9 kg/ha) and T3 (98.71 kg/ha) and the profit incurred from these channels leniently depended on the percentage occurrence of larger prawns in the final population. In T3, the contribution of 120-230 gm and >230gm weight classes were in the order of 30.13 and 10.17% respectively. Even though the production from T3 was less, the economic return from this channel was high due to the presence of larger prawns belonging to the terminal stages in the morphotypic transformation, which belongs to the higher weight classes.

The prawns at the final harvest were marketed 'head-on' based on the market tariff prevailing in Kochi. The market structure includes categorizing the prawns into 5 different groups based on their individual weight. The price packages offered by the seafood processing plants located at Cochin are given in Chapter 7. While computing the revenue from the various treatments based upon the above tariff, total income worked out to be of Rs. 13,370/- in T1, which was on a lower side to their counterparts in T2 (Rs. 20,524/-). On the contrary, among modified batch graded groups better results were obtained in T3 (Rs. 21,720/-) when compared to that of T4 (Rs. 12,000/-). In spite of better production rates, T6 did not perform well in terms of economic returns (Rs. 12, 425/-), whereas revenue wise, T5 emerged as more economical and profitable (Rs. 15,235/-). This could be attributed to the predominance of slow growing undersized males (SM and WOC) in the harvested population in T6. Operation cost in T6 was highest when compared to other channels, among them cost of feed and seed accounted for more than 50% of the total expenditure. The profit incurred from

channels maintained as control was very low (Rs. 16,260/-), when compared to other treatments.

4. Discussion

M. rosenbergii expresses perceptible individual variation in growth rates and consequently in size soon after metamorphosis of the larvae into the post-larval form (Wickins, 1972; Forster and Beard, 1974). Though the average growth is inversely related to density, however, the wide variation in growth rate is believed to be induced primarily by intrinsic factors associated with social hierarchy rather than environmental factors (Sandifer and Smith, 1975; Ra'anan, 1982). Most of the earlier works related to improving the yield and revenue from scampi farming have basically focused on management practices in the form of segregating batches of post larvae prior to stocking or soon after the nursery phase (Daniels *et al.*, 1995; Malecha *et al.*, 1981b; Karplus *et al.*, 1986b; 1987; D'Abramo *et al.*, 1991; Daniels and D'Abramo, 1994). The intensity of Heterogenous Individual Growth (HIG), which has been a major obstacle in the sustainable culture of this species, could be reduced to some extent by exercising some of these management strategies. In the present study, the grading experiment of post larvae soon after the hatchery phase has been attempted to understand to what extent the differential growth can be minimized in the grow-outs. 'Jumpers', which had relatively low survival rate were adequately compensated by a better mean weight. Whereas, the 'Laggards' had the highest retrieval rate, however, their average weight was very low. Increase in population number might have aggravated to intraspecific competition for food and space. With a percentage increase of males in 'jumpers', there are

chances for increased predation through cannibalism among males soon after the molting. As a result, the final population was affected. Whereas, in 'Laggards' where the percentage of males were less, the chances for competition was reduced and reciprocally the survival rates showed an increase. In the present study, all animals were of the same age and were maintained under similar grow-out conditions.

Although growth suppression through competition has been well documented (Wilbur and Collins, 1973; Wohlfarth, 1977), stimulation of growth, which is observed under communal conditions but not in isolation, is still a puzzling biological phenomenon. Ra'anan and Cohen (1983) asserted that the appearance of Jumpers in *M. rosenbergii* is not primarily as a result of competition, if at all, but of factors intrinsically associated with population development. The result of ANOVA of mean weight of prawns in the present study did not follow any specific pattern as predicted. In neither of the trials the mean weight of Small males (SM) differed significantly ($P>0.01$) nor did the level of significance increased as the morphotypes proceeded to their terminal stage of growth. The difference in mean weight of prawns was significantly higher among the transitional morphotypes such as t-SOC, WBC and SBC. The effects of higher density at the final harvest on mean whole body weight and total yield are the result of changes in the mean wet weight of particular morphotypes rather than percentage of morphotypes (Daniels *et al.*, 1995). Karplus *et al.* (1986a) and Daniels *et al.* (1995) observed a decrease in mean weight of males (except for SM) and female morphotypes commensurating with the increase in stocking density. The results of the present study were also in concordance with

the earlier findings. The mean weights of morphotypes within the five groups were significantly different. Owing to lesser number, greater space for survival and lesser competition for food the 'jumpers' turned out to be larger in size when compared to their counterparts in other channels, especially in morphotypes like SOC, t-SOC, SBC and OBC. The results were complementary to the findings of Daniels *et al* (1995), who reported that it is not only density but the morphotypic composition also, which are playing important roles in determining the mean wet weight

The proportion of male morphotypes in the different channels differed significantly. The diverse pattern of morphotypic transformation and the consequent population structure registered at the time of final harvest can be correlated to the pattern of growth undergone by the post larvae in each set of trials. Ranjeet and Kurup (1999) observed that the larvae that hatched first from the egg cluster were comparatively weaker and took much more time to attain the post larval stage than the later hatched clutch. The trend continued even in the grow-outs as observed in the present study. The proportion of weaker undersized males in the channels stocked with first hatched larvae in the present study could be the aftermath of this process. Ra'anan and Cohen (1985) showed that the ontogeny contributes as a major factor in determining the social structure of a population of freshwater prawn *M. rosenbergii*. The results obtained from the above set of adaptive trials were also found complimentary to the findings of Ra'anan and Cohen (1985). The better performance of the 'Second hatched' found in the present study were complimentary to that of Mashiko (1987) who

reported a direct relationship between egg size and incubation time on the growth and metamorphosis in *M. rosenbergii*.

In the second sets of experiments including modified batch graded population, the sorting of post larvae was basically performed prior to stocking based on the performance of the post larvae at the PL-15 stage. The post larvae, which showed better growth, faster movement and better feed intake during the hatchery phase were expected to perform well as the whole group were destined to be emerged as fast growing "Jumpers". As expected, the final population was contributed by larger prawns, which had attained terminal stage contrary to 'laggards', which were dominated by female prawns and undersized small males. The results obtained on the population characteristics and weight distribution pattern of different male morphotypes were in accordance to the earlier results of Karplus *et al.* (1986a) and Tidwell *et al.* (1999) who reported that the average individual weight of prawns were significantly reduced by the predominance of undersized males and females in the final population. Grading at stocking affects both the mean weight and the population structure. Difference in the mean weight and yield of the graded fractions are presumably due to changes in population structure rather than differences in absolute weight (Karplus *et al.*, 1986a). Larvae from a single brood may transform into the post-larval stage within a range of 3 to 7 days (Ra'anan and Cohen, 1982). Sandifer and Smith (1979) reported that early metamorphosis confers no growth or development advantage, nor late metamorphosis with any disadvantage in *M. rosenbergii* post-larval population. But in total contrast to this, the present findings show a well-defined differentiation in the performance of batch graded and modified batch

graded post larvae. The occurrences of a higher frequency of males among Jumpers in the present study were in lines with that of Howlader and Kiortsis (1978) and Karplus *et al.* (1990).

At the final harvest, net profit from the channels was very less when compared to that of polders (Kurup *et al.*, 2000). Stunting in males of *M. rosenbergii* expressed by a high frequency of the small male morphotypes in the population is a major obstacle to the viability of prawn culture due to the fact that prawn prices are size dependant. Revenue obtained from each channel was different mainly due to the varied population structure and weight distribution of prawns under each set of trials. The fragile market structure prevailing in Kuttanad has also adversely affects the profit from scampi farming in this area. The structure is basically focussed on the presence of premium or larger sized prawns, as a result the farmers are compelled to continue their farming period for another two more months. In the present study, higher proportion of larger prawns belonging to 120-230 g and >230g category were held responsible for the overall production and profit in the channels stocked with 'jumpers' and 'later hatched' larval groups. Since the survival rates 'jumpers' were relatively less, its culture was found not much economically profitable. Whereas, the later hatched larval group showed good survival results. And therefore it can be reasonably be concluded that there are every possibility for the increased net production and profitability in the farming of *M. rosenbergii* by way of incorporating appropriate pre-stocking management measures such as stocking with batch graded and modified size graded post larvae.

Table 9.1 Stocking details and yield characteristics of first hatched post larval groups reared under batch grading in three channels (Treatment 1)

	CHANNELS		
	1	2	3
STOCKING			
Number per ha.	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4
HARVEST			
Number per square meter	0.23	0.24	0.25
Number per ha.	2387	2415	2573
Mean weight (g)	44.58	43.76	41.85
Gross production (Kg/ha)	56	62	70
Net production (Kg/ha)	53.14	60.78	66.45
Survival (%)	19.89	20.12	21.44
Mean male weight (g)	54.81	52.16	49.35
Mean female weight (g)	39.58	38.43	36.45
% by number of males in population	42.85	38.83	35.21
% by number of females in population	57.15	61.17	64.79
Sex ratio	1:1.33	1:1.57	1:1.84

Table 9.2 Comparison of mean weight of morphotypes in three channels of Treatment 1 (T1)

Morphotypes	F-value
SF	1.9547
WOF	2.0147
SOF	1.3581
TOF	1.6852
WBF	2.0654
SBF	2.1458
SM	1.3249
WOC	2.1364
SOC	1.5863
t-SOC	1.9842
WBC	2.0745
SBC	2.0987
OBC	1.9783

SF = Small female
WOF= Weak orange clawed female
SOF = Strong orange clawed female
TOF = Transforming strong orange clawed female
WBF= Weak blue clawed female
SBF = Strong blue clawed female
SM = Small male
WOC = Weak orange clawed male
SOC = Strong orange clawed male
t-SOC = Transforming strong orange clawed male
WBC = Weak blue clawed male
SBC = Strong blue clawed male
OBC = Old blue clawed male

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes

Table 9.3 Stocking details and yield characteristics of later hatched post larval groups reared under batch grading in three channels (Treatment 2)

	CHANNELS		
	1	2	3
STOCKING			
Number per ha.	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4
HARVEST			
Number per square meter	0.2	0.19	0.22
Number per ha.	2092	1968	2214
Mean weight (g)	67.62	69.25	61.89
Gross production (Kg/ha)	90	85	98
Net production (Kg/ha)	88.91	81.26	96.58
Survival (%)	17.43	16.4	18.45
Mean male weight (g)	75.28	78.15	70.53
Mean female weight (g)	58.11	56.24	60.14
% by number of males in population	42.92	43.58	40.68
% by number of females in population	57.08	56.42	59.32
Sex ratio	1:1.32	1:1.29	1:1.45

Table 9.4 Comparison of mean weight of morphotypes in three channels of Treatment 2 (T2)

Morphotypes	F-value
SF	1.8432
WOF	1.9032
SOF	1.2466
TOF	1.5737
WBF	1.9539
SBF	2.0343
SM	1.2134
WOC	2.0249
SOC	1.4748
t-SOC	1.8727
WBC	1.9630
SBC	1.9872
OBC	1.8668

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 9.1

Table 9.5 Stocking details and yield characteristics of first settled post larvae reared under modified batch grading in three channel (Treatment 3)

	CHANNELS		
	1	2	3
STOCKING			
Number per ha.	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4
HARVEST			
Number per square meter	0.18	0.18	0.18
Number per ha.	1842	1856	1895
Mean weight (g)	66.38	69.52	71.08
Gross production (Kg/ha)	94	100	105
Net production (Kg/ha)	91.23	98.71	102.5
Survival (%)	15.35	15.47	15.79
Mean male weight (g)	78.15	78.53	75.24
Mean female weight (g)	49.35	56.38	60.14
% by number of males in population	49.35	47.88	52.1
% by number of females in population	50.65	52.12	47.9
Sex ratio	1:1.02	1:1.08	1:0.91

Table 9.6 Comparison of mean weight of morphotypes in three channels of Treatment 3 (T3)

Morphotypes	F-value
SF	1.6927
WOF	1.7932
SOF	1.4760
TOF	1.4403
WBF	1.6912
SBF	2.2967
SM	0.7618
WOC	1.5161
SOC	1.2648
t-SOC	1.7965
WBC	1.8935
SBC	2.2085
OBC	1.9040

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 9.1

Table 9.7 Stocking details and yield characteristics of second settled post larvae reared under modified batch grading in three channels (Treatment 4)

	CHANNELS		
	1	2	3
STOCKING			
Number per ha.	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4
HARVEST			
Number per square meter	0.22	0.19	0.21
Number per ha.	2236	1964	2170
Mean weight (g)	45.98	56.27	48.74
Gross production (Kg/ha)	86	64	78
Net production (Kg/ha)	84.26	61.38	74.81
Survival (%)	18.63	16.37	18.08
Mean male weight (g)	48.39	58.21	54.49
Mean female weight (g)	35.21	40.25	37.11
% by number of males in population	36.25	42.37	38.11
% by number of females in population	63.75	57.63	61.89
Sex ratio	1:1.75	1:1.36	1:1.62

Table 9.8 Comparison of mean weight of morphotypes in three channels of Treatment 4 (T4)

Morphotypes	F-value
SF	2.3241
WOF	1.5247
SOF	1.6584
TOF	2.0358
WBF	0.5894
SBF	0.8424
SM	1.247
WOC	1.3524
SOC	1.2255
t-SOC	1.8459
WBC	2.0145
SBC	1.4553
OBC	1.5873

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 9.1

Table 9.9 Stocking details and yield characteristics of Jumpers reared under size grading experiments in three channels (Treatment 5)

	CHANNELS		
	1	2	3
STOCKING			
Number per ha.	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4
HARVEST			
Number per square meter	0.15	0.15	0.14
Number per ha.	1503	1572	1456
Mean weight (g)	83.11	80.26	88.58
Gross production (Kg/ha)	60	68	54
Net production (Kg/ha)	58.71	64.89	50.24
Survival (%)	12.53	13.10	12.13
Mean male weight (g)	110.27	102.45	118.42
Mean female weight (g)	65.72	59.85	70.54
% by number of males in population	58.23	56.41	60.33
% by number of females in population	41.77	43.59	39.67
Sex ratio	1:0.71	1:0.77	1:0.65

Table 9.10 Comparison of mean weight of morphotypes in three channels of Treatment 5 (T5)

Morphotypes	F-value
SF	1.9331
WOF	1.6039
SOF	1.6819
TOF	1.6714
WBF	1.0090
SBF	1.7007
SM	0.7786
WOC	1.1799
SOC	1.1402
t-SOC	1.7831
WBC	1.9193
SBC	1.9426
OBC	1.7642

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 9.1

Table 9.11 Stocking details and yield characteristics of Laggards reared under size grading in three channels (Treatment 6)

	CHANNELS		
	1	2	3
STOCKING			
Number per ha.	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4
HARVEST			
Number per square meter	0.18	0.29	0.18
Number per ha.	2541	2895	3098
Mean weight (g)	59.52	56.53	51.21
Gross production (Kg/ha)	100	98	96
Net production (Kg/ha)	98.71	96.81	93.45
Survival (%)	21.18	24.11	25.82
Mean male weight (g)	68.53	63.94	57.3
Mean female weight (g)	46.38	44.27	38.41
% by number of males in population	47.88	42.83	38.52
% by number of females in population	52.12	57.17	61.48
Sex ratio	1:1.08	1:1.33	1:1.59

Table 9.12 Comparison of mean weight of morphotypes in three channels of Treatment 6 (T6)

Morphotypes	F-value
SF	1.8882
WOF	1.7536
SOF	1.4642
TOF	1.6225
WBF	1.4814
SBF	1.8675
SM	0.9960
WOC	1.6024
SOC	1.3075
t-SOC	1.8279
WBC	1.9411
SBC	1.9649
OBC	1.8155

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 9.1

Table 9.13 Stocking details and yield characteristics from three channels maintained as control (Treatment 7)

	CHANNELS		
	1	2	3
STOCKING			
Number per ha.	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4
HARVEST			
Number per square meter	0.36	0.31	0.33
Number per ha.	3681	3128	3380
Mean weight (g)	54.58	59.52	58.72
Gross production (Kg/ha)	145	121	130
Net production (Kg/ha)	142.68	118.24	127.23
Survival (%)	30.68	26.07	28.2
Mean male weight (g)	65.27	68.53	68.41
Mean female weight (g)	40.85	46.38	43.03
% by number of males in population	31.12	35.52	32.83
% by number of females in population	68.88	64.48	67.17
Sex ratio	1:2.21	1:1.81	1:2.04

Table 9.14 Comparison of mean weight of morphotypes in three channels of Treatment 7 (T7)

Morphotypes	F-value
SF	2.2967
WOF	0.7618
SOF	1.5161
TOF	1.2648
WBF	1.5247
SBF	1.6584
SM	2.0358
WOC	0.5894
SOC	0.8424
t-SOC	1.247
WBC	1.1402
SBC	1.7831
OBC	1.9193

P>0.05 (Non significant)
F-value non-significant among channels for morphotypes
For expansion of morphotypes refer Table 9.1

Table 9.15 Summary of fortnightly water quality parameters in treatments 1 - 7 (Coconut garden channels)

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7
Surface Temperature	29.7	29.1	28.4	29.3	31.4	30.4	29.9
(deg C)	(26.1 - 32.5)	(27.2 - 31.8)	(24.7 - 32.8)	(26.1 - 32.2)	(28.6 - 33.1)	(24.4 - 34.2)	(27.4 - 32.6)
Surface Dissolved Oxygen	5.4	5.4	3.62	5.5	5.06	4.49	4.3
(mg/l)	(4.4 - 6.3)	(4.2 - 6.0)	(3.0 - 6.2)	(2.8 - 5.8)	(2.3 - 7.1)	(3.6 - 6.1)	(3.2 - 6.2)
Water pH range	5.6 - 7.2	5.8 - 7.2	5.4 - 7.8	6.2 - 7.9	6.4 - 8.1	5.9 - 7.4	5.9 - 7.4
Soil pH range	5.8 - 6.8	4.3 - 6.5	5.3 - 7.8	5.6 - 7.9	5.2- 6.8	5.2 - 6.9	5.2 - 7.1
Transparancy	21.3	20.5	12.4	18.6	21.4	28.4	22.4
(cm)	(15 - 45)	(12 - 38)	(8 - 35)	(10 - 38)	(15 - 38)	(10 - 45)	(10 - 42)
Total Alkalinity	43.2	40.5	68.4	52.14	86.74	64.29	46.7
(mg/l)	(18 - 56)	(20 - 59)	(32 - 87)	(35 - 92)	(56 - 114)	(44 - 102)	(23 - 78)
Nitrite-N	0.8	0.23	0.1	0.1	0.1	0.1	1.07
(mg/l)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)
Total Ammonia	0.02	0.07	0.1	0.05	0.05	0.1	0.05
(mg/l)	(0 - 0.08)	(0 - 0.12)	(0 - 0.2)	(0 - 0.1)	(0 - 0.1)	(0 - 0.4)	(0 - 0.1)
Hydrogen sulphide	0.01	0.01	0.01	0.01	0.01	0.01	0.01
(mg/l)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)

Figures are means of two replicates and 22 sampling dates (N = 44). The range of observed values are given in parenthesis

Table 9.16 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared under different batch grading, size grading and modified batch grading management systems in seven treatments (coconut garden channels)

	TREATMENTS						
	Batch graded		Modified batch graded		Size graded		Control
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6	Treatment 7
STOCKING							
Number per ha.	12,000	12,000	12,000	12,000	12,000	12,000	12,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	2.4	2.4	2.4	2.4	2.4	2.4	2.4
HARVEST							
Number per square meter	0.24	0.2	0.18	0.21	0.15	0.29	0.34
Number per ha.	2415	2092	1856	2170	1503	2895	3380
Mean weight (g)	43.76	67.62	69.52	48.74	83.11	56.53	58.72
Gross production (Kg/ha)	62	90	100	78	60	98	130
Net production (Kg/ha)	60.78	88.91	98.71	74.81	58.71	96.81	127.23
Survival (%)	20.12	17.43	15.47	18.08	12.53	24.11	28.2
Mean male weight (g)	52.16	75.28	78.53	54.49	110.27	63.94	68.41
Mean female weight (g)	38.43	58.11	56.38	37.11	65.72	44.27	43.03
% by number of males in population	38.83	42.92	47.88	37.11	58.23	42.83	32.83
% by number of females in population	61.17	57.08	52.12	62.89	41.77	57.17	67.17
Sex ratio	1:1.57	1:1.32	1:1.08	1:1.69	1:0.71	1:1.33	1:2.04

Table 9.17. Analysis of Variance in net production from seven treatments

ANOVA

Source of Variation	*Sum of Squares*	*df*	*Mean sum of squares*	*calculated F*
Between Groups	13632	6	2272	11.6031
Within Groups	4112	14	195.8095	
Total	17744	20		

Table 9.18 t-Test comparison on the net production between the treatments

	Treatment 1 vs 7	Treatment 2 vs 7	Treatment 1 vs 2	Treatment 3 vs 7	Treatment 4 vs 7	Treatment 3 vs 4	Treatment 5 vs 7	Treatment 6 vs 7	Treatment 5 vs 6
t- value	6.0845*	4.1297*	3.3954*	2.1145**	4.3328*	1.667	6.8984*	3.5729**	6.5817*

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

Table 9.19 Percentage contribution of male and female morphotypes in the harvested population by weight in seven treatments (1 - 7)

Treatment		Mean weight of male morphotype							Mean weight of female morphotype					
		SM	WOC	SOC	t-SOC	WBC	SBC	OBC	SF	WOF	SOF	TOF	WBF	SBF
	Number sampled	50	40	29	157	26	32	59	34	19	5	41	36	35
	% by weight	3.61	0.76	0.23	10.24	15.45	12.85	9.81	3.75	0.43	0.12	22.15	14.00	6.60
1	Mean weight	14.10	30.70	86.00	107.20	81.30	119.03	119.80	10.76	26.42	45.65	46.67	49.68	63.91
(n=2)	Standard deviation	6.60	4.65	19.41	18.87	19.86	15.43	26.03	4.72	32.73	11.39	28.52	25.92	31.09
	Coeff. of variation	39.08	11.34	21.14	18.48	20.63	12.07	17.78	44.82	61.62	20.35	56.34	48.84	41.30
	Number sampled	30	20	13	51	54	41	58	30	54	7	21	30	30
	% by weight	2.26	0.45	0.14	13.55	9.52	17.52	21.57	1.34	0.84	0.13	12.45	9.45	10.78
2	Mean weight	12.89	41.01	91.81	102.10	96.26	127.88	146.39	10.53	53.12	55.98	50.62	53.07	75.28
(n=2)	Standard deviation	10.73	20.16	26.93	46.83	26.98	21.87	16.11	6.90	11.83	3.24	15.98	17.30	17.29
	Coeff. of variation	76.10	65.67	31.31	43.68	33.19	18.37	13.45	64.13	44.78	21.60	34.24	34.82	27.05
	Number sampled	25	36	27	47	43	32	47	36	60	0	24	28	94
	% by weight	0.87	0.24	5.34	23.30	13.76	13.19	9.72	1.06	0.14	0.00	14.85	8.24	9.29
3	Mean weight	19.55	37.75	69.82	77.69	79.44	103.77	112.77	17.36	32.82	0.00	73.31	62.27	71.32
(n=2)	Standard deviation	18.57	4.92	16.38	21.88	21.41	29.51	22.52	2.87	14.23	0.00	13.63	15.49	9.11
	Coeff. of variation	94.99	13.03	23.46	28.16	26.95	28.44	19.97	16.53	43.36	0.00	18.59	24.88	12.77
	Number sampled	26	41	25	22	63	45	52	65	60	30	41	29	24
	% by weight	3.29	1.23	0.34	14.89	14.82	12.05	8.74	3.55	0.89	0.95	22.08	11.54	5.63
4	Mean weight	14.56	31.46	44.60	60.56	59.99	94.33	102.44	12.49	60.19	63.39	57.39	60.13	85.01
(n=2)	Standard deviation	11.75	13.59	28.58	24.21	75.32	8.68	80.08	25.08	23.08	13.28	18.87	16.27	21.44
	Coeff. of variation	80.70	43.20	64.08	39.98	125.56	9.20	78.17	200.86	38.35	20.95	32.88	27.06	25.22
	Number sampled	75	9	6	147	129	36	158	44	14	0	49	105	15
	% by weight	1.14	0.28	0.76	23.73	16.67	7.66	34.26	0.76	0.16	0.00	3.65	8.19	1.65
5	Mean weight	10.61	21.67	83.33	112.96	90.43	148.89	151.72	12.05	54.64	0.00	52.14	54.59	76.80
(n=2)	Standard deviation	4.20	8.66	17.22	34.80	46.14	38.71	33.53	3.94	31.95	0.00	27.74	25.14	30.31
	Coeff. of variation	39.61	39.97	19.50	30.81	51.03	26.00	22.10	32.67	58.48	0.00	53.21	46.05	39.46
	Number sampled	29	2	10	147	110	41	59	24	10	0	137	82.00	10.00
	% by weight	0.91	0.70	0.12	11.69	13.09	6.14	24.84	0.55	0.79	0.00	12.54	21.81	6.81
6	Mean weight	12.93	40.00	92.00	113.36	106.91	171.22	172.07	16.04	31.50	0.00	71.99	60.95	70.00
(n=2)	Standard deviation	4.91	0	15.49	31.96	39.21	27.22	39.71	5.31	16.67	0.00	16.07	17.93	11.55
	Coeff. of variation	37.99	0.00	16.84	28.19	36.68	15.90	23.08	3.11	52.94	0.00	22.33	29.41	16.50
	Number sampled	34	11	50	74	84	29	88	19	12	27	110	180	44
	% by weight	0.59	0.13	1.45	26.31	18.57	11.08	16.03	0.50	0.77		15.57	7.89	1.11
7	Mean weight	1.32	26.82	50.00	66.76	65.61	89.45	119.24	12.26	27.92	61.95	48.17	51.18	65.41
(n=2)	Standard deviation	3.63	9.56	0.00	20.98	25.02	28.08	42.74	3.66	8.59	14.34	12.74	14.06	14.05
	Coeff. of variation	32.08	35.64	0.00	31.42	38.62	31.39	35.84	29.88	30.76	23.15	27.47	21.48	32.08

For expansion of morphotypes refer Table 9.1

Table 9.20 Comparison of mean weight of morphotypes among seven treatments (T1 - T7)

Morphotypes	F-value
SF	3.7864
WOF	6.7948*
SOF	0.0599
TOF	14.1421*
WBF	6.3253*
SBF	1.8018
SM	2.0007
WOC	1.8047
SOC	9.0054*
t-SOC	27.8798*
WBC	10.015*
SBC	29.5026*
OBC	32.675*

Table 9.21 Comparison of mean individual weight between the seven treatments by applying t-Test

Morpotype	Treatment 1 vs 7	Treatment 2 vs 7	Treatment 1 vs 2	Treatment 3 vs 7	Treatment 4 vs 7	Treatment 3 vs 4	Treatment 5 vs 7	Treatment 6 vs 7	Treatment 5 vs 6
SF	0.4232	3.2332*	1.6838**	2.7540*	0.2305	2.0261**	2.7540*	0.5329	2.4162*
WOF	0.734	2.3058*	1.8021**	1.7709**	0.8868	0.7321	0.9841	0.6155	0.615
SOF	0.6426	0.4372	0.3267	-	0.7258	-	-	-	-
TOF	0.7514	2.8716*	1.2383**	3.2111*	2.1417**	0.7289	3.7853*	0.9812	1.1104**
WBF	1.0534**	1.2571**	1.3218	2.4740*	2.1129**	0.7315	3.4715*	2.2114*	1.1786**
SBF	0.7915	0.3257	0.3589	1.1305**	0.9217	0.5241	2.3173*	0.3597	1.2134**
SM	0.1960	2.3817*	2.1618*	0.4541	1.6418*	1.9301**	1.3880	0.7748	0.7648
WOC	0.3579	1.7216*	0.7158	1.6163**	0.9217	1.2316**	1.4345**	0.7916	1.1248
SOC	0.3573	0.9715	1.1247**	0.7310	0.4256	1.3211**	1.0216	0.5421	2.3793*
t-SOC	0.3231	2.5132*	2.9624*	3.2588*	1.6586	7.8059*	11.6927*	4.5231*	3.1997*
WBC	0.5954	2.4391*	1.8727**	1.2480**	5.7771*	5.7396*	1.1697**	1.6672**	1.9949**
SBC	0.8309	2.8900**	2.2540*	3.5883*	6.1868*	11.4542*	3.1908*	1.2416	5.3550*
OBC	0.8041	3.3358*	1.6071**	2.8741*	1.4349**	8.3123*	6.4453*	2.8741*	2.4647*

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

For expansion of morphotypes refer Table 9.1

Fig: 9.1 Morphotypic composition of harvested population in Channels stocked with batch graded post larvae of *M. rosenbergii*

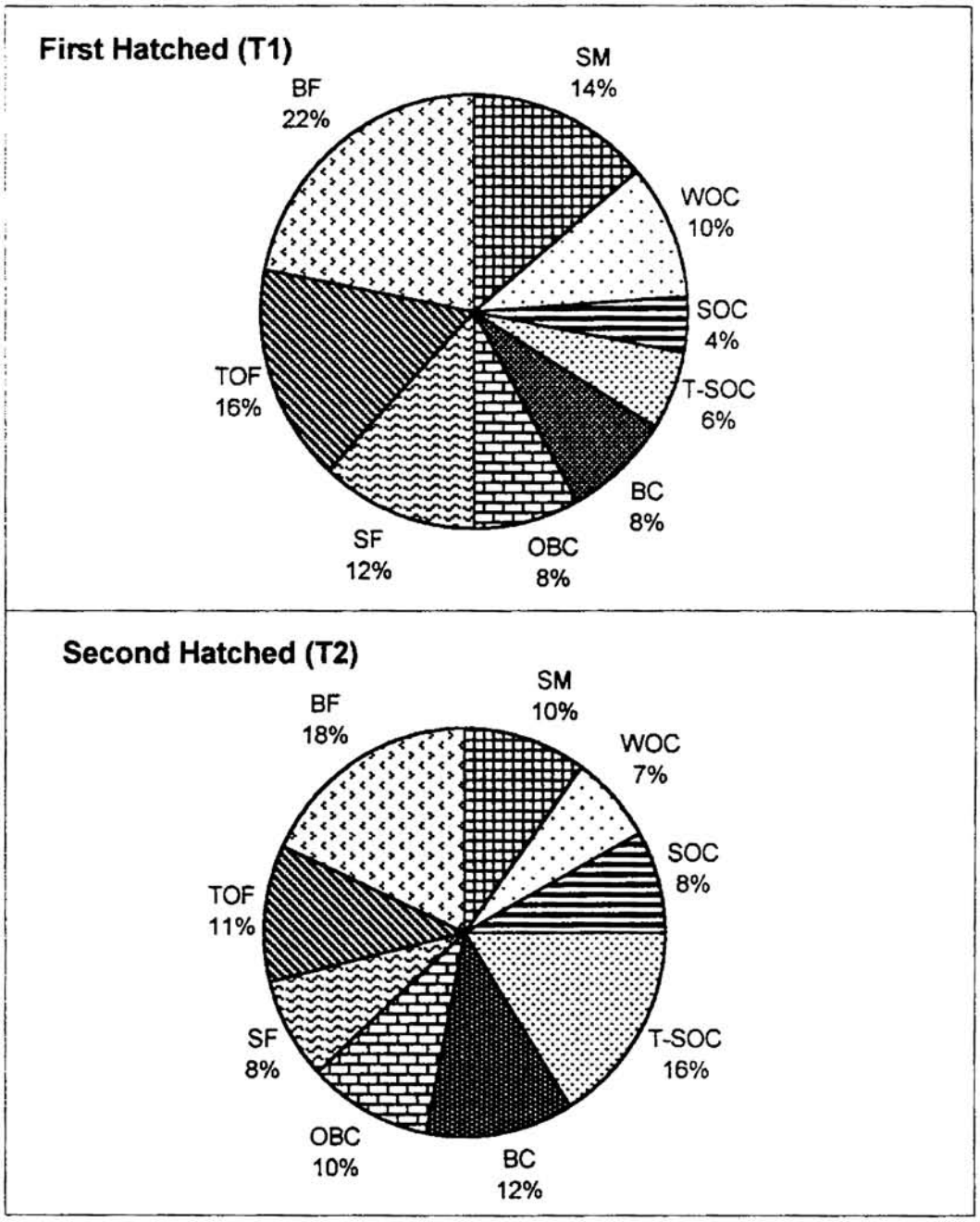


Fig: 9.2. **Morphotypic composition of harvested population in channels with modified batch graded post larvae of *M. rosenbergii***

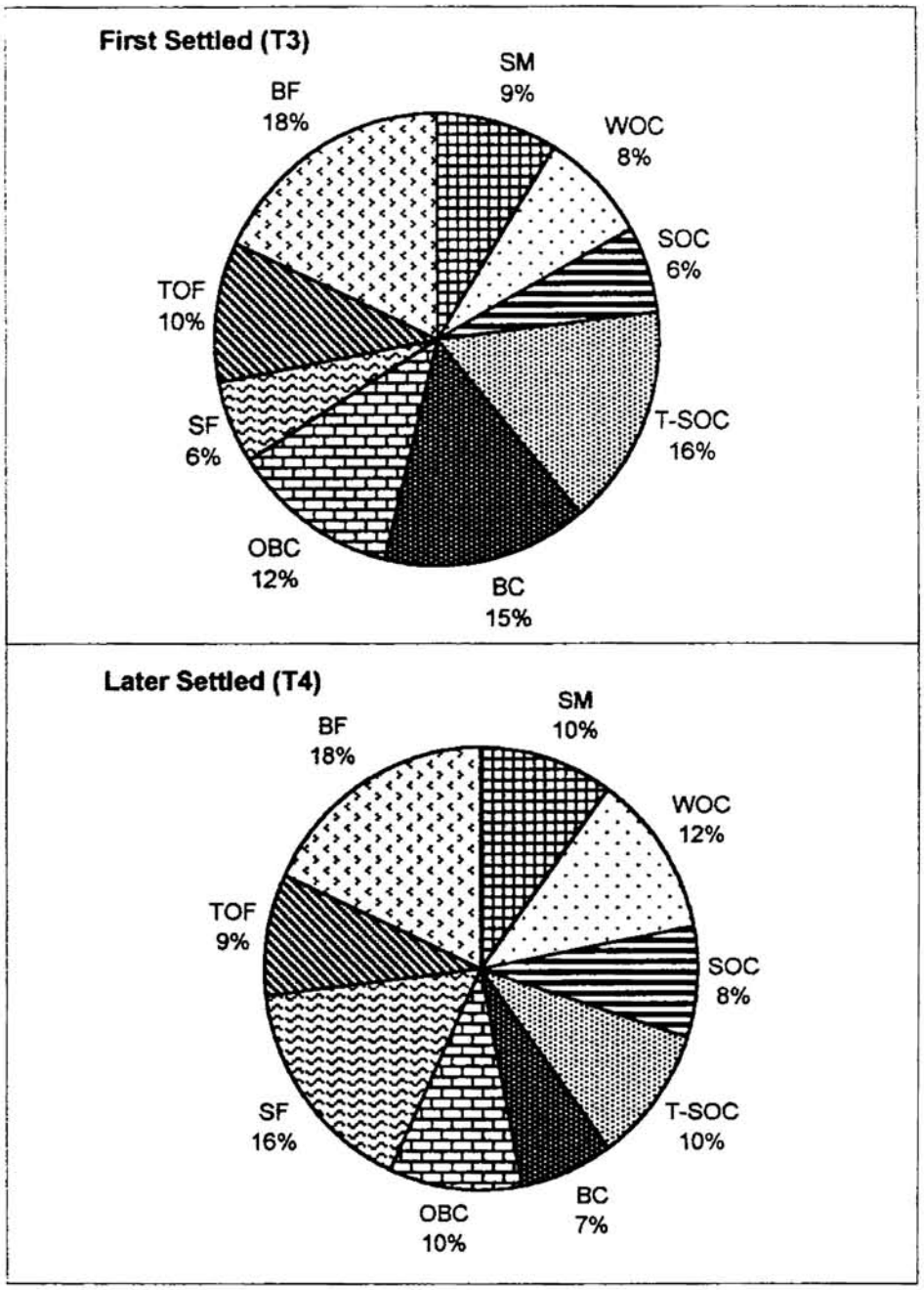


Fig:9.3 Population characteristics of Channels stocked with batch graded post larvae

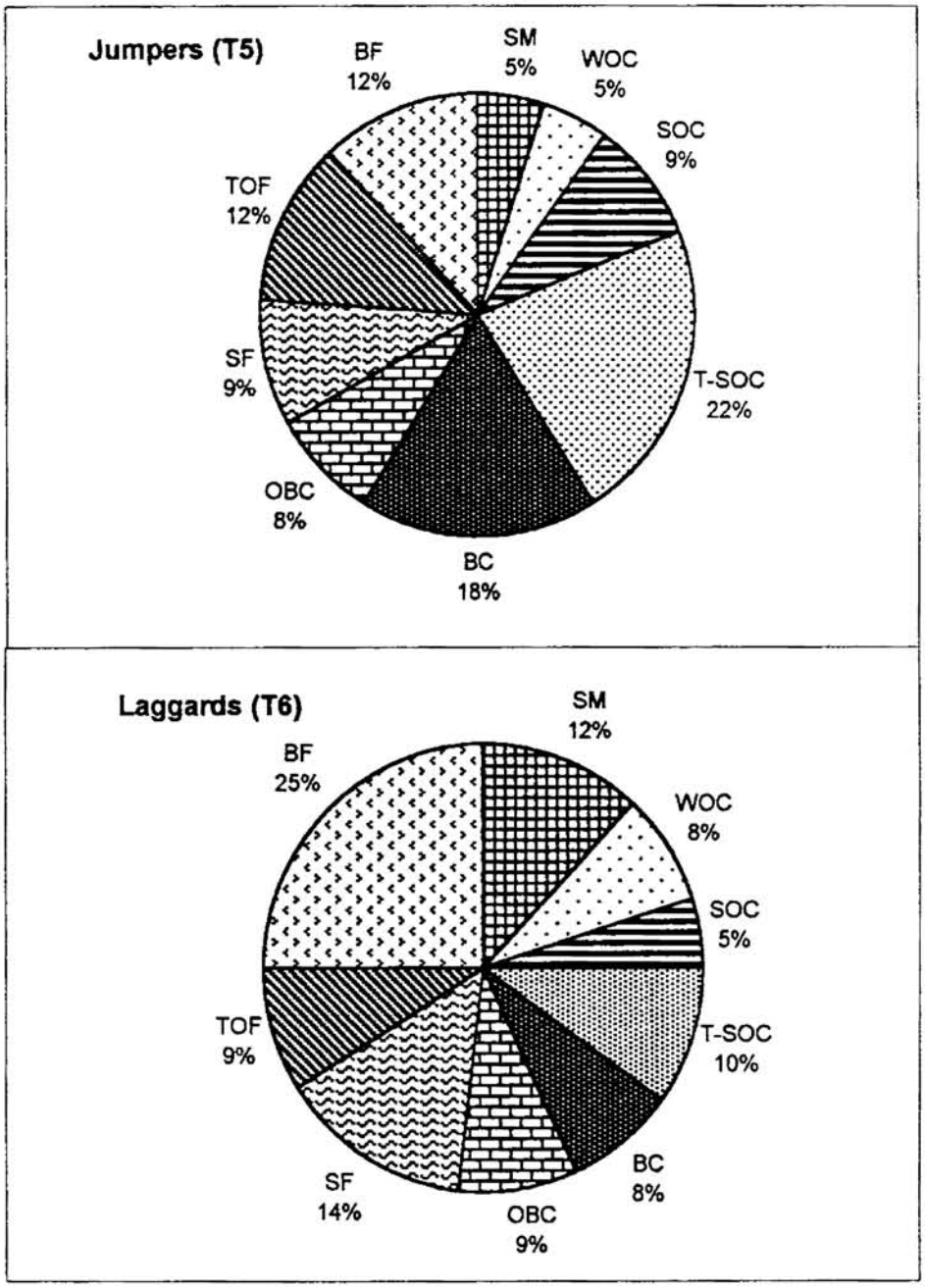


Fig 9.4. Marketable yield structure of harvested population of *M. rosenbergii* in batch graded post larvae

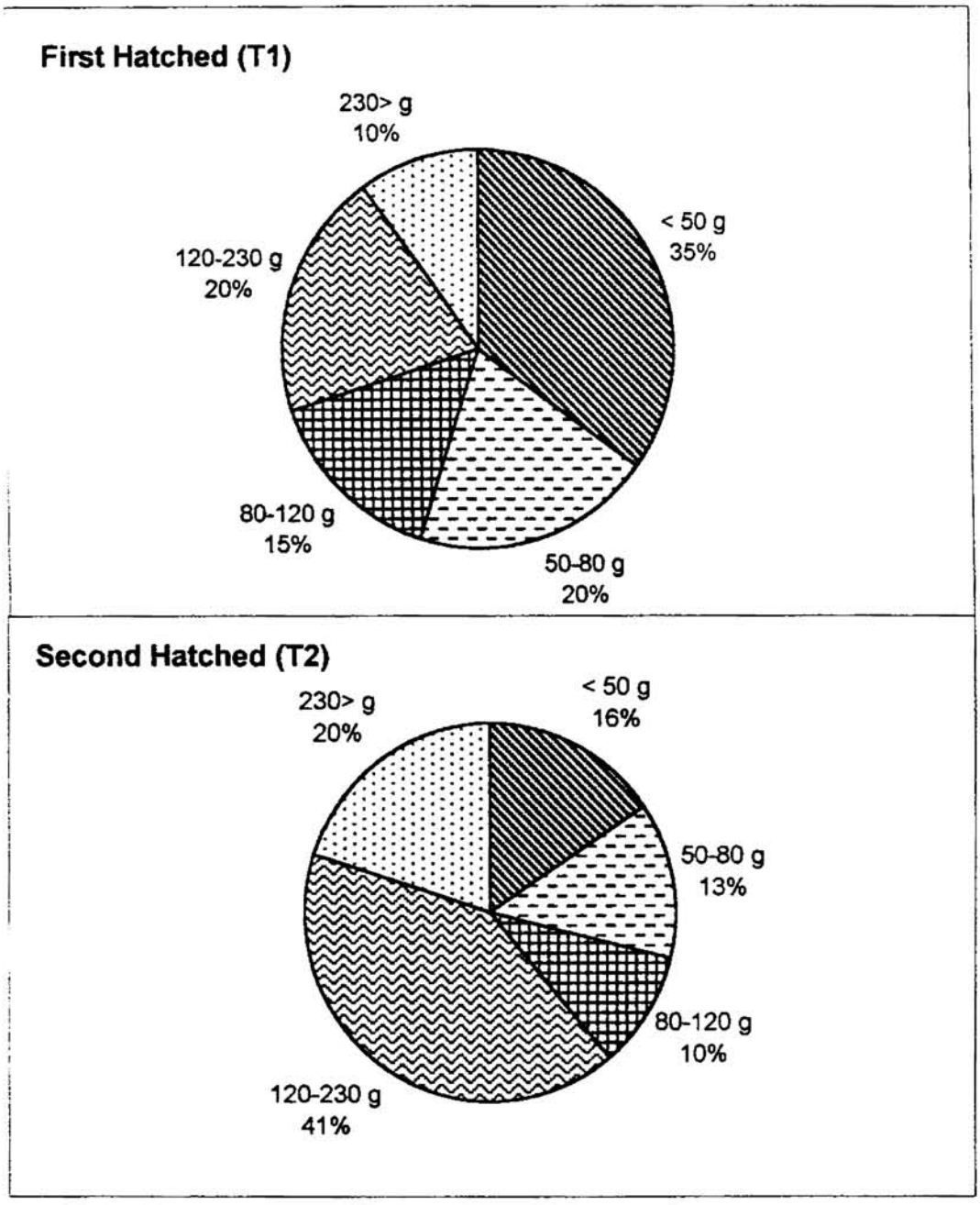


Fig 9.5. Marketable yield structure of harvested population of *M. rosenbergii* in modified batch graded post larvae

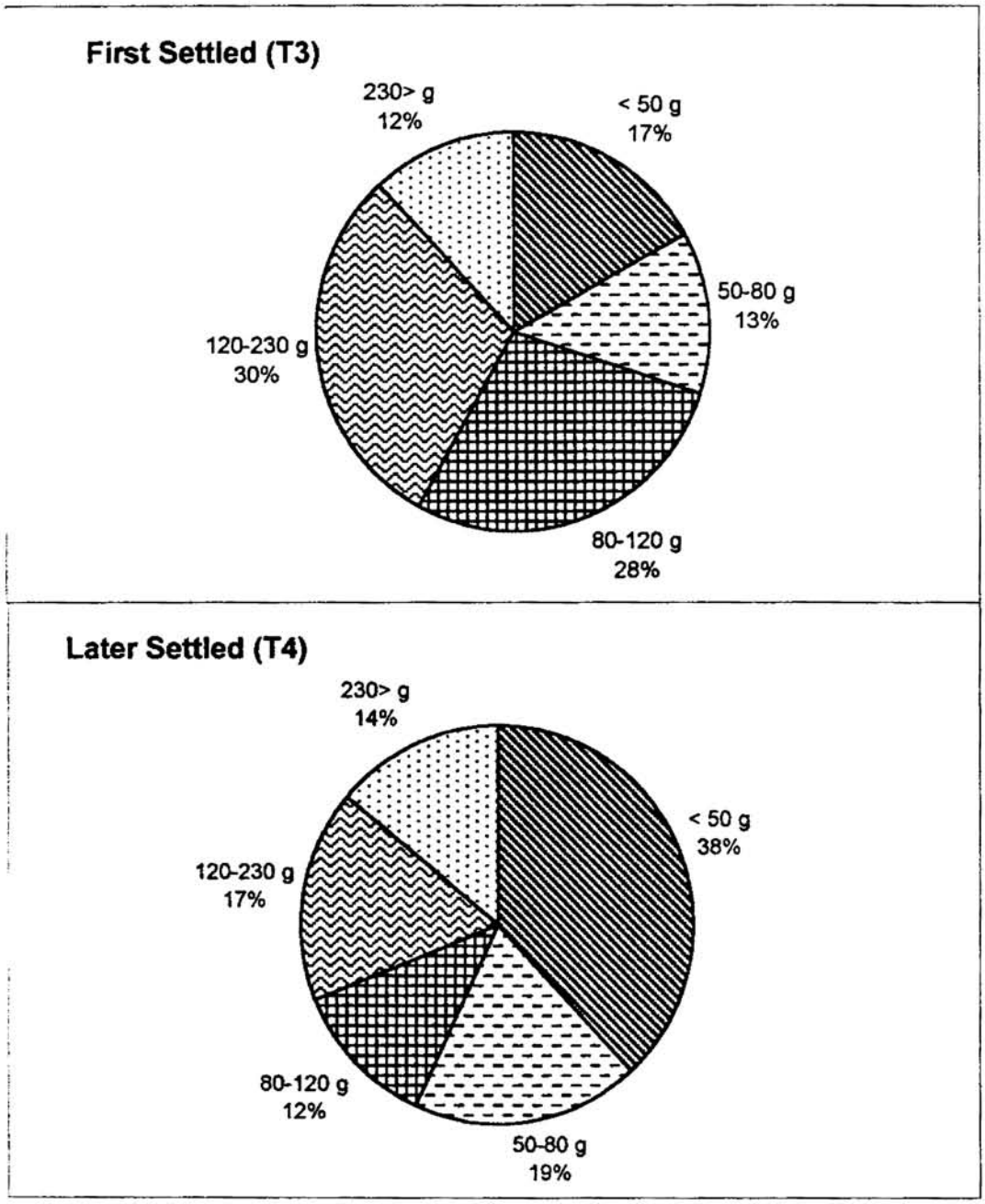
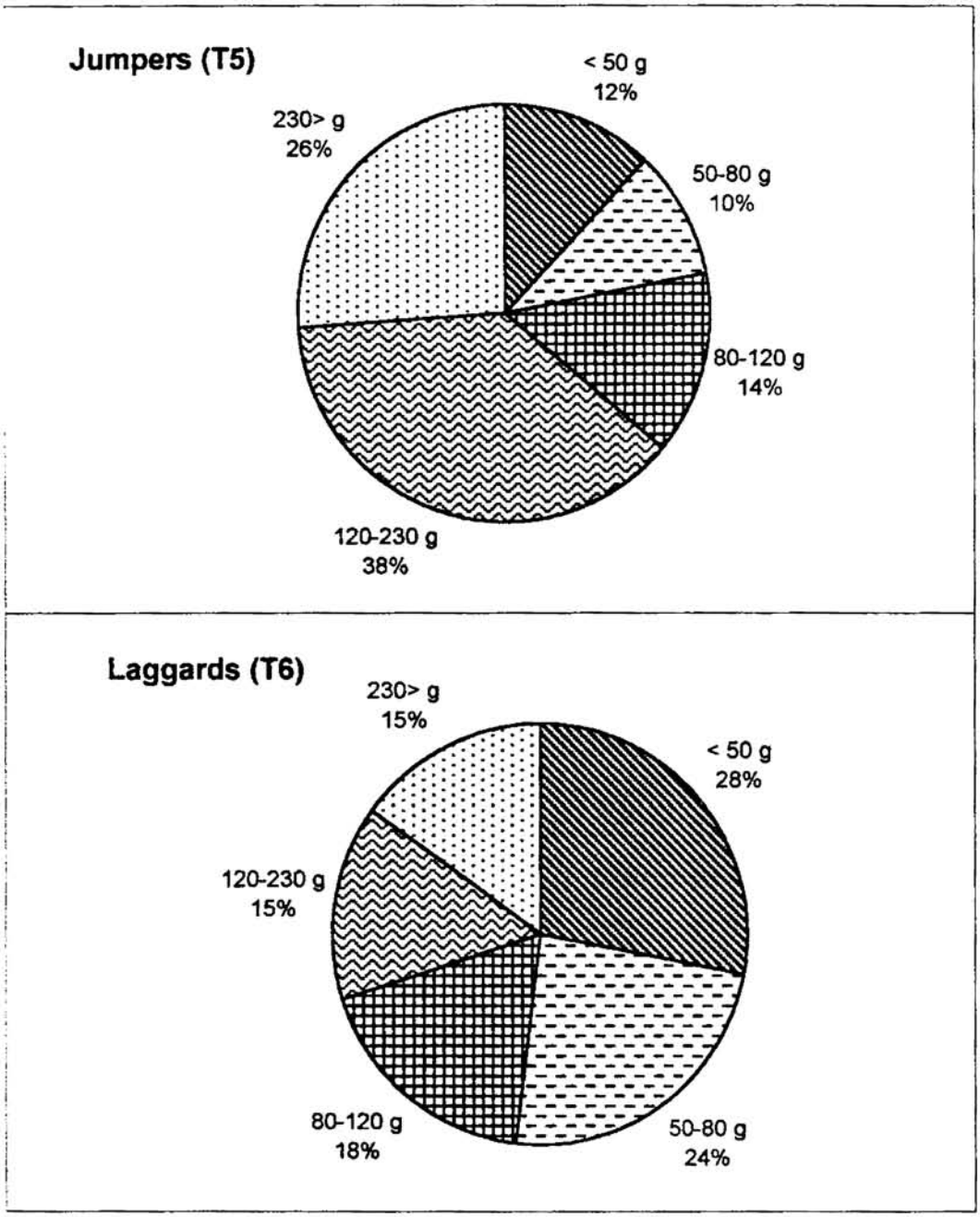


Fig 9.6. Marketable yield structure of harvested population of *M. rosenbergii* in batch graded post larvae



CHAPTER 10

Effect of added substrates on the production and population characteristics of *Macrobrachium rosenbergii*

1. Introduction:

Macrobrachium rosenbergii (de Man), the giant freshwater prawn with the trade name "Scampi" is widely accepted as the most suitable cultivable prawn among the freshwater prawn species. Development and sustenance of prawn farming based on *M. rosenbergii* on a commercial scale has now become widespread, especially in Southeast Asian countries. Availability of quality seeds of *M. rosenbergii* in required quantity from hatcheries triggered a further momentum in the farming of this species. Recent set backs experienced in the land based agriculture in Kerala owing to the prevailing socio-economic conditions have further prompted many farmers to switch over to the farming of 'scampi' in the derelicted water bodies and fallow polders by resorting to either monoculture or polyculture in combination with freshwater fishes. The intricate coconut garden channels and homestead ponds, which are available in plenty in Kerala, also provide impetus for the successful farming operations of scampi. However, the wide disparity in growth and resulting differential size structure among the single aged male population of *M. rosenbergii* act as the major bottleneck in its successful aquaculture. Males exist in three basic morphotypes (Cohen *et al.*, 1981; Kuris *et al.*, 1987) – viz., Small male (SM), Orange clawed male (OC) and Blue clawed male (BC). The harvested populations from the grow-outs show diverse weight distribution structure with the individual weight ranging from 5 to 250 gm. Preponderance of undersized small male prawns

makes the culture highly uneconomical, and therefore, it is not the total production that decides the economic viability of farming, but the marketable yield structure of the harvested population. Adding to this, the cannibalistic nature inherent with this species causes reduction in the survival rate significantly, thereby, affecting the net revenue from the culture.

Attempts were made to enhance the net yield and income from *M. rosenbergii* culture by resorting to various management strategies such as trials with different stocking densities, changes made effective in the duration of culture, providing nursery rearing, stocking of batch graded and size graded populations, etc. (D'Abramo *et al.*, 1989; Karplus *et al.*, 1986a, 1987; Smith *et al.*, 1981; Kurup *et al.*, 1996), however, the importance of additional shelters and added substrates were realized only very recently (Tidwell *et al.*, 1999). Tidwell *et al.* (1998a) studied the effect of artificial substrates on the ponds for increasing the production. However, very few attempts have been made to standardize the percentage of artificial substrates required for ensuring highest survival and growth of this species without compromising to the economic viability. In their natural habitat, the blue clawed males occupy a distinct territory without allowing the subordinate males to approach at its vicinity. This results in a struggle for space, which is clearly visible under higher stocking densities. The retrieval rates in these ponds differ significantly with a sizeable number of prawns being killed during their moulting periods thus reducing the survival percentage. Being primarily a benthic animal, prawns are constrained by the availability of two-dimensional space rather than three-dimensional volume. This limitation on production is exacerbated by the territorial nature of male prawns

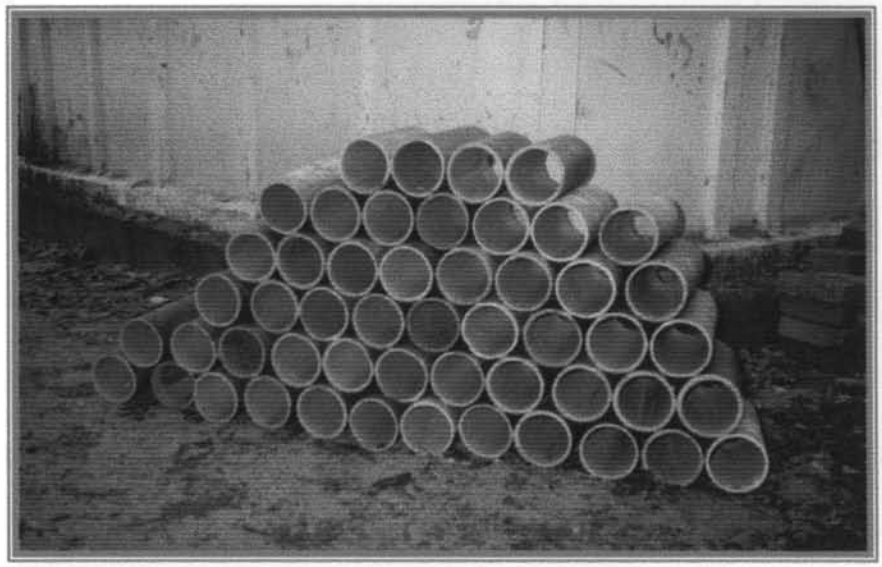
(Cohen *et al.*, 1981). Sandifer and Smith (1977) reported that addition of substrate in nursery tanks allowed prawns to utilize the entire water column and reduces mortality.

In the present study, an attempt was made to delineate the effect of different types of artificial shelters on the growth, morphotypic differentiation and transformation of *M. rosenbergii*. Effort was also made to bring out the dynamics of the interactions and transformation among the various morphotypes under varying proportions of additional substrates and this would contribute to the knowledge on social, ecological and management strategies on population dynamics in grow-outs. The effect of different types of artificial substrates on the survival rate, growth, marketable yield structure and gross economics from the grow-outs were evaluated. The standardisation of the type and percentage of artificial shelters aiming at stocking at higher densities would be helpful in improving the marketable yield structure, economics and consequently the revenue from the harvested population. An attempt was also made to optimise the percentage of additional substrate needed per m^2 of the tank for maximising production as well as marketable yield structure without affecting the capital investment.

2. Materials and Methods

Two sets of experiments were conducted in the present study. In the first set of trials, the standardization of the percentage of additional shelters in the form of broken pipes were attempted in the outdoor ferrocement tanks. Two tanks each with a dimension of 4m x 3m x 1m and three tanks of 5m

x 4m x 1m were used for the present study. Mud at 5 inches thickness was filled at the tank bottom. Two weeks prior to the anticipated date of stocking, the tanks were drained, allowed to dry and raked to remove organic debris (dried leaves and sludge). One week prior to stocking, tanks were filled with water and the average water depth in each tank was maintained at 1 m. Urea at a rate of 0.25kg / 10 tonnes of water was added. Organic fertiliser in the form of cow dung was also applied in desired intervals as per requirement in order to maintain the bloom. Water was exchanged on a weekly basis. The tanks were aerated at night using blowers of 2 HP capacity. Post larvae at PL 20 stage were stocked in all the five tanks at a stocking density of 8 nos./m^2. Four tanks were provided with additional substrates in the form of 6 inches broken earthen pipes of one feet length, while fifth tank was maintained as control tanks without providing any additional substrates. The pipes were so arranged that the first tank had an additional surface area of 10% more than the tank bottom surface area. In the same way, the subsequent tanks were provided with pipes that had additional area coverage in the order of 20, 30 and 40% respectively. On the day of stocking, the mean weight of the post larvae destined to be stocked was determined from a sample of 20 post larvae that were blotted free of water and were weighed individually. The prawns were fed with a 35% protein rich sinking pellet feed of 2-mm thickness prepared from locally available ingredients. The daily ration was assessed, based on the body weight of the prawns, which were collected in the check trays. One half of the daily ration was distributed over the entire surface area in the morning at 7 AM and the next half during night at 9 PM. Regular observations were made on physico-chemical parameters of the



A. Earthen pipes used as additional substrates in polders of Kuttanad

B. Earthen pipes provided as additional substrates in outdoor tank experiments

water (AOAC, 1965) at fortnightly intervals. Monitoring of the growth of prawns in the different tanks were done by observing the specimens collected from the check trays. The tanks were aerated with the help of a 2 HP blower during night. Water quality parameters such as dissolved oxygen, pH, temperature and transparency were observed daily following AOAC (1985), while Ammonia-nitrogen, nitrite-nitrogen and hydrogen sulphide were estimated on a weekly interval using Aquakit from MERCK. At the termination of the experiment after 4 months, the tanks were drained and the length and weight of individual prawns were recorded (Ra'anan, 1982). Data on growth, survival rates, yield characteristics and population structure of each tank was analysed (Harikrishnan and Kurup, 1997). The whole set of experiments were repeated twice for the same period again (triplicates) and the data generated from these were grouped in such a way that treatments containing 40% additional substrate was demarcated as treatment 1 (OT1) and subsequent treatments with 30%, 20%, 10% and no substrates were designated as OT2, OT3, OT4 and OT5 respectively.

Second sets of experiments were carried out in twelve separate polders of Kuttanad. The areas of these polders ranged from 0.4 to 2 ha. The data for the present study was computed from these 12 polders that were grouped under three separate treatments based on the type and availability of additional shelters. Each set of treatment represents data collected from four separate polders (quadruplicates). The first set of treatments (T1) represent data collected from polders without any additional shelters (control), while T2 and T3 represents data collected from polders having shelters in the form of earthen pipes and nylon nets respectively. After harvesting the 'Punja' paddy crop in the

month of February these polders were totally dried and scientifically prepared (Kurup *et al.*, 1996). Lime was applied @ 500 kg/ha and cow dung was applied for phased manuring @ 1000 kg/ha. Post larvae (PL-20) were stocked @ 35,000/ha in nine polders and @ 30,000/ha in rest three polders after a brief nursery period of one month. In these polders two different combinations of artificial substrates were tried in duplicates. T1 was devoid of any additional substrates and was maintained as the control treatment. In T2 artificial shelter in the form of broken earthen pipes @ 25% of the total pond surface area was provided, while in T3 nylon nets of 2 cm mesh size were erected in a two-tier form throughout the sides of the polder and were used as an alternative substrate. Substrates in the form of two horizontal layers or tiers of nylon mesh (2 m breadth and 60 m length) with corrugated bamboo poles (8 cm diameter, 1 m long) attached to them were tied with the help of nylon wires through out the entire stretch of the pond. The total added surface area supplied by the nets was 240 m^2, which works out to 25% of the total surface area. Prawns were fed with an on-farm made feed with 35-40% protein ratio @ 5% of the body weight on a daily basis in two installments, 40% in the early morning and 60% in the evening. Water was exchanged once in a week with the help of pumps. Water quality parameters such as temperature, dissolved oxygen, transparency, water and soil pH were monitored on a daily basis following AOAC (1985), while levels of total ammonia-nitrogen (TAN), nitrite-nitrogen and hydrogen sulphide in water samples collected from each pond at approximately 1300 h were determined on a fortnightly basis using the Aquakit from MERCK. Upon termination of the culture after seven months, the polders were harvested by

pumping it dry and the prawns were hand picked from the bottom. Random samples between 500-1000 prawns each from the six polders were examined on the day of harvest. All the prawns were sorted to sex and morphotype wise (Kuris *et al.* 1987 and measured up to nearest millimeter and weighed up to nearest gram. Statistical analysis for both the experiments were done using SPSS 7.5 and Excel 98 package for Windows and the results were statistically analysed following Snedecor and Cochran (1961). To test whether there is any difference in average weight gain, final mean weight and total biomass among the treatments, ANOVA was applied followed by *t*-test.

3. Results

3.1 Outdoor tank trials:

The details on yield characteristics at final harvest from the outdoor tanks for the five sets of treatments (OT-1 to OT-5) are shown in Tables 10.1, 10.3, 10.5, 10.7 and 10.9 respectively. Mean weight, survival rate and net production within each treatment did not show any significant difference ($P>0.05$). Mean weight ranged from 8.8 to 9.2g in OT-1, 9.8 to 13.2g in OT-2, 5.6 to 7.0g in OT-3, 6.1 to 7.0g in OT-4 and 3.9 to 5.2 in OT-5 respectively. Similarly survival rate and net production also reduced along with lowering of added shelter among the treatments. Survival rate, however, ranged from 71.21 to 82.58% in OT-1, 65.15 to 77.27% in OT-2, 46.36 to 56.82% in OT-3, 26.36 to 32.73% in OT-4 and 19.09 to 27.27% in OT-5 respectively. While the net production ranged between 628.3 and 732.05 Kg/ha in OT-1, 761.68 and 863.52 Kg/ha in OT-2, 275.4 and 354.2 Kg/ha in OT-3, 185.11 and 204.2 Kg/ha in OT-4 and 91.8 and 127.9 Kg/ha in OT-5 respectively. Comparison of mean weight of

morphotypes for the five sets of treatments (OT-1 to OT-5) is given in Table 10.2, 10.4, 10.6, 10.8 and 10.10. F value shows that the mean weight within the treatment (OT1 to OT5) did not vary significantly (P>0.05).

No significant differences (P > 0.05) could be recorded in the water quality parameters in any of the twelve tanks studied. Table 10.11 shows the mean values observed for different water quality parameters and the range within each treatment. Surface water temperature among the treatments ranged between 26.1-32.5°C (OT1), 27.2-31.8°C (OT2), 24.7-32.8°C (OT3), 26.1-32.2°C (OT4) and 28.6-33.1°C (OT5). Mean values for dissolved oxygen ranged between 4.2 mg/l in OT3 to 5.6 mg/l in OT5. Total alkalinity ranged between 20.5 mg/l in OT2 to 38.4 mg/l in OT3. Water pH in all the treatments was in the range of 6.2-8.1. In all the treatments, total ammonia-nitrogen recorded below 0.1 mg/l and nitrite-nitrogen observed below 0.2 mg/l.

Details of population density at stocking and harvesting, mean weight of the prawns at various levels of final density, percentage of survival and net production in the four tanks are given in Table 10.12. The results of the present study clearly showed that the addition of substrates have a direct bearing on the improving the survival rate. Highest percentage of retrieval rate was encountered from OT-2 (75.75%) and OT-1 (71.21%), wherein an additional substrate @ 30 and 40% respectively were provided. In total contrast, the least survival rates were observed in OT-4 (29.09%), which was provided with an additional substrate level at 10%, and OT-5 (23.63%) in which no substrate was provided. The mean weight of prawns in the outdoor trials were far below when

compared to grow-outs and ranged from 4.712 g in OT-5 to 10.487 g in OT-1. Production was highest in OT-1 (821.48 kg/ha) while least was observed in OT-5 (111.37 kg/ha). The results of ANOVA on net production from the fifteen tanks (Table 10.13) showed a significant difference in production (F= 279.266, $P<0.01$) within the treatments, showing that the incorporation of additional substrates was useful in the improvement of net production in the treatments. The pair wise comparison of the performance of the 5 sets of treatments at different levels of additional substrates is given in Table 10.14. There was significant difference in the production from the five sets of treatments studied. From the results of t-test it could be understood that the difference in production from OT-1 and OT-2 is significant only at 5% level. Interestingly an increment in production by over 400% could be registered between the tanks which were provided with least and maximum substrates respectively (OT-5 and OT-1). The structure of male, female and juvenile prawn populations under the four separate treatments are given in Table 10.15. Majority of the morphotypes were seen in OT-1, while in the successive tanks the presence of intermediate morphotypes were low, whereas least differentiation could be seen in OT-5. Except in OT-2, immature juveniles that contributed substantially in weight to the total production dominated all the other treatments. The population structure of the harvested population in the five treatments of outdoor tanks is depicted in Fig 10.1 to 10.5. The results showed a clear predominance of juvenile population in all the five treatments, although their representation did not follow any particular trend. Another noteworthy feature was the presence of fast growing orange-clawed males in the population. The highest percentage of OC males was

encountered in OT-2 (13.5%) followed by OT-1 (11.5%) and OT-3 (10.3%) respectively. OT-4 was totally devoid of any OC male morphotypes. It would thus appear that a direct relationship exists between the percentage contribution of OC males and percentage of substrate, however, no such trend could be discernible with the percentage of juveniles in the final harvested population.

3.2 Grow-out trials:

3.2.1 Population structure:

Results of the initial stocking and corresponding yield characteristics at final harvest from Treatment 1 (Polders) without any additional substrate are shown in Table 10.16. None of the parameters evaluated showed any significant variation among the three sets of polders studied. Low values in net production, which ranged from 118.2 to 152.6 Kg/ha, survival rate (26.1% to 28.2%) and mean weight for prawns (34.5 g to 42.1 g) were registered in T1. Females were the dominant group during the final harvest in all the polders. Results of statistical comparison of mean weight of morphotypes in three channels of T1 are given in Table 10.17. The result of F value suggests that the mean weight within the treatment (T1) did not vary significantly ($P>0.05$).

Stocking details and yield characteristics of polders that were provided with additional shelter in the form of earthen pipes (Treatment 2) are given in Table 10.18. No significant difference in the mean weight of prawns could be noted within the three polders. Net production ranged between 435.1 to 508.5 Kg/ha, while survival ranged from 45.2 and 49.7%. Mean weights of prawns, however, were comparatively better than that of T1 and ranged between 42.1 and 59.5 g respectively. The males dominated the final population and

comparison of mean weight of morphotypes among the three polders (T2) by applying ANOVA is shown in Table 10.19. The results revealed that the mean weight for morphotypes within the treatment (T2) did not vary significantly ($P>0.05$).

Details on the initial stocking and corresponding yield characteristics of *M. rosenbergii* from polders containing additional shelter in the form of tiers of nylon nettings (Treatment 3) are shown in Table 10.20. Significantly higher values for net production and survival rates were recorded from these polders, which ranged from 565.2 to 752.5 Kg/ha and 55.16% and 61.8% respectively. In all the polders, males dominated the final population and as a result the mean weight of prawns from these polders were also high ranging between 49.5 g and 62.1 g respectively. Comparison of mean weight of morphotypes among the three polders (T3) by applying ANOVA is shown in Table 10.21. The results revealed that the mean weight for morphotypes within the treatments (T3) did not vary significantly ($P>0.05$).

No significant difference ($P>0.05$) in water quality parameters was found among the treatments. Mean values calculated for different water quality parameters and the range is shown in Table 10.22. Surface water temperature among the treatments ranged between 27.1-33.2°C (T1), 26.5-34.2°C (T2) and 25.8-34.5°C (T3). Mean values for dissolved oxygen ranged between 4.82 mg/l in T2 to 6.25 mg/l in T3. Total alkalinity ranged between 48.61 mg/l in T2 to 75.58 mg/l in T3. Water pH was in the range of 6-8.5 and soil pH between 5-6.5. In all the treatments, the total ammonia-nitrogen recorded below 0.1 mg/l and nitrite-nitrogen observed below 0.2 mg/l.

Details regarding the stocking and yield characteristics of prawns during the final harvest in the three treatments are given in Table 11.23. Difference in the survival rate, mean weight of prawns and net production in all the three treatments studied could be observed. Polders provided with tiers of net as additional substrate showed better survival rate (58.86%), higher mean weight for prawns (68.6 g) and enhanced net yield (605.5 kg/ha). Results of ANOVA on the net production from the three different treatments is given in Table 10.2 and it shows that incorporation of additional substrates was found useful in increasing the net production within the treatment (F= 311.132, P<0.01). On further examination of the production performance between treatments following pair wise analysis (t- test), it could be seen that there was significant difference (P<0.01) in the net production between T1 and T2 and T1 and T3 was discernible, while comparison between T2 and T3 showed only significance at 5% level. The structure of male and female morphotypes in the final harvested population is depicted in Table 10.26. The percentage contribution by weight of various male and female morphotypes to the respective harvested yield from the three treatments is given in Table 10.26. Mean weight of individual morphotypes increased with the incorporation of additional substrates. Variation was highly perceptible in the mean weight of larger male morphotypes (SBC and OBC), which increased from 91.54 g and 122.03 g in T1 to 172.66 g and 173.51 g in T3 respectively. The percentage contribution of BC morphotypes among the three treatments was recorded in the order of 44.54% in T1, 76.58% and 52.75% in T2 and T3 respectively. In total contrast to this, the representation of SOC and its intermediate morphotypes, WOC and t-SOC showed an inverse trend with higher

values in T1 (29.93%) that reduced to 17.92 and 14.12% in T2 and T3 respectively. Figs 10.6 to 10.8 represent the morphotypic composition of males of *M. rosenbergii* in the three treatments. In T1 OC (44%) was the dominant morphotype, while in T2 and T3 the BC showed predominance (60% and 56% respectively). From the results of population structure it is clearly evident that the undersized male morphotypes such as SM and WOC were more in T1 (18%). In total contrast, the proportion of larger BC males were higher in T2 and T3 that were provided with 25% additional substrate in the form of broken pipes or tiers of nets, whereas the same in T1 was only 41%. The results are complimentary to the earlier findings from the outdoor tank experiments showing an inverse relation between the proportion of SM and WOC with decrease in the percentage of additional substrates. It would thus appear that addition of substrates was utmost helpful in increasing the frequency of terminal BC morphotypes. In females, variations in the morphotypic composition of harvested population were discernible and was almost identical to their male counterparts. The morphotypic composition of females in the three treatments is depicted in Fig 10.9 to 10.11. It could be seen that in T2 and T3, the percentage of SOF and its advanced stages viz. TOF, WBF and SBF accounted for 81 and 83% respectively. The representation of lower morphotypes such as SF and WOF in T1 was 22%, when compared to 19 and 17% recorded in T2 and T3 respectively.

3.2.2 Yield characteristics

Stocking densities and yield characteristics of *M. rosenbergii* reared in three treatments are given in Table 10.23. In T1 and T2, the stocking was done @ 3.5/ m^2, while in T3 it was 3/ m^2. However, a remarkable difference

could be noticed at the time of harvest, especially in T2 and T3. The retrieval varied from 26.84 in T1 to 58.78 % in T3. The net production varied from 111.62 to 605.56 Kg/ha/7 months, the lowest being in T1 while T3 recorded the highest. Effect of additional substrate on the mean weight of the population could also be observed in the three treatments, showing a highest mean weight of 58.78 g in T3 against 38.46 g registered in T1. Mean weight, standard deviation, coefficient of variance and skewness in respect to various male and female morphotypes are given in Table 10.26. Invariably, the mean weight of all male and female morphotypes was comparatively higher in T2 and T3 that were provided with additional substrates. In order to test the differences, if any, in the weight attained by various morphotypes of *M. rosenbergii* and their yield from the three treatments studied, the data was statistically analysed using one-way ANOVA. The results revealed that there was no significant difference existing between treatments ($F= 1.7789$), however, the weight attained by various morphotypes showed significant difference ($F= 39.836$, $P< 0.01$). Table 10.27 shows the result of one-way ANOVA of various morphotypes under different levels of management. Significant difference ($P<0.01$) in the F- values was recorded among larger morphotypes such as TOF, t-SOC, WBC, SBC and OBC showing the diverse nature of their representation by weight in the three sets of treatments studied. A significant difference at 0.05% level ($P<0.05$) was also seen in smaller morphotypes such as SF, WBF and WOC. Nevertheless, the non-significance of SM, SBF and SOC by weight in any of the three sets of polders was noteworthy. From the "t" test it could be seen that the mean weight attained by male and female morphotypes differed significantly between each other ($t= 1.84$). The

result of pair wise analysis of the different morphotypes under three different sets of management level is shown in Table 10.28. From the results of 't-test,' it was obvious that the mean weight attained by males and female morphotypes among the three treatments differed significantly ($P<0.05$). The difference was more pronounced between treatments without any additional substrates (T1) and those provided with earthen pipes or net tiers as additional substrates (T2 and T3 respectively). However, results of pair-wise analysis on the mean weight of prawns between the treatments having additional substrates did not show any significant differences ($P>0.05$).

The weight distribution pattern and marketable yield structure of prawns in the three treatments at different levels of artificial substrates are depicted in Fig 10.12 to 10.14. The predominance of undersized prawns (< 50g) was remarkably high in T1 (26%), which showed a significant reduction in the treatments with additional substrates and was in the range of 11 and 14% in T2 and T3 respectively. The dominance of weight group 120 – 230 g and >230 g was very apparent in T2 and T3. Whereas, in T1, the weight distribution of the total population was found to be influenced by the intermediary morphotypes and female population as a result of which 60-80 g group showed a distinct predominance. The price packages offered by the seafood processing plants located at Cochin for *M. rosenbergii* per Kg were as those discussed in Chapter 7. In this respect, the percentage of highly profitable weight group i.e., > 120 gm was less in T1 (24%), while, in contrast, the treatments provided with 25% of additional shelters showed a better performance with 44% in T2 and 41% in T3. Of the total biomass produced from the three treatments, 89 and 86% of the yield

from T2 and T3 belonged to > 50 g group and therefore, was marketable. While, only 74% was marketable in T1. While computing the revenue of the three treatments, a total income of Rs. 19,966, Rs. 91,520/- and Rs. 1,19,678/- could be worked out in T1, T2 and T3 respectively. It may, therefore be seen that by providing additional substrate in the form of nets, the net revenue could be increased by about 500%. The slight difference in the density between T2 and T3 did not affect the production adversely and the net revenue from these two treatments was almost similar.

4. Discussion

The economic success of prawn production in any country depends on: (a) improvement in the production of prawn characterized by an increased growth potential at a minimal cost (Ra'anan *et al.*, 1984); and (b) the improvement of pond management procedure during the grow-out stage, aimed at increasing both growth rates and chances of survival. In respect of addressing the second issue the present work was attempted with a basic assumption that by increasing the available submerged surface area and by supplying prawns with hiding places, the antagonistic behavior inherent to *M. rosenbergii* can be reduced, thereby reducing growth inhibition and improving survival. With this objective, an attempt was made to study the response of prawns stocked at a fixed density to varying amounts of substrates. The mean retrieval rate, mean weight of prawns, net production and marketable yield structure of the population was found to be governed much by the artificial substrates provided to each treatment. The results of the outdoor tank experiments revealed that an additional substrate level @ 30% was useful to increase the production by over

400%. Moreover, 30% additional substrate was found to be more profitable than to 40% substrate level, since the difference of 10% of substrate could not contribute significantly to the net production, therefore, it can well be concluded that inclusion of 30% substrate is more economically viable in the farming of *M. rosenbergii*. Results obtained from ferro-cement tanks in the present study are comparable with the findings of Evans (1975) and Peebles (1979) in earthen tanks under high density. The occurrence of large number of adult morphotypes in Tanks 1 and 2 which had an additional substrate level of 40 and 30% respectively, indicate that the conditions in these tanks were most conducive for the prawn to undergo further transformation, On the other hand, tanks provided with least level of substrates (Tank 1) showed an overall dominance of juvenile prawns, and this may be due to the effect of complex social hierarchy prevailing in this tank (Cohen and Ra'anan, 1985). The competition for food and space in Tanks 4 and 5 was reduced considerably with the introduction of added substrates, which ultimately resulted in better mean weight for individual prawn, greater survival rate and in turn high net yield.

As observed in outdoor tank trial, result also advocates the necessity of added substrates in improving the yield and income in the culture of *M. rosenbergii*. A dynamic shift in the proportion of advanced male and female morphotypes with difference in the quantity of shelters could also be discernible. In T2 and T3 which were provided with an additional shelter cover of 25% in the form of broken pipes and nylon nets respectively, relatively high percentage of OBC and its transitional morphotypes were seen and this would indicate the fact that morphotypic transformation in these treatments were comparatively faster

when compared to the one without any substrate (T1), the latter was characterized by the presence of undersized non-marketable male morphotypes such as SM and WOC in appreciable numbers. The difference in the transformation pattern as manifested by the percentage of male morphotypes in these treatments may be attributed to a complex social organizational hierarchy shown by *M. rosenbergii*. By providing an additional substrate level of 25% the density is reduced considerably. At relatively higher density (T1) the percentage of SM and WOC were very high and this would suggest the chances of inhibition of growth of SM by BC due to the proximity of the latter. BC represents the final morphotypic stage of the developmental pathway, originated from the transformation of OC which originated from SM. Hence, an irreversible morphotypic transformation pathway of SM to OC to BC occurs (Cohen *et al.*, 1981; Sagi and Ra'anan, 1988). The predominance of intermediary morphotypes in the T1 in the present study well corroborated with the findings of Tidwell *et al.* (1999) who reported that at higher density the proportion of OC males in the final population increased significantly. Brody *et al.* (1980) and Cohen *et al.* (1981) reported that the relative proportion of the three male morphotypes SM, OC and BC remain nearly constant at 5:4:1. However, the present finding is totally in totally at variance with the above findings since the ratio was found to be ranging from 1: 2.9: 2.7 (T1) to 1: 3.4: 6.6 (T2). In ponds without substrate the number of small males (SM) was significantly higher while, Blue clawed males (BC) showed an increase at significant levels in ponds with added substrates.

In general, substrate may improve yield either by resolving growth inhibition, which is apparent when prawns are in close proximity with each other, or by reducing the mortality rates by providing hiding places to newly molted and small individuals. The present findings show a very strong agreement with that of Tidwell *et al.* (1998 a and b, 1999). Cohen *et al.* (1983) reported that adding substrate to ponds allowed for an increase in prawn production of 14%, while Tidwell *et al.* (1998a) assessed the effect of added substrate under temperate conditions and reported that in prawns stocked at relatively low densities, production and average size were increased @ 20 and 23% respectively. Ra'anan *et al.* (1984) reported that substrate was more effective in intensely stocked systems. However, Tidwell *et al.* (1999) added fixed amount of substrate to ponds stocked at different densities and found no significant interactions between stocking density and presence of substrate, though substrate was helpful in increasing production significantly without decreasing the average weight. *M. rosenbergii* is primarily a benthic organism which spends very little time in the water column (Peebles, 1979), thus, the actual density of a prawn population is governed by the available surface area (Evans, 1975). Density becomes a major limiting factor in prawn production mainly due to the territorial nature of the dominant males, which appear at a relatively early stage of the population development (Cohen *et al.*, 1982). The presence of these large dominant males inflicts a growth suppressive effect on the other individuals in their proximity (Ra'anan, 1982). These large males are occupying the terminal stage with respect to further growth (Ra'anan, 1982). Providing a niche in the

form of additional shelters to these dominant males would directly reduce their free territory, which would thus indirectly provide other submissive prawns a better environment to undergo further transformation and growth as they are getting free from social hierarchy. The difference in the growth and population structures of prawns stocked at the same density, with or without substrate, would seem to support the theory that it is primarily the two-dimensional space available to each prawn which limits growth and influences morphotype ratios (Karplus *et al.*, 1986a; Tidwell *et al.*, 1998a).

The yield of *M. rosenbergii* obtained from T2 and T3 provided with shelters was found comparable with results of Ra'anan *et al.* (1984) who found an increase in the marketable prawn number @ 17.7% in ponds which were provided with substrates. Although the survival rate registered in the present study were slightly less than that observed by Ra'anan *et al.* (1985) the net production did not vary considerably. This may be due to the fact that average individual weight of prawn in the present study was higher to earlier reports and also that proportion of BC (56%) males largely contributed to the slight increase in the net production. Based on the results of the present study it can be asserted that tier system is more beneficial as an additional substrate both from production as well as economic points of view. Moreover, nets are easy for replacement, which can be removed with least labor when compared to the earthen pipes. The results of t-test also support the fact that the net production from T3 was more than that of T2. Increase in the mean weight of prawns with increase in substrate level as observed in the present study show disagreement

with that of Tidwell *et al.* (2000), who reported that no consistent impact ($P>0.05$) on average weight or number within each individual male morphotypes would be possible. In the present study, a steady increase in the mean weight of prawns was noticed for both male and female morphotypes with the inclusion of additional substrates. Ra'anan *et al.* (1985) could achieve a production of 2,879 kg/ha and 2,064 kg/ ha in *M. rosenbergii* during their trials with added substrates, which was on a higher side when compared to the present findings. However, a direct comparison of the results of the present study with the above may not hold good in view of the fact that the above production figures are extrapolated ones based on experimental culture undertaken in 0.1 – 0.2 ha. But comparable results were achieved in yield characteristics, which were 24% higher in ponds with substrates. When compared to those without substrates. Furthermore, the percentage of marketable prawns was also found to be 17.7% higher in treatment with substrates. The better yield characteristics envisaged in the present study may be due to the better utilization of feed as reported by Tidwell *et al.* (2000). In summary, addition of increasing percentage of artificial substrates in grow-outs results in linear increase in total production without the normal decrease in average weights. The results of outdoor tank experiments show that increasing of an additional substrate by 30% would definitely be helpful in increasing the survival rate, mean weight and net production in the tanks. Performance of nylon nets provided in the form of tiers gave promising results as substrates in terms of net yield and percentage of marketable prawn groups besides having the advantage of easy handling at the time of harvest. Therefore, information on the effect of additional substrate, type of additional

substrate, influence of various types of additional substrates, their percentage requirements etc. will certainly influence on the dynamics of male and female morphotypes in a population, these information are of paramount importance in improving the economic yield from the farming of *M. rosenbergii*.

Table 10.1 Stocking details and yield characteristics of post larvae reared in tanks containing 40% additional substrates (Treatment 1)

	TANKS			
	1	2	3	Mean values
Capacity (tonnes)	12	12	12	12
Percentage shelter provided	30	30	30	30
Duration of culture (days)	150	150	150	150
Initial stocking density(PL/m2)	10	10	10	10
No.of post larvae stocked	132	132	132	132
No.of prawns harvested	94	109	96	100
Survial rate (%)	71.21	82.58	72.73	75.75
Net production (Kg/ha)	628.38	732.05	672.09	675.25
Average weight of prawns (g)	8.824	8.8652	9.2413	8.103

Table 10.2 Comparison of mean weight of morphotypes in three tanks of Treatment 1

Morphotypes	F-value
Juveniles	0.5714
SF	0.2838
WOF	0.2402
SOF	-
TOF	0.6238
WBF	1.0507
SBF	0.9801
SM	1.2838
WOC	1.613
SOC	0.8727
t-SOC	0.6772
WBC	0.5735
SBC	1.0512
OBC	0.2508

SF = Small female
WOF= Weak orange clawed female
SOF = Strong orange clawed female
TOF = Transforming strong orange clawed female
WBF= Weak blue clawed female
SBF = Strong blue clawed female
SM = Small male
WOC = Weak orange clawed male
SOC = Strong orange clawed male
t-SOC = Transforming strong orange clawed male
WBC = Weak blue clawed male
SBC = Strong blue clawed male
OBC = Old blue clawed male

P>0.05 (Non significant)
F-value non-significant among tanks for morphotypes

Table 10.3 Stocking details and yield characteristics of post larvae reared in tanks containing 30% additional substrates (Treatment 2)

	TANKS			
	1	2	3	Mean values
Capacity (tonnes)	12	12	12	12
Percentage shelter provided	40	40	40	40
Duration of culture (days)	150	150	150	150
Initial stocking density(PL/m2)	10	10	10	10
No.of post larvae stocked	132	132	132	132
No.of prawns harvested	102	86	92	94
Survial rate (%)	77.27	65.15	69.70	71.21
Net production (Kg/ha)	761.68	863.52	804.44	821.48
Average weight of prawns (g)	9.857	13.254	11.542	10.487

Table 10.4 Comparison of mean weight of morphotypes in three tanks of Treatment 2

Morphotypes	F-value
Juveniles	0.6628
SF	0.3292
WOF	-
SOF	-
TOF	0.7236
WBF	-
SBF	1.1369
SM	1.4892
WOC	1.8711
SOC	-
t-SOC	0.7856
WBC	0.6653
SBC	1.2194
OBC	-

P>0.05 (Non significant)
F-value non-significant among tanks for morphotypes
For expansion of morphotypes refer Table 10.1

Table 10.5 Stocking details and yield characteristics of post larvae reared in tanks containing 20% additional substrates (Treatment 3)

	TANKS			
	1	2	3	Mean values
Capacity (tonnes)	20	20	20	20
Percentage shelter provided	20	20	20	20
Duration of culture (days)	150	150	150	150
Initial stocking density(PL/m2)	10	10	10	10
No.of post larvae stocked	220	220	220	220
No.of prawns harvested	102	111	125	118
Survial rate (%)	46.36	50.45	56.82	53.63
Net production (Kg/ha)	275.49	354.26	322.86	317.28
Average weight of prawns (g)	5.942	7.0214	5.6824	5.916

Table 10.6 Comparison of mean weight of morphotypes in three tanks of Treatment 3

Morphotypes	F-value
Juveniles	1.4050
SF	1.1420
WOF	1.1467
SOF	-
TOF	-
WBF	1.6421
SBF	-
SM	1.4071
WOC	2.0037
SOC	-
t-SOC	-
WBC	1.3699
SBC	1.6590
OBC	-

P>0.05 (Non significant)
F-value non-significant among tanks for morphotypes
For expansion of morphotypes refer Table 10.1

Table 10.7 Stocking details and yield characteristics of post larvae reared in tanks containing 10% additional substrates (Treatment 4)

	TANKS			
	1	2	3	Mean values
Capacity (tonnes)	20	20	20	20
Percentage shelter provided	10	20	20	10
Duration of culture (days)	150	150	150	150
Initial stocking density(PL/m2)	10	10	10	10
No.of post larvae stocked	220	220	220	220
No.of prawns harvested	66	58	72	64
Survial rate (%)	30.00	26.36	32.73	29.09
Net production (Kg/ha)	184.35	185.11	204.25	189.31
Average weight of prawns (g)	6.145	7.0214	6.241	6.359

Table 10.8 Comparison of mean weight of morphotypes in three tanks of Treatment 4

Morphotypes	F-value
Juveniles	0.8275
SF	0.5521
WOF	-
SOF	-
TOF	0.8109
WBF	-
SBF	-
SM	1.1847
WOC	-
SOC	-
t-SOC	-
WBC	-
SBC	-
OBC	-

P>0.05 (Non significant)
F-value non-significant among tanks for morphotypes
For expansion of morphotypes refer Table 10.1

Table 10.9 Stocking details and yield characteristics of post larvae reared in tanks containing no additional substrates (Treatment 5)

	TANKS			
	1	2	3	Mean values
Capacity (tonnes)	20	20	20	20
Percentage shelter provided	0	20	20	0
Duration of culture (days)	150	150	150	150
Initial stocking density (PL/m2)	10	10	10	10
No.of post larvae stocked	220	220	220	220
No.of prawns harvested	42	60	54	52
Survial rate (%)	19.09	27.27	24.55	23.63
Net production (Kg/ha)	91.87	108.93	127.97	111.37
Average weight of prawns (g)	4.812	3.9942	5.2136	4.712

Table 10.10 Comparison of mean weight of morphotypes in three tanks of Treatment 5

Morphotypes	F-value
Juveniles	1.0835
SF	0.8205
WOF	-
SOF	-
TOF	0.8829
WBF	1.3206
SBF	-
SM	1.0856
WOC	-
SOC	-
t-SOC	-
WBC	-
SBC	-
OBC	-

P>0.05 (Non significant)
F-value non-significant among tanks for morphotypes
For expansion of morphotypes refer Table 10.1

Table 10.11 Summary of fortnightly water quality parameters in treatments 1-5 of outdoor tanks

Parameters	Treatment 1 (OT1)	Treatment 2 (OT2)	Treatment 3 (OT3)	Treatment 4 (OT4)	Treatment 5 (OT5)
Surface Temperature	29.7	29.1	28.4	29.3	31.4
(deg C)	(26.1 - 32.5)	(27.2 - 31.8)	(24.7 - 32.8)	(26.1 - 32.2)	(28.6 - 33.1)
Surface Dissolved Oxygen	5.4	5.4	4.2	5.5	5.6
(mg/l)	(4.4 - 6.3)	(4.2 - 6.0)	(3.0 - 6.2)	(2.8 - 5.8)	(2.3 - 7.1)
Water pH range	6.6 - 7.2	6.8 - 7.2	6.4 - 7.8	6.2 - 7.2	6.4 - 8.1
Transparancy	11.3	10.5	12.4	18.6	12.5
(cm)	(15 - 25)	(12 - 28)	(8 - 35)	(10 - 38)	(15 - 38)
Total Alkalinity	23.2	20.5	38.4	22.14	36.74
(mg/l)	(18 - 34)	(20 - 39)	(12 - 47)	(25 - 42)	(26 - 54)
Nitrite-N	0.8	0.23	0.1	0.1	0.1
(mg/l)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)	(0.01 - 0.2)
Total Ammonia	0.02	0.07	0.1	0.05	0.05
(mg/l)	(0 - 0.08)	(0 - 0.12)	(0 - 0.2)	(0 - 0.1)	(0 - 0.1)
Hydrogen sulphide	0.01	0.01	0.01	0.01	0.01
(mg/l)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)	(0 - 0.02)

Figures are means of three replicates and 11 sampling dates (N = 33). The range of observed values is given in parentheses

Table 10.12 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared under different percentage of additional substrates

Treatments	OT1	OT2	OT3	OT4	OT5
Capacity (tonnes)	12	12	20	20	20
Percentage shelter provided	40	30	20	10	0
Duration of culture (days)	150	150	150	150	150
Initial stocking density(PL/m2)	10	10	10	10	10
No.of post larvae stocked	132	132	220	220	220
No.of post larvae harvested	94	100	118	64	52
Survial rate (%)	71.21	75.75	53.63	29.09	23.63
Net production (Kg/ha)	821.48	675.25	317.28	189.31	111.37
Average weight of prawns (g)	10.487	8.103	5.916	6.359	4.712

OT = Outdoor treatments (refer Table 10.11)

Table 10.13. Analysis of Variance on the net production from the five treatments

ANOVA

Source of Variation	Sum of Squares	df	Mean sum of Squares	Calculated F
Between Groups	925569.2	4	231392.3	279.2665*
Within Groups	5800	10	828.57	
Total	931369.2	14		

Table 10.14 t-Test comparison on the net production between the treatments

Net Production	Treatment 1 vs 2	Treatment 1 vs 3	Treatment 1 vs 4	Treatment 1 vs 5	Treatment 2 vs 3	Treatment 2 vs 4	Treatment 2 vs 5	Treatment 3 vs 4	Treatment 3 vs 5	Treatment 4 vs 5
t- value	5.1700**	15.779*	22.3505*	28.3956*	10.609*	17.1805*	22.707*	6.5714**	10.6234*	3.1385**

* Significant at 1% level (P<0.01)
** Significant at 5% level (P<0.05)

Table 10.15 Percentage contribution of Juvenile, male and female morphotypes by weight in the seven treatments

Treatment		Mean weight of *M. rosenbergii* from the tanks											
		Juveniles	SM	WOC	SOC	t-SOC	OBC	SF	WOF	SOF	TOF	WBF	SBF
	Number sampled	161	12	18	11	11	11	12	12	0	17	12	0
	% by weight	16.03	2.02	15.81	6.67	11.83	13.77	1.83	12.05	0.00	14.74	5.27	0.00
	Mean weight	2.44	9.37	18.37	62.38	83.78	128	5.39	24.41	-	28.48	54.83	-
1	Standard deviation	1.75	0.26	7.75	-	-	-	0.57	12.15	-	12.86	6.26	-
(n=3)	Coeff. of variation	71.57	2.86	42.17	-	-	-	10.75	49.78	-	45.16	11.42	-
	Number sampled	142	14	19	0	11	10	13	2	0	13	6	0
	% by weight	8	4.36	7.75	0.00	12.92	19.45	3.32	12.06	0.00	16	16.12	0.00
	Mean weight	1.53	8.86	17.79	-	105	158	9.06	12.45	-	21.64	21.79	-
2	Standard deviation	1	3.91	9.38	-	-	-	5.06	3.54	-	7.83	2.17	-
(n=3)	Coeff. of variation	65.66	44.11	52.77	-	-	-	55.9	45.34	-	36.22	9.95	-
	Number sampled	141	14	12	4	1	0	32	14	1	11	0	0
	% by weight	22.25	6.82	5.04	24.17	3.48	0.00	19.47	10.78	5.04	2.96	0.00	0.00
	Mean weight	3.13	9.8	14.52	34.77	20.48	-	9.32	15.47	29.28	17.42	-	-
3	Standard deviation	2	3.49	0.19	4.7	-	-	2.31	2.63	-	-	-	-
(n=3)	Coeff. of variation	64.03	35.65	1.36	13.52	-	-	23.94	17.04	-	-	-	-
	Number sampled	44	13	0	0	0	0	17	1	0	14	5	0
	% by weight	39.93	5.78	0.00	0.00	0.00	0.00	14.54	2.33	0.00	19	18.42	0.00
	Mean weight	6.62	9.95	-	-	-	-	10.71	11.84	-	24.5	18.98	-
4	Standard deviation	30.79	7.71	-	-	-	-	4.39	-	-	6.11	12.22	-
(n=3)	Coeff. of variation	464.98	77.53	-	-	-	-	40.97	-	-	24.95	64.39	-
	Number sampled	138	5	1	0	0	0	18	1	0	12	0	0
	% by weight	56.41	4.92	1.37	0.00	0.00	0.00	19.68	1.87	0.00	15.75	0.00	0.00
5	Mean weight	6.95	12.72	16.21	-	-	-	14.27	18.15	-	21.59	-	-
(n=3)	Standard deviation	2.75	1.32	-	-	-	-	3.15	-	-	0.85	-	-
	Coeff. of variation	39.56	10.37	-	-	-	-	22.07	-	-	3.93	-	-

For expansion of morphotypes refer Table 10.1

Table 10.16 Stocking details and yield characteristics of *M. rosenbergii* from four polders without any additional substrate (Treatment 1)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35,000	35,000	35,000	35,000	35,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	0.95	0.95	0.93	0.91	0.94
Number per ha.	9538	9524	9384	9135	9394
Mean weight (g)	34.58	39.52	38.72	42.14	38.46
Gross production (Kg/ha)	145	121	130	156	125
Net production (Kg/ha)	142.68	118.24	127.23	152.65	111.62
Survival (%)	27.25	27.21	28.2	26.1	26.84
Mean male weight (g)	45.27	48.53	48.41	53.48	50.54
Mean female weight (g)	30.85	36.38	33.03	32.86	32.58
% by number of males in population	41.12	45.52	42.83	43.84	41.92
% by number of females in population	58.88	54.48	57.17	56.16	58.08
Sex ratio	1:1.43	1:1.19	1:1.33	1:1.28	1:1.38

Table 10.17 Comparison of mean weight of morphotypes in four polders of Treatment 1

Morphotypes	F-value
SF	2.0470
WOF	1.4094
SOF	1.8535
TOF	1.9165
WBF	1.3233
SBF	1.5166
SM	1.9076
WOC	1.1310
SOC	1.5790
t-SOC	1.8493
WBC	1.9846
SBC	1.5386
OBC	1.9839

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 10.1

Table 10.18 Stocking details and yield characteristics of *M. rosenbergii* from four polders with substrate in the form of earthen pipes (Treatment 2)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35000	35000	35000	35000	35000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.74	1.65	1.58	1.7	1.69
Number per ha.	17425	16548	15832	17025	16920
Mean weight (g)	42.25	59.52	58.72	42.14	52.25
Gross production (Kg/ha)	512	456	438	489	486
Net production (Kg/ha)	508.56	452.41	435.11	477.41	453.11
Survival (%)	49.79	47.28	45.23	48.64	48.35
Mean male weight (g)	47.97	58.53	62.41	53.48	57.97
Mean female weight (g)	40.97	36.38	43.03	36.86	39.97
% by number of males in population	52.14	51.89	58.62	54.21	54.11
% by number of females in population	47.86	48.11	41.38	45.79	45.89
Sex ratio	1:0.91	1:0.92	1:0.70	1:0.84	1:0.85

Table 10.19 Comparison of mean weight of morphotypes in four polders of Treatment 2

Morphotypes	F-value
SF	1.7972
WOF	2.0571
SOF	2.1908
TOF	2.5682
WBF	1.1218
SBF	1.3748
SM	1.7794
WOC	1.6726
SOC	2.3155
t-SOC	2.4517
WBC	2.8291
SBC	1.2942
OBC	2.0485

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 10.1

Table 10.20 Stocking details and yield characteristics of *M. rosenbergii* from four polders with additional substrate in the form of net tiers (Treatment 3)

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	30,000	30,000	30,000	30,000	30,000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	6.0	6.0	6.0	6.0	6.0
HARVEST					
Number per square meter	1.68	1.85	1.65	1.82	1.76
Number per ha.	16853	18542	16549	18201	17598
Mean weight (g)	62.15	49.52	58.72	52.14	58.78
Gross production (Kg/ha)	605	757	569	621	630
Net production (Kg/ha)	600.25	752.5	565.2	617.2	605.56
Survival (%)	56.18	61.81	55.16	60.67	58.66
Mean male weight (g)	72.14	58.53	62.34	53.48	68.6
Mean female weight (g)	36.75	30.38	37.03	30.86	34.25
% by number of males in population	50.8	53.6	55.4	51.15	52.49
% by number of females in population	49.2	46.4	44.6	48.85	47.51
Sex ratio	1:0.96	1:0.86	1:0.80	1:0.95	1:0.91

Table 10.21 Comparison of mean weight of morphotypes in four polders of Treatment 3

Morphotypes	F-value
SF	1.2648
WOF	1.5247
SOF	1.6584
TOF	2.0358
WBF	0.5894
SBF	0.8424
SM	1.2470
WOC	1.1402
SOC	1.7831
t-SOC	1.9193
WBC	2.2967
SBC	0.7618
OBC	1.5161

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 10.1

Table 10.22 Summary of fortnightly water quality parameters in treatments 1-3 (Polders)

Parameters	Treatment 1 (T1)	Treatment 2 (T2)	Treatment 3 (T3)
Surface Temperature	30.85	30.4	31.26
(deg C)	(27.1 - 33.2)	(26.5 - 34.2)	(25.8 - 34.5)
Surface Dissolved Oxygen	5.26	4.82	6.25
(mg/l)	(2.5 - 6.4)	(2.3 - 6.1)	(2.6 - 8.1)
Water pH range	6.3 - 8.4	6.0- 7.8	6.5 - 8.2
Soil pH range	4.8 - 6.5	5.2 - 6.6	5.2 - 6.2
Transparancy	32.5	35	28.7
(cm)	(12 - 55)	(15 - 68)	(10 - 55)
Total Alkalinity	69.24	48.61	75.58
(mg/l)	(35 - 85)	(36 - 90)	(34 - 132)
Nitrite-N	0.1	0.2	0.1
(mg/l)	(0.02 - 0.2)	(0.01 - 0.5)	(0.01 - 0.2)
Total Ammonia	0.05	0.05	0.1
(mg/l)	(0 - 0.1)	(0 - 0.1)	(0 - 0.4)
Hydrogen sulphide	0.01	0.01	0.01
(mg/l)	(0 - 0.02)	(0 - 0.02)	(0 - 0.05)

Figures are means of four replicates and 18 sampling dates (N = 72). The range of observed values is given in parenthesis

Table 10.23 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared in polders under different types of additional substrates

	TREATMENTS		
	T1	T2	T3
STOCKING			
Number per ha.	35,000	35000	30,000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	6.0
HARVEST			
Number per square meter	0.94	1.69	1.76
Number per ha.	9394	16920	17598
Mean weight (g)	38.46	52.25	58.78
Gross production (Kg/ha)	125	486	630
Net production (Kg/ha)	111.62	453.11	605.56
Survival (%)	26.84	48.35	58.66
Mean male weight (g)	50.54	57.97	68.6
Mean female weight (g)	32.58	39.97	34.25
% by number of males in population	41.92	54.11	52.49
% by number of females in population	58.08	45.89	47.51
Sex ratio	1:1.38	1:0.85	1:0.91

Table 10.24. Analysis of Variance in net production from the three treatments

Source of Variation	Sum of Squares	df	Mean sum of Squares	Calculated F
Between Groups	427518.95	2	213759.47	311.132*
Within Groups	6183.33	9	687.037	
Total	433702.29	11		

Table 10.25 t-Test comparison on the net production between the treatments

Net Production	Treatment 1 vs Treatment 2	Treatment 1 vs Treatment 3	Treatment 2 vs Treatment 3
t- value	21.6614*	22.4600*	6.3841**

* Significant at 1% level (P<0.01)
** Significant at 5% level (P<0.05)

Table 10.26 Percentage contribution of male and female morphotypes of *M. rosenbergii* by weight from the three treatments (T1- T3)

Treatment		Mean weight of male morphotype							Mean weight of female morphotype					
		SM	WOC	SOC	t-SOC	WBC	SBC	OBC	SF	WOF	SOF	TOF	WBF	SBF
	Number sampled	46	23	62	86	96	41	100	31	24	12	122	192	56
	% by weight	0.80	0.26	1.80	27.87	21.22	15.10	8.22	0.81	1.53	0.12	12.04	8.42	1.80
	Mean weight (g)	11.35	27.45	51.17	68.32	67.15	91.54	122.03	12.55	28.57	0.00	49.30	52.38	66.94
1	Standard deviation	2.61	8.54	8.34	19.96	24.00	27.06	41.72	2.64	7.57	1.02	11.72	13.04	13.03
(n=4)	Coeff. of variation	23.76	27.32	23.35	23.10	30.30	23.07	27.52	21.56	22.44	28.34	19.15	13.16	23.76
	Standard error	1.49	3.75	0.87	3.31	3.60	6.08	5.43	1.71	3.35	0.87	2.08	1.92	2.99
	Skewness	0.72	0.51	0.31	2.14	0.62	0.22	1.06	0.66	1.45	0.31	0.61	0.40	0.82
	Number sampled	79	13	10	151	133	40	162	48	18	6	53	109	19
	% by weight	0.20	0.12	1.26	16.54	16.13	12.66	35.13	0.62	0.21	2.74	3.80	8.50	2.09
	Mean weight (g)	11.88	24.27	93.33	126.52	101.28	166.76	169.93	13.50	61.20	64.40	58.40	61.14	86.02
2	Standard deviation	3.57	8.03	16.59	34.17	45.51	38.08	32.90	3.31	31.32	9.98	27.11	24.51	29.68
(n=4)	Coeff. of variation	43.15	43.51	23.04	34.35	54.57	29.54	25.64	36.21	62.02	21.99	56.75	49.59	43.00
	Standard error	0.44	2.84	6.98	2.82	4.01	6.40	2.62	0.54	8.49	7.45	3.91	2.40	7.78
	Skewness	1.88	2.04	3.56	0.87	1.34	1.95	1.18	1.75	0.88	0.84	1.30	0.72	1.70
	Number sampled	42	15	23	160	123	54	72	37	23	21	150	95	23
	% by weight	1.32	1.12	0.27	12.73	9.02	13.42	30.31	0.79	1.82	1.23	8.92	10.33	8.71
	Mean weight (g)	14.37	41.44	93.44	114.80	108.35	172.66	173.51	17.48	32.94	62.07	73.43	62.39	71.44
3	Standard deviation	4.20	8.66	17.22	34.80	46.14	38.71	33.53	3.94	31.95	10.61	27.74	25.14	30.31
(n=4)	Coeff. of variation	39.61	39.97	19.50	30.81	51.03	26.00	22.10	32.67	58.48	18.45	53.21	46.05	39.46
	Standard error	0.49	2.89	7.03	2.87	4.06	6.45	2.67	0.59	8.54	7.50	3.96	2.45	7.83
	Skewness	1.04	1.20	2.72	0.03	0.50	1.11	0.34	0.91	0.04	0.00	0.46	-0.12	0.86

For expansion of morphotypes refer Table 10.1

Table 10.27 Comparison of mean weight of individual morphotypes among the three treatments

Morphotypes	F-value
SF	4.2698**
WOF	1.3067
SOF	0.1409
TOF	80.7642*
WBF	5.9058**
SBF	0.8657
SM	1.8725
WOC	6.6329**
SOC	1.4715
t-SOC	26.3055*
WBC	11.0462*
SBC	24.5735*
OBC	14.9119*

Table 10.28 Comparison of mean weight of morphotypes of *M. rosenbergii* between the three treatments by applying t-Test

Morpotype	Treatment 1 vs Treatment 2	Treatment 1 vs Treatment 3	Treatment 2 vs Treatment 3
SF	2.0674**	1.5927	0.1144
WOF	1.3902	0.0186	2.2667*
SOF	0.4760	0.0859	0.1602
TOF	3.5198*	8.6156*	0.4137
WBF	3.2046*	5.8711**	1.1095
SBF	1.9779	1.9022	3.2246*
SM	3.329**	1.7760	1.9047
WOC	10.6204*	7.6487**	2.1119
SOC	1.1432	2.4967	2.1707
t-SOC	0.0413	21.417*	20.188*
WBC	5.0707**	14.846*	8.4106**
SBC	4.8329**	20.3251*	11.9821*
OBC	5.8508**	12.8215*	10.1682*

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

For expansion of morphotypes refer Table 10.1

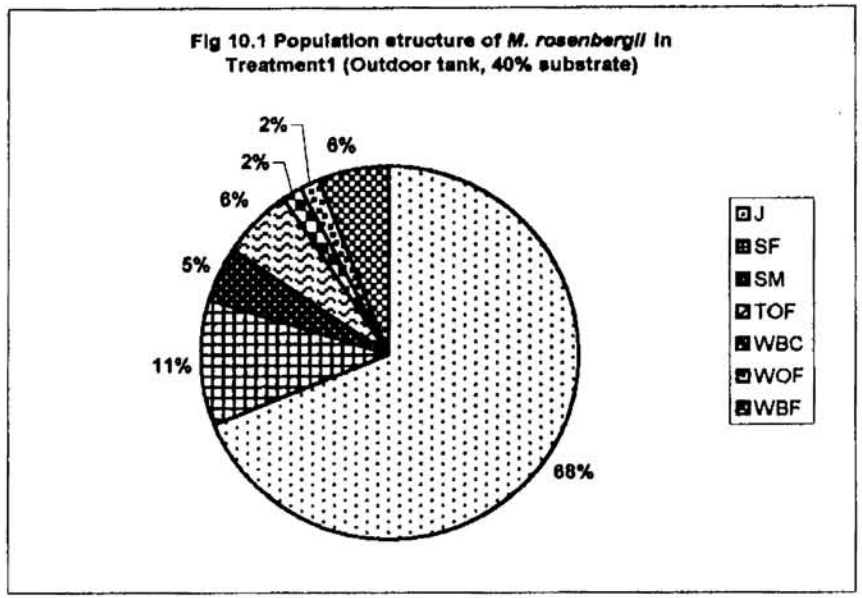


Fig 10.1 Population structure of M. rosenbergii in Treatment1 (Outdoor tank, 40% substrate)
2%
6%
2%
6%
5%
11%
68%
J
SF
SM
TOF
WBC
WOF
WBF

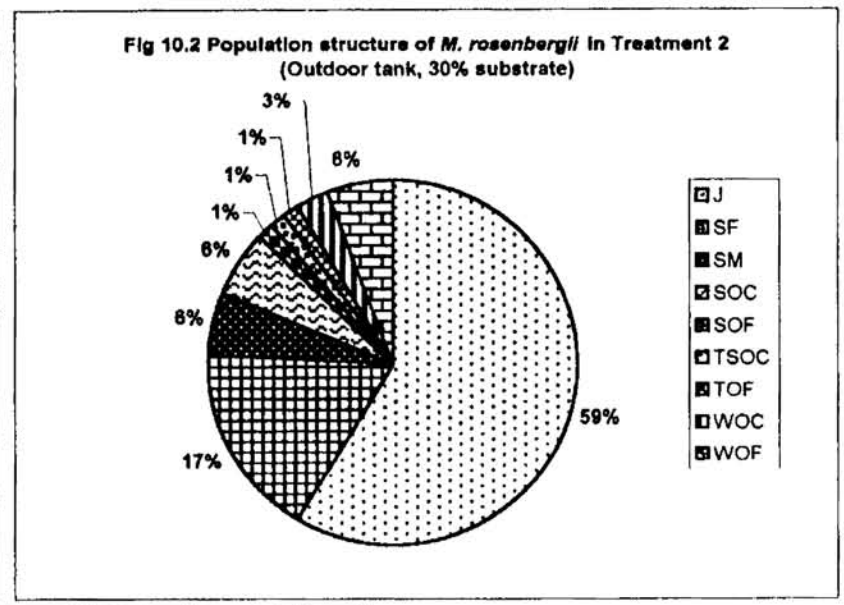


Fig 10.2 Population structure of M. rosenbergii in Treatment 2 (Outdoor tank, 30% substrate)
3%
1%
1%
1%
6%
8%
17%
59%
J
SF
SM
SOC
SOF
TSOC
TOF
WOC
WOF

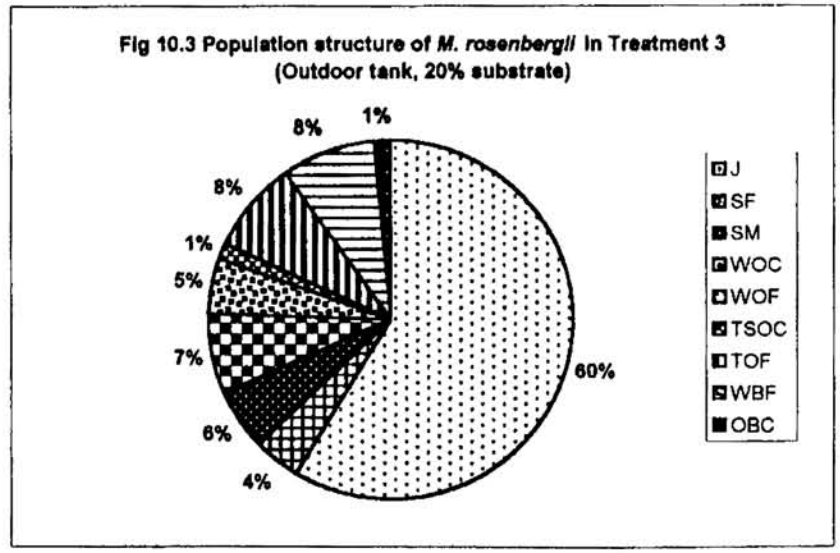


Fig 10.3 Population structure of M. rosenbergii in Treatment 3 (Outdoor tank, 20% substrate)
8%
1%
8%
1%
5%
7%
6%
4%
60%
J
SF
SM
WOC
WOF
TSOC
TOF
WBF
OBC

Fig 10.4 Population structure of *M. rosenbergii* in Treatment 4 (Outdoor tank, 10% substrate)

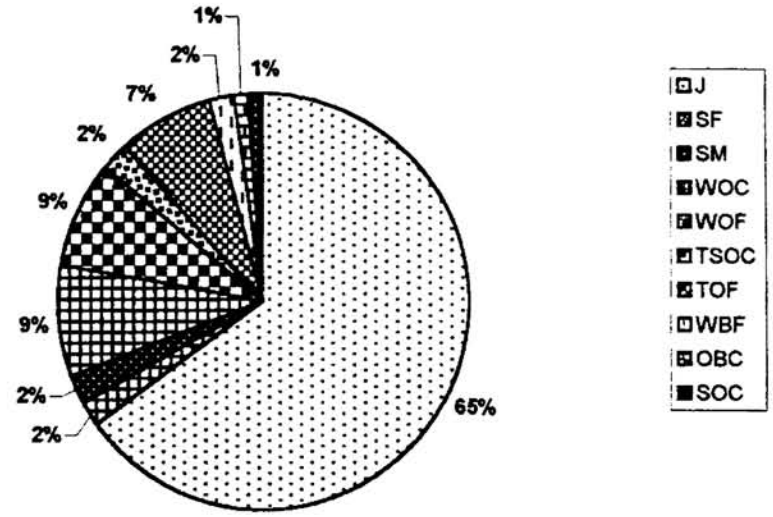


Fig 10.5 Population structure of *M. rosenbergii* in Treatment 5 (Outdoor tank, No substrate)

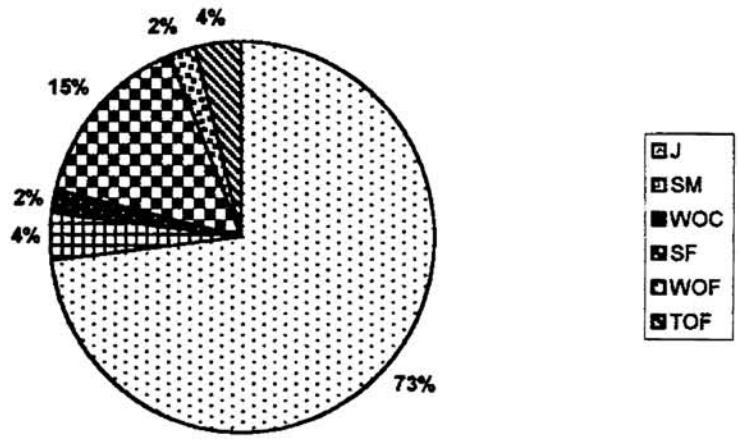


Fig 10.6 Morphotypic composition of males of *M. rosenbergii* in Treatment 1 (Polders, No Substrate)

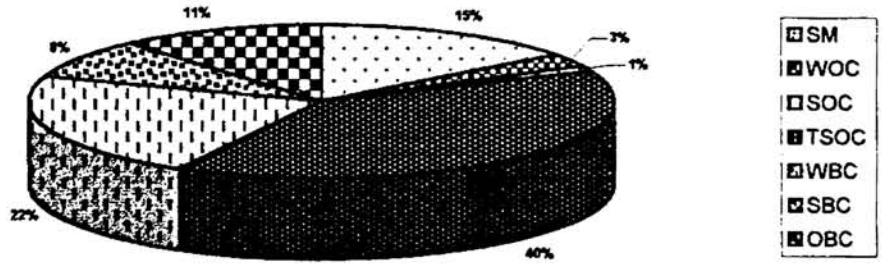


Fig 10.7 Morphotypic composition of males of *M. rosenbergii* in Treatment 2 (Polders, Pipe substrates)

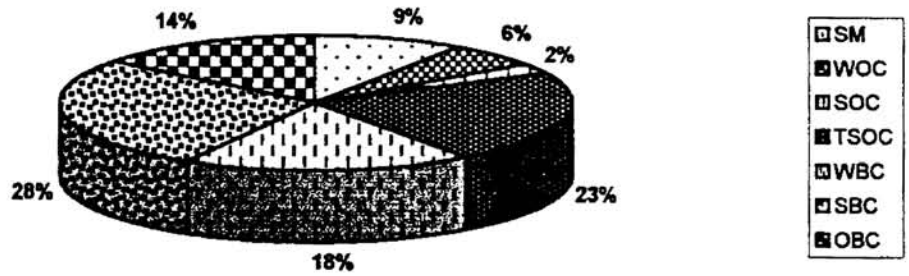


Fig 10.8 Morphotypic composition of males of *M. rosenbergii* in Treatment 3 (Polders, Net substrate)

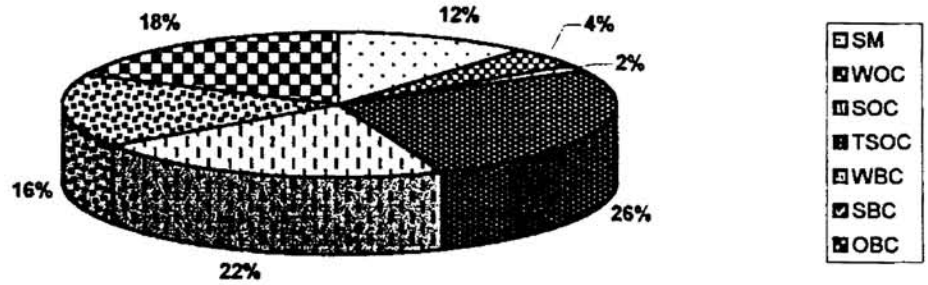


Fig 10.9 Morphotypic composition of females of *M. rosenbergii* in Treatment 1 (Polders, No Substrate)

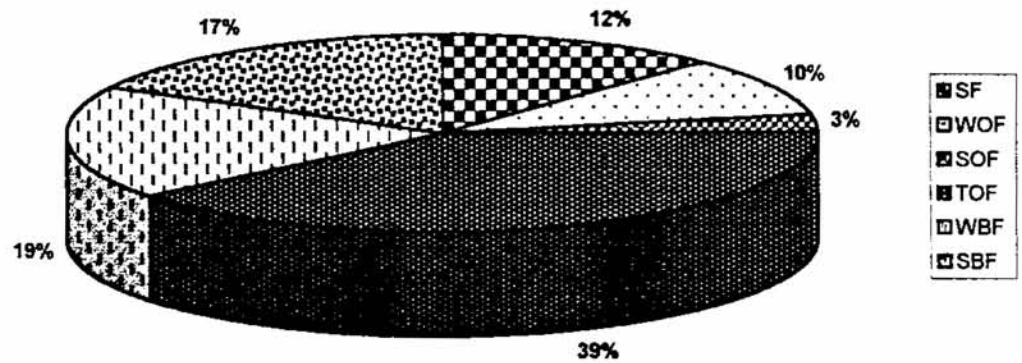


Fig 10.10 Morphotypic composition of females of *M. rosenbergii* in Treatment 2 (Polders, Pipe substrates)

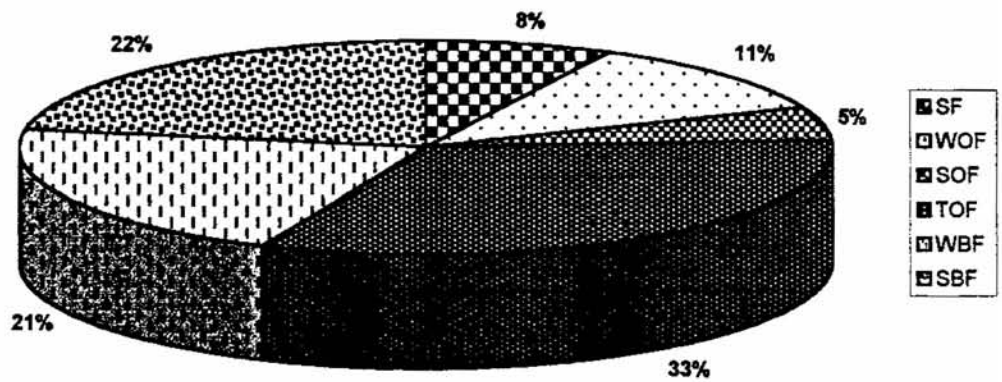


Fig 10.11 Morphotypic composition of females of *M. rosenbergii* in Treatment 3 (Polders, Net substrate)

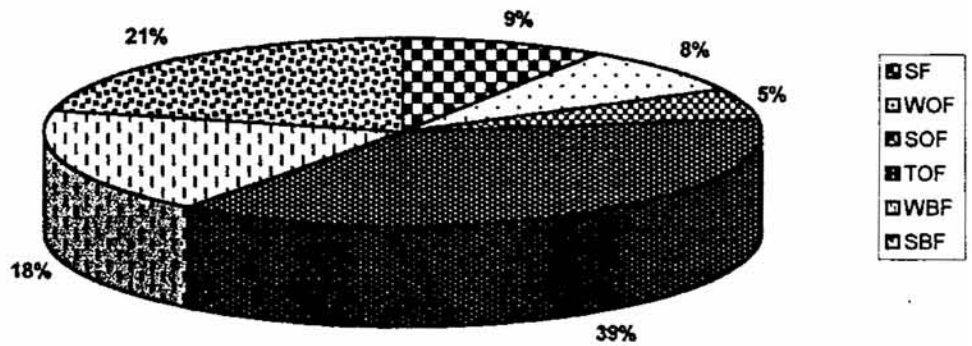


Fig 10.12 Market structure of harvested population of *M. rosenbergii* in Treatment 1 (Polders, No Substrate)

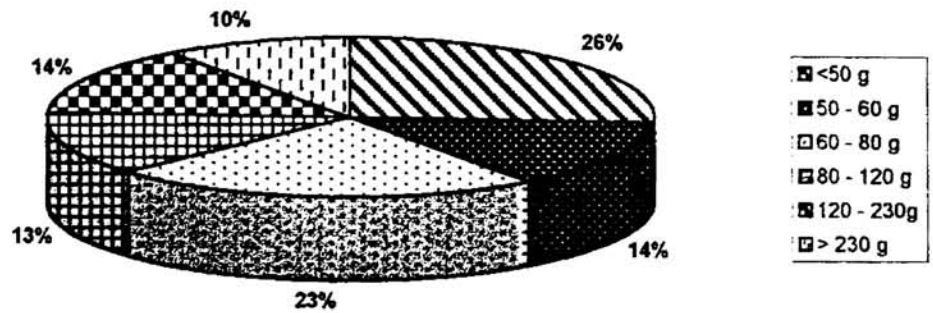


Fig 10.13 Market structure of harvested population of *M. rosenbergii* in Treatment 2 (Polders, Pipe substrates)

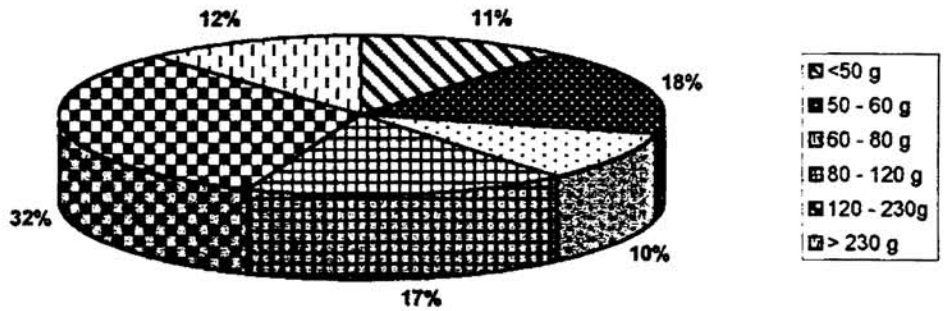
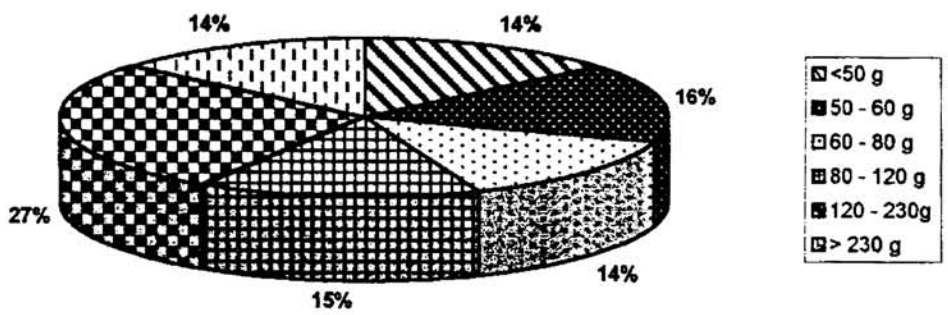


Fig 10.14 Market structure of harvested population of *M. rosenbergii* inTreatment 3 (Polders, Net substrate)



Chapter 11

Effect of cull of harvesting on the production and population structure of *Macrobrachium rosenbergii*

1.Introduction

In a tropical wetland ecosystem such as Kuttanad, the potential for culture of *M. rosenbergii* is immense. Although paddy cultivation in these polders has been practiced on a regular basis, its integration with scampi culture has given a new thrust to economy of traditional farming in Kuttanad. Integration of paddy with prawn/fish has turned out to be a viable alternative to effectively utilize the vast amount of fertile water available in Kuttanad. In spite of these high potentialities, scampi farming in Kuttanad faces a number of impediments that has affected the sustainability of its culture in these regions. The major problems among them are differential growth among cultured population, insurgence of flood in the low lying paddy lands during culture periods, and low returns during the culture operations (Kurup *et al.*, 2001). In a way all these factors are related to the geography of Kuttanad ecosystem. Since this region lies below the mean sea level (MSL) draining water from the prawn farms usually is a laborious process. Hence the culture seasons are so adjusted that complete harvesting takes place only after the monsoon season. Harvesting usually is done following complete draining or pumping of water from the pond and prawns are hand picked. It has been reported by earlier authors that partial harvesting or cull harvesting during the mid of the culture period allows to selectively harvest larger males and low sized undesirable female prawns from the population

(Malecha *et al.*, 1981a; Siddiqui *et al.*, 1995; Lin and Boonyaratpalin, 1988). This would not only provide a source of additional income for the farmers during the culture period but also provide a suitable environment for the subordinate males to grow faster (Peterson, 1982; Losordo *et al.*, 1986). This would also improve the mean weight of prawns and ultimately the net yield from its culture.

Through out the world two types of cull harvesting has been practiced in *M. rosenbergii* farms, one that involves dewatering and reducing the water level to half followed by cast netting and other, a modified cull harvesting technique involving seining of the water column using nylon nets. Regardless of farm size, one of the most costly activities involved in prawn grow-out operation is selective harvesting of market size prawns by cast netting (Fujimoto *et al.*, 1977). Moreover, the harvesting efficiency in cull harvesting is less when compared with that of selective harvesting (McGinty and Alston, 1993). Harvest efficiency is defined as the number of market size prawns caught in a single harvesting operation divided by the total number of market size prawns present in the pond prior to harvesting (Losordo *et al.*, 1986). It is with this end view that a study was conducted in the polders of Kuttanad to understand the role of selective harvesting through cull harvesting and modified cull harvesting technique in improving the market structure and net yield from *M. rosenbergii* farming in Kuttanad. Effort is also made to compare different types of harvesting techniques suitable in offering high mean weight and reducing the extent of size disparity in the culture of *M. rosenbergii* with special reference to the polders of Kuttanad.

2. Materials and Methods

The final harvest data for the present study were collected from the twelve polders of Kuttanad that followed modified extensive monoculture of *M. rosenbergii* under a single stocking density of 35,000/ha during 1999-2001. These polders were grouped under three sets of treatments (T1 to T3) depending on the nature of harvesting being conducted in the polders. Each set of treatment represents data collected from four separate polders (quadruplicates). Hence Treatment 1 (T1) included harvest data from polders that did not follow any selective harvesting, while Treatment 2 (T2) included data from polders that followed cull harvesting through dewatering and cast netting after 4 months of initial stocking. Cull harvesting by cast netting in the shallow areas along the entire pond stretch was done on the 4^{th} and 6^{th} months of culture. Similarly, Treatment 3 (T3) included data from polders that followed a modified cull harvesting technique through seining after the 4^{th} and 6^{th} months of culture. Modified cull harvesting through seines were done with a 20m x 2.1m x 3.8 cm seines. The bridle attached to this acts as a towline and serves to relieve the strain on the seine lead line and enables the seine to make a better seal against the pond sides and bottom. The middle portion of the seine is enlarged to form a shallow bag. To harvest a pond two men each at the two ends of the bridle pull the bridle/seine assembly along the entire stretch of the farm, while two men hold the lead line and net intact at the bottom. As a result with the help of 6 men the seine can be towed which takes less than 2 hrs for catching the prawns. The larger male and female prawn (> 50 g) caught during cull harvesting are collected and the rest were restocked to the pond. Final harvesting in all the polders was

Plate 11.1

A Total harvesting of *M .rosenbergii* by resorting to hand picking

B. Modified Cull harvesting of *M .rosenbergii* by resorting to seining.

carried out after 8 months of culture. Post larvae at PL-15 stage were procured from a local hatchery and stocked soon after the 'Punja' paddy season in February. Artificial substrates in the form of broken pipes were introduced into each polder @ 25% of the total pond bottom (Tidwell *et al.*, 1998a). Prawns were initially fed with a high protein content feed comprising of groundnut oil cake, rice bran and boiled butchery waste @ 10% of the total body weight and later with an on-farm made feed with 35-40% protein ratio @ 5% of the body weight on a daily basis in two installments, 40% in the early morning and 60% in the evening. Water was exchanged once in a week with the help of pumps. During partial harvest the data on individual length, weight of prawns, morphotypic composition and yield from each polder was recorded and these data were combined with the final harvest data to arrive at the final population structure and market yield from each polder. On the termination of farming after eight months, the polders were totally harvested by pumping it dry and the prawns were hand picked from the bottom. Random samples between 500-1000 prawns each from the twelve polders were examined on the day of harvest and sorted to sex and morphotypes (Kuris *et al.* 1987). All the prawns were measured up to nearest millimeter and weighed up to nearest gram. The weight of individual morphotypes from polders within each treatment was compared following ANOVA. In order to compare the effect of a different harvesting system on population characteristics and yield structure the cumulative mean values of each treatment (T1 to T3) was compared. Statistical analysis was done using SPSS 7.5 and Excel 98 package for Windows and the results were statistically evaluated following Snedecor and Cochran (1961). Average weight

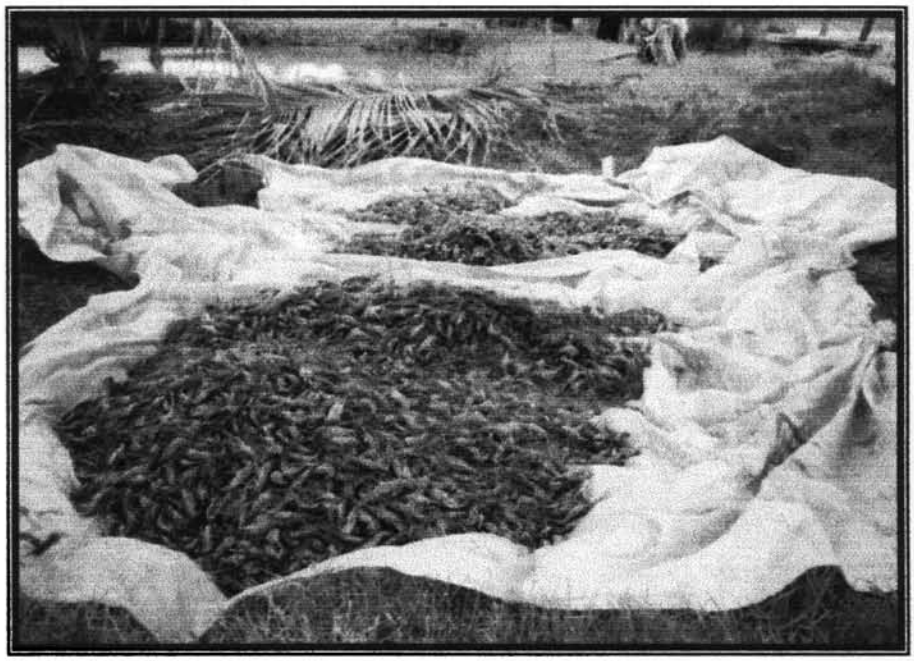
gain, final mean weight and total biomass were analysed by ANOVA followed by *F*-test.

3. Result

3.1 Population structure

The details on yield characteristics from Treatment 1 (Polders) where no cull harvesting was introduced till the end of culture is shown in Table 11.1. None of the parameters computed showed significant variation among the four sets of polders studied. Variation in net production, survival rate and mean weight of prawns in T1 did not show significant difference (P>0.05). While net production ranged between 112.4 to 161.0 Kg/ha, the survival rate were registered within the range 32.1 to 48.3% and mean weight ranged from 38.5 to 52.2 g. Sex ratio within the polders did not follow any particular pattern. Comparison of mean weight of individual morphotypes in polders of T1 through ANOVA (Table 11.2) did not show any significant difference (P>0.05) within the treatment (T1).

Table 11.3 shows the details on yield characteristics of polders that were subjected to cull harvesting through dewatering and cast netting (Treatment 2). Mean weight of prawns within the four polders did not show any significant variation (P>0.05). Net production ranged between 452.8 to 482.6 Kg/ha, the survival rate ranged between 46.7 and 48.3% and mean weight also did not differ considerably ranging between 52.2 and 55.1 g respectively. Males dominated the final population in almost all the polders. Comparison of mean weight of individual morphotypes among the four polders (T2) is shown in Table 11.4. The



A Heap from a total harvest of *M.rosenbergii* from a polder of Kuttanad

B. Size grading of the harvested population of *M.rosenbergii* in Kuttanad

results of ANOVA suggest that the mean weight for morphotypes within the treatment (T4) did not vary significantly (P>0.05).

Yield characteristics of polders that were subjected to modified cull harvesting through seining are shown in Table 11.5 (Treatment 3). The mean weight among prawn in T3 prawns did not vary significantly (P>0.05). The net productions in this treatment ranged between 463.1 to 562.8 Kg/ha, the survival rate ranged between 52.7% and 54.9% and mean weight between 62.25 and 65.1 g respectively. In almost all the channels males dominated the final population. Comparison of mean weight of individual morphotypes among the four polders (T3) is shown in Table 11.6. The results of ANOVA suggest that the mean weight for morphotypes within the treatment (T3) did not vary significantly (P>0.05).

The mean value of yield characteristics of various treatments from the above analysis is given in Table 11.7. From the table it can be seen that the type of harvesting pattern has a profound influence on the final marketable yield structure and population characteristics of *M. rosenbergii*. Among the treatments lowest values for mean weight, survival rate and net production were encountered from T1 that did not follow any partial harvesting strategies prior to complete harvest. The average values of these parameters in the tune of 44.6 g, 39.1% and 140.2 Kg/ha respectively. In total contrast to this better results were registered for mean weight and survival in T2 and T3, which ultimately resulted in better production. The mean weight and survival rate of prawn in these treatments (T2 and T3) were on a higher side (53.4 and 63.5g and 47.7% and

53.9% respectively). Correspondingly, an increase in the net production from these two treatments was also seen (461.7 and 515.2 Kg/ha respectively). The sex ratio in the three treatments also varied considerably, with the predominance of females in the final population in T1 (1:1.10). In contrast males were dominant in T2 and T3 (1:0.92 and 1: 0.94 respectively). Similar to the results of mean weight of prawns, the mean weight of males and females in T2 and T3 were also higher than that in T1. The percentage contribution of different male and female morphotypes under the three treatments is shown in Table 11.10. From the table it can be seen that percentage contribution by weight of lower order male morphotypes (SM and WOC) was considerably high (3.7%) in T1, in total contrast, their representation in T2 and T3 were reduced to 1.4% and 1.6% respectively. Conversely, the percentage of higher market weight morphotypes (SBC and OBC) was alarmingly less in T1 (8%) when compared to T2 (35%) and T3 (25.3%). The mean weight of individual morphotypes also followed a similar pattern within the treatments. Better results on mean weight was registered in treatments T2 and T3. Significant among them were the mean weights of SM and OBC that ranged from 11.35 g to 14.37 g and 122 g to 173.5 g respectively in T1 and T3. The results on the coefficient of variance showed wide disparity between cull harvested and non cull harvested population.

The population structure for various male and female morphotypes is depicted in Fig 11.1 and 11.2. The results showed that a clear difference in the percentage representation of individual morphotypes among the three treatments was visible at the end of the culture period. While the contribution of SM and

WOC was high in T1 (12% and 8%), the representation of larger morphotypes (SBC and OBC) was high in T2 (12% and 8%) and T3 (12% and 11%) respectively. The contribution of SM, OC and BC morphotypes among the treatments were in the order 12%, 20% and 16% in T1, 4%, 25% and 32% in T2 and 5%, 21% and 33% in T3, similarly, female morphotypes also followed almost identical pattern. Their representation in the final catch was in the order 8% by SF, 22% by OF and 22% by BF in T1, whereas in T2 and T3 their occurrence was 4%, 16% and 19% in T2 and 6%, 14% and 21% in T3. It is worth noting that the percentage of low weight grade prawns showed a significant reduction in those treatments where the partial harvest was introduced during the culture period.

3.2 Yield Characteristics

Variations in the mean weight of population could be observed among the three treatments, showing highest mean weight of 63.55 g in T3 against 44.64 g registered in T1 (Table 11.7). Mean weight, standard deviation and coefficient of variation in respect of various male and female morphotypes are given in Table 11.10. Invariably, the mean weights of male and female morphotypes were highest in T3. On the contrary to this, coefficient of variation suggests a more heterogeneous nature of population in T2 and T3 in which cull harvesting was exercised during the culture period. The net production also varied significantly within the treatments. The lowest mean net production was recorded in T1 (140.2 kg/ha), whereas the maximum production was registered in T3 (515.2 kg/ha). Results of ANOVA on net production (Table 11.8) from the

three sets of treatments show a significant difference at 1% level (F= 35.4552). Further analysis on variation in production among the treatments applying t- test (Table 11.9) showed maximum variations (P<0.01) between treatments T1 and T2 and between T1 and T3. It may also be noted that the results of t-test showed that the mean weight of larger OC and BC morphotypes did not vary significantly between treatments 2 and 3. Moreover, the net production between treatments 2 and 3 was found to be insignificant (Table 11.9) (P>0.05). This indicates that irrespective of the pattern of partial harvest, there was no difference in the net production. But, in terms of mean weight and net production, modified cull harvesting through seining was found more effective. A detailed economic analysis of the different harvesting techniques on the economic feasibility of *M. rosenbergii* culture is given in Chapter 12. Results of ANOVA on mean weight of individual morphotypes is shown in Table 11.11. The results show a diverse nature of weight distribution (P<0.01) among the three treatments for TOF, WBF, SBF, t-SOC, WBC, SBC and OBC morphotypes. On further analysis by applying t-test (Table 11.12) it was found that the difference in weight distribution among various morphotypes was significant (P<0.01) among the treatments T1andT2 as well as T1andT3. It would thus appear that the differences in weight distribution in the three treatments were well reflected in the net production registered from them.

The weight distribution patterns of prawns in the three sets of treatments at different levels of harvesting strategies are depicted in Fig 11.3 to 11.5. In T2 and T3 it appeared that percentage of males in the final population

influenced the weight distribution pattern profoundly, which is evident from the preponderance of >120g-weight group in the treatments T2 and T3 (47% and 40% respectively). On the contrary, weight class constituted only 26% in treatment 1 where the predominant size group was 50-80 g (36%), the harvested population being strongly influenced by female morphotypes. The cumulative percentage of undersized male and female morphotypes (<50 g) in the final population was perceptibly high in treatment that did not follow a partial harvest (22% in T1), while in treatments were cull harvesting was carried out (T2 and T3) the same was worked out to be only 6% and 8% respectively. It could also be seen that out of the total biomass produced from the three treatments, 94% of the yield from T2 and 92% of the yield from T3 belonged to >50g group and therefore were marketable whereas in T1 only 78% was marketable.

4. Discussion

Communally cultured *M. rosenbergii* post larvae exhibit a rapid increase in size variation following metamorphosis, accompanied by an increased skewness in size distribution, due to large difference in growth rates of different individuals in the population, a condition referred to as "heterogeneous individual growth" or HIG (Sandifer and Smith, 1975, 1985; Malecha, 1977, 1981a; Ra'anan and Cohen, 1984, 1985; Smith *et al.*, 1978a; Daniels and D'Abramo, 1994). Various management approaches designed to minimize HIG have concentrated on selective harvesting or size grading of pond population. In temperate climates selective harvest of "market size" prawns prior to a final drain down harvest has been performed (Willis and Berrigan, 1977; Cohen and

Ra'anan, 1983). Malecha *et al.* (1981b) proposed a management practice that included pre-harvest size grading and restocking of non-market size prawns after complete harvesting by pond draining. In the present study an attempt was made to evaluate the role of two cull harvesting techniques in improving the production and marketable yield structure from the polders. The two commonly used partial harvesting techniques viz., draining cum cast netting and seining have also been evaluated for their economic sustainability in Kuttanad ecosystem where complete draining of polders during monsoon season is next to impossible. With the advent of cull harvesting techniques in Hawaii (Fujimura, 1974), most prawn farmers have opted for year round stocking and production. In Kuttanad where the culture fields lie below the mean sea level, possibility of continuous harvesting and stocking on a year round basis would not be a viable proposition. Moreover, the negative impact of size disparity also was found high in the culture systems of this region (Ranjeet and Kurup, 2001a), which was evident from the high proportion (25%) of non-marketable grade in the final harvested population. Therefore, development of a definite culture and harvesting system was found highly imperative that would not only improve the net yield by increasing the survival rate but also produce greater number of marketable prawns (>50 g).

In *M. rosenbergii* a social stimulus i.e., the presence or absence of a 'bull' (larger males) results in stunning or accelerating growth of 'runts' (smaller males). Growth suppression in 'runt' is based on reduced size increment, while the molting rate remains the same. The social interactions among the different male morphotypes regulate their growth and morphotype

stage (Karplus and Hulata. 1992). In order to attain the dominant position in the population, the 'runts' immediately transforms to successive stages and becomes a dominant male when given a chance. As a result, the subordinate males instead of following a definite path of SM → OC → BC through its intermediary stages, skips up some of the transitional stages to attain a subterminal position in the transformation pathway (Karplus *et at.*, 1991,1992a). The OC transforms into BC only after becoming larger than the largest BC in its vicinity (Karplus *et al.*, 1992a). This phenomenon termed "Leap frog " growth pattern results in a series of differently sized BC males whose size is positively correlated with the time of their transformation (Karplus *et al.*, 1991). The results from the present study showed a clear differentiation in the mean weight, survival and net production among the three treatments. Better results were registered from T2 and T3 where either cull harvesting or modified cull harvesting system was adopted. Since most of the larger sized prawn (>50 g) was selectively harvested at the end of 4^{th} month of culture, the larger prawns never got a chance to develop a social hierarchy in these treatments. This resulted in faster morphotypic transformation and growth for the other prawns that were deprived of food and space at first instance. Hence cull harvesting takes advantage of the fact that individual prawns in a pond population exhibit highly variable growth rates so that by culling large animals from the population, smaller animals could grow into size classes 'vacated' by the larger animals and market-sized animals could be marketed during the culture period (Malecha, 1986b). Similarly, the predominance of male morphotypes during the final harvest in T2 and T3 point out to the fact that before a particular morphotype could post its dominance in the pond, whereby

increasing the competition for food and space among males. they were selectively harvested. This not only maintained the number of males above the normal range but also produced another set of dominant males that initiated the transformation pathway faster only to culminate finally into larger males. The predominance of males in T2 and T3 may be a strong cause whereby net production from these two treatments increased considerably due to the high preponderance of large sized prawns, which also increased the compensatory growth of smaller animal. The present results show that small prawns retain an inherent capacity for compensatory growth; consequently their sizes are largely transient brought on by the social hierarchy prevailing in the pond. This means that HIG is best managed by completely removing all the dominant males since their presence restricts the transformation of subdominant males into dominant males and small males into subdominant ones. Inefficient removal of large animals by inefficient harvesting diminishes the desired effect. In the present study, the population structure at the end of the culture could be taken as a benchmark for this. The preponderance of undersized SM and WOC in T1 of the present study was mainly due to this inefficient mode of harvesting.

The results drawn from the population structure among the three treatments show that better morphotypic transformation could be seen in T2 and T3. This was confirmed by the predominance of larger male and female morphotypes in appreciable numbers at the time of final harvest. In these two treatments larger OC and BC morphotypes showed their dominance in the final catch. This was found complimentary to that of Malecha (1986b) who reported

that production per unit time and area are higher in cull harvested systems due to the high preponderance of larger size prawns on account of increased compensatory growth of smaller animals. Siddiqui *et al.* (1995) evaluated the effects of harvesting methods (cull and batch) on population structure, growth, and yield of freshwater prawn, *M. rosenbergii*, cultured at two densities. They found that cull harvesting had a direct bearing on the specific growth rate (SGR) of prawns but did not affect the sex ratio. In contrast to this finding, in the present study, males showed dominance in treatments that followed cull harvesting. This may primarily be due to selective harvesting of male prawns and restocking of females back to the ponds, and this can alleviate to mortality due to cannibalism among dominant males, thereby their percentage in the final population can be increased. On comparison of the performance of two cull harvesting techniques, adopted in the present study, it could be seen that in the present study the net production was not considerably affected by the difference in cull-harvesting pattern. Average values of the expenses towards harvesting under the three treatments were worked out to be Rs. 8300/- for T1, Rs. 23,430/- for T2 and Rs. 10,800/- for T3. Although the net production from T2 and T3 did not vary significantly, the additional expense spent in T2 for draining and netting the pond twice during the culture period was found to be expensive and the net expense worked out to be twice as much as that spent for seining. Hence, from an economic point of view modified cull harvesting technique was found more profitable. However, the reduced harvesting efficiency was found to be the only drawback in this technique when compared to the other cull harvesting techniques. Harvesting efficiency would get greatly reduced due to improper

seining owing to a number of factors such as uneven pond bottom, improper pulling force and presence of rooted weeds and grasses.

Market yield structure of harvested prawns observed in the present study also showed a diverse pattern similar to their population structure. The preponderance of prawns > 120 g class in T2 and T3 may be due the presence of a conducive habitat that enabled prawns to grow to their maximum marketable size. A shift in the weight class so attained might have helped in improving the overall mean weight of the prawn from these treatments. Conversely, a complex social hierarchy was found pronounced in T1 whereby, smaller prawns were deprived of food and space, which ultimately culminated in the preponderance of non-marketable undersized prawns (<50 g) in this treatment. There was a definite shift of mean weight of female prawns from 40 g to over 50 g in both T2 and T3. Most of the 'scampi' processing companies operating in and around Cochin procure and export prawns above 50 g weight. Prawns, which fall below this weight class, are sold only in local markets where they fetch only very low prices (Rs. 80/Kg), in contrast to the market price of Rs.130/- for >50 g. It is worth noticing that, nearly 16% of the prawns that otherwise would have fallen below 50 g weight class, were pushed above the 50 g barrier only through the incorporation of cull harvesting techniques. It would thus appear that, the cull harvest technique not only facilitates greater percentage of prawns to be marketed for export purpose, but also allows the farmer to have an option of receiving an intermediate revenue during the course of culture. The practice of intermediate, partial harvest in Kuttanad is definitely a sound management

strategy for two reasons. Firstly, the females that matured relatively early (berried females) could be selectively removed in the early harvest. This apart from providing an additional income to the farmer, also reduces the population density in the pond thus helpful in reducing the cost on feed. Secondly, intermediate harvesting also removes large males and allows the production to shift gradually to males that commanded a good price. The interval between each harvest was also found very crucial, in the above realities. Because of their aggressive sexual and territorial behavior, which influences the final production and yield, the removal of Blue-clawed males from the culture pond is highly warranted. In contrast, the Small-clawed males will have to be allowed to grow to the Orange-clawed stage since they demands higher value in export market because of their greater tail weight (Sandifer and Smith, 1977). Thus, through cull harvesting production of larger OC males can be improved substantially, which would be a viable means of augmenting biomass and economic yield of prawn farming in Kuttanad and similar areas across the world.

Table 11.1 Stocking and Harvest details of *M. rosenbergii* in the four polders of Kuttanad (Treatment 1) where no cull harvesting was adopted

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35000	35000	35000	35000	35000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.69	1.23	1.37	1.28	1.39
Number per ha.	16920	11247	13716	12842	13681
Mean weight (g)	52.25	43.05	38.54	44.72	44.64
Gross production (Kg/ha)	486	116.58	165.58	146.58	228.69
Net production (Kg/ha)	145.3	112.45	161.06	142.23	140.26
Survival (%)	48.35	32.13	39.18	36.69	39.09
Mean male weight (g)	57.97	49.58	48.21	44.68	50.11
Mean female weight (g)	39.97	36.2	31.45	36.07	35.92
% by number of males in population	54.11	47.86	43.58	51.79	49.34
% by number of females in population	45.89	52.14	56.42	48.21	50.67
Sex ratio	1:0.85	1:1.08	1:1.29	1:0.93	1:1.10

Table 11.2 Comparison of mean weight of morphotypes in four polders of Treatment 1 (T1) where no cull harvesting was employed

Morphotypes	F-value
SF	1.5643
WOF	1.9709
SOF	1.7042
TOF	1.3627
WBF	1.4669
SBF	1.1727
SM	0.5083
WOC	1.1059
SOC	1.0668
t-SOC	2.2086
WBC	2.0118
SBC	1.6670
OBC	2.1122

SF = Small female
WOF= Weak orange clawed female
SOF = Strong orange clawed female
TOF = Transforming strong orange clawed female
WBF= Weak blue clawed female
SBF = Strong blue clawed female
SM = Small male
WOC = Weak orange clawed male
SOC = Strong orange clawed male
t-SOC = Transforming strong orange clawed male
WBC = Weak blue clawed male
SBC = Strong blue clawed male
OBC = Old blue clawed male

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes

Table 11.3 Stocking and Harvest details of *M. rosenbergii* in the four polders of Kuttanad (Treatment 2) where partial harvesting by draining and cast netting was carried out

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35000	35000	35000	35000	35000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.69	1.68	1.67	1.63	1.67
Number per ha.	16920	16850	16724	16358	16713
Mean weight (g)	52.25	55.14	52.78	53.47	53.41
Gross production (Kg/ha)	486	487	462	457	473
Net production (Kg/ha)	453.11	482.65	458.17	452.87	461.70
Survival (%)	48.35	48.14	47.78	46.73	47.75
Mean male weight (g)	57.97	54.68	55.87	59.58	57.03
Mean female weight (g)	39.97	41.07	37.58	41.2	39.96
% by number of males in population	54.11	56.42	48.21	53.87	53.15
% by number of females in population	45.89	43.58	51.79	46.13	46.85
Sex ratio	1:0.85	1: 0.77	1:1.07	1:0.86	1:0.92

Table 11.4 Comparison of mean weight of morphotypes in four polders of Treatment 2 (T2) where partial harvesting by draining and cast netting was adopted

Morphotypes	F-value
SF	1.6582
WOF	2.0892
SOF	1.8065
TOF	1.4445
WBF	1.5549
SBF	1.2431
SM	0.5388
WOC	1.1723
SOC	1.1308
t-SOC	2.3411
WBC	2.1325
SBC	1.7670
OBC	2.2389

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 11.1

Table 11.5 Stocking and Harvest details of *M. rosenbergii* in the four polders of Kuttanad (Treatment 3) where modified cull harvesting through seining was carried out

	POLDERS				
	1	2	3	4	Mean values
STOCKING					
Number per ha.	35000	35000	35000	35000	35000
Mean weight (g)	0.2	0.2	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0	7.0	7.0
HARVEST					
Number per square meter	1.92	1.84	1.89	1.88	1.88
Number per ha.	19247	18445	18970	18825	18872
Mean weight (g)	62.25	65.14	63.32	63.47	63.55
Gross production (Kg/ha)	486	507	557	574	531
Net production (Kg/ha)	463.11	492.65	542.45	562.87	515.27
Survival (%)	54.99	52.70	54.20	53.78	53.92
Mean male weight (g)	57.97	64.68	62.05	59.58	61.07
Mean female weight (g)	39.97	51.07	47.51	41.2	44.94
% by number of males in population	53.58	53.17	46.13	53.87	51.69
% by number of females in population	46.42	46.83	53.87	46.13	48.31
Sex ratio	1:0.86	1: 0.88	1:1.16	1:0.86	1:0.94

Table 11.6 Comparison of mean weight of morphotypes in four polders of Treatment 3 (T3) where modified cull harvesting through seining was adopted

Morphotypes	F-value
SF	1.6857
WOF	2.1045
SOF	1.8298
TOF	1.4780
WBF	1.5854
SBF	1.2823
SM	0.5980
WOC	1.2135
SOC	1.1733
t-SOC	2.2260
WBC	2.0233
SBC	1.6682
OBC	2.1267

P>0.05 (Non significant)
F-value non-significant among polders for morphotypes
For expansion of morphotypes refer Table 11.1

Table 11.7 Stocking details and yield characteristics of *Macrobrachium rosenbergii* reared under different harvesting practices in polders of Kuttanad

	TREATMENTS		
	1 (No cull harvest)	2 (Cull harvest by Draining & Castnetting)	3 (Modifed cull harvest by seining)
STOCKING			
Number per ha.	35000	35000	35000
Mean weight (g)	0.2	0.2	0.2
Biomass per ha. (Kg)	7.0	7.0	7.0
HARVEST			
Number per square meter	1.39	1.67	1.88
Number per ha.	13681	16713	18872
Mean weight (g)	44.64	53.41	63.55
Gross production (Kg/ha)	228.69	473	531
Net production (Kg/ha)	140.26	461.70	515.27
Survival (%)	39.09	47.75	53.92
Mean male weight (g)	50.11	57.03	61.07
Mean female weight (g)	35.92	39.96	44.94
% by number of males in population	49.34	53.15	51.69
% by number of females in population	50.67	46.85	48.31
Sex ratio	1:1.10	1:0.92	1:0.94

Table 11.8. Analysis of Variance in net production from the three sets of harvesting practices

Source of Variation	*Sum of square*	*df*	*Mean sum of Square*	*Calculated F*
Between Groups	454924.65	2	224654.5	35.4552
Within Groups	34646.56	9	28354.98	
Total	489571.21	11		

Table 11.9 t-Test comparison on the net production between the treatments

Net Production	Treatment 1 vs Treatment 2	Treatment 1 vs Treatment 3	Treatment 2 vs Treatment 3
t- value	24.545*	28.558*	1.5644

* Significant at 1% level (P<0.01)
** Significant at 5% level (P<0.05)

Table 11.10 Percentage contribution of male and female morphotypes by weight in the harvested population three treatments under three harvesting strategies

Pond		Mean weight of male morphotype							Mean weight of female morphotype					
		SM	WOC	SOC	t-SOC	WBC	SBC	OBC	SF	WOF	SOF	TOF	WBF	SBF
	Number sampled	25	16	16	45	15	46	55	14	15	15	24	45	28
	% by weight	1.25	2.49	1.49	15.56	16.13	5.64	4.82	3.25	2.64	1.09	18.61	14.65	12.38
	Mean weight	11.35	27.45	51.17	68.32	67.15	91.54	122.03	12.55	28.57	0.00	49.30	52.38	66.94
T1	Standard deviation	2.61	8.54	8.34	19.96	24.00	27.06	41.72	2.64	7.57	1.02	11.72	13.04	13.03
(n=4)	Coeff. of variation	23.76	27.32	23.35	23.10	30.30	23.07	27.52	21.56	22.44	28.34	19.15	13.16	23.76
	Standard error	0.62	2.88	0.00	2.44	2.73	5.21	4.56	0.84	2.48	0.00	1.21	1.05	2.12
	Skewness	0.41	0.20	0.00	1.83	0.31	-0.09	0.75	0.35	1.14	0.00	0.30	0.09	0.51
	Number sampled	30	21	21	50	20	51	60	19	20	20	29	50	33
	% by weight	1.14	0.28	0.76	23.73	16.67	7.66	34.26	0.76	0.16	1.09	3.65	8.19	1.65
	Mean weight	11.88	24.27	93.33	126.52	101.28	166.76	169.93	13.50	61.20	64.40	58.40	61.14	86.02
T2	Standard deviation	3.57	8.03	16.59	34.17	45.51	38.08	32.90	3.31	31.32	9.98	27.11	24.51	29.68
(n=4)	Coeff. of variation	43.15	43.51	23.04	34.35	54.57	29.54	25.64	36.21	62.02	21.99	56.75	49.59	43.00
	Standard error	0.91	0	4.9	2.64	3.74	4.25	5.17	1.08	5.27	5.93	1.37	1.98	3.65
	Skewness	0.47	0.00	1.38	0.45	0.73	0.06	0.32	0.26	0.91	0.66	0.25	0.23	0.91
	Number sampled	21	12	24	25	48	24	14	32	25	18	62	28	24
	% by weight	0.91	0.70	0.12	11.69	13.09	6.14	24.84	0.55	0.79	0.00	12.54	21.81	6.81
	Mean weight	14.37	41.44	93.44	114.80	108.35	172.66	173.51	17.48	32.94	62.07	73.43	62.39	71.44
T3	Standard deviation	4.20	8.66	17.22	34.80	46.14	38.71	33.53	3.94	31.95	10.61	27.74	25.14	30.31
(n=4)	Coeff. of variation	39.61	39.97	19.50	30.81	51.03	26.00	22.10	32.67	58.48	18.45	53.21	46.05	39.46
	Standard error	0.49	2.89	7.03	2.87	4.06	6.45	2.67	0.59	8.54	7.50	3.96	2.45	7.83
	Skewness	1.04	1.20	2.72	0.03	0.50	1.11	0.34	0.91	0.04	0.00	0.46	-0.12	0.86

For expansion of morphotypes refer Table 11.1

Table 11.11 Comparison of mean weight of morphotypes among the three harvesting strategies

Morphotypes	F-value
SF	1.2145
WOF	1.6652**
SOF	2.1516**
TOF	26.6546*
WBF	12.871*
SBF	5.5465*
SM	1.6454
WOC	1.4214
SOC	1.0354
t-SOC	19.6455*
WBC	15.5654*
SBC	14.0657*
OBC	11.6058*

Table 11.12 Comparison of mean weight between the three treatments by applying t-Test

Morpotype	Treatment 1 vs Treatment 2	Treatment 1 vs Treatment 3	Treatment 2 vs Treatment 3
SF	2.353*	1.8035**	1.3954**
WOF	2.5096*	1.0145	2.8061
SOF	0.1425	0.1776	0.2799
TOF	3.2541*	1.2654**	1.324**
WBF	4.7988*	2.8101*	2.86878
SBF	3.7778*	1.7891**	1.8477**
SM	1.1443	0.2161	0.2926
WOC	5.5044*	3.7274*	0.4165
SOC	0.499	0.1781	0.2751
t-SOC	6.9787*	1.5761**	1.5026**
WBC	5.9862*	1.1835	2.7935*
SBC	5.844*	1.2464	1.0119
OBC	6.4527*	1.0621	1.6123

For expansion of morphotypes refer Table 11.1

* Significant at 1% level (P<0.01)

** Significant at 5% level (P<0.05)

Fig 11.1 Population structure of male morphotypes of *M. rosenbergii* in the harvested population in three treatments (Polders)

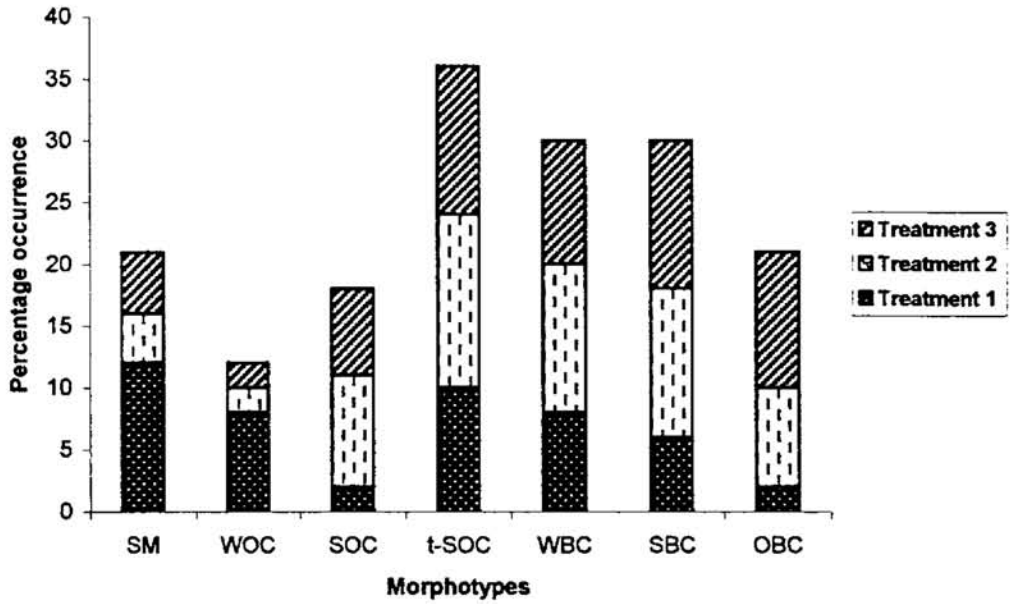


Fig 11.2 Population structure of female morphotypes of *M. rosenbergii* in harvest population in three treatments (Polders)

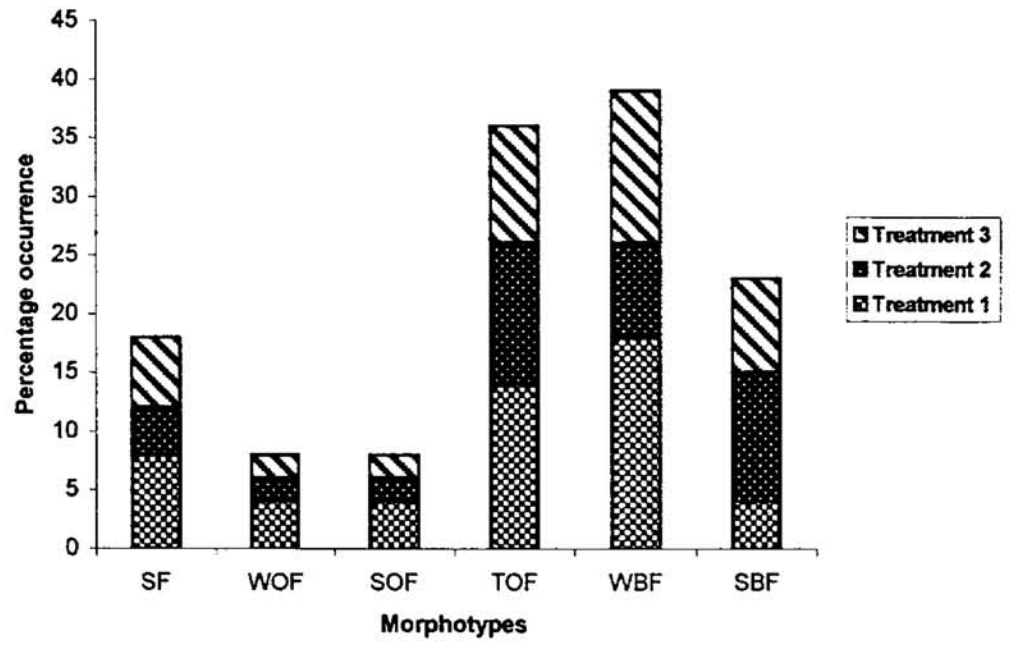


Fig 11.3 Market yield structure of harvested population in Treatment 1 (Full harvest)

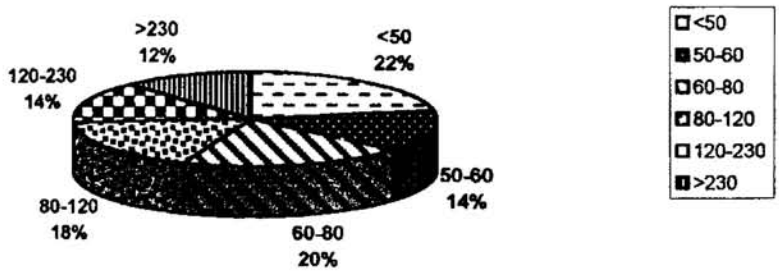


Fig 11.4 Market yield structure of harvested population on Treatment 2 (Cull harvest)

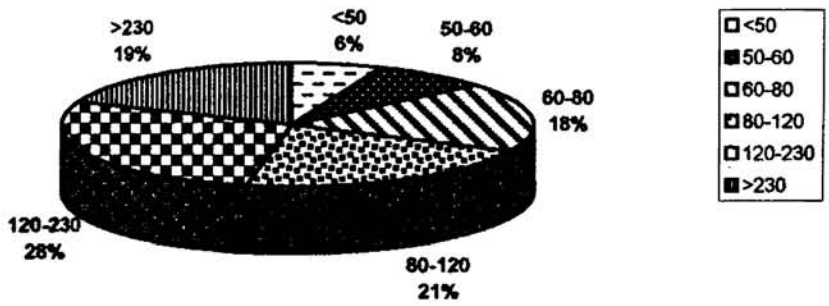
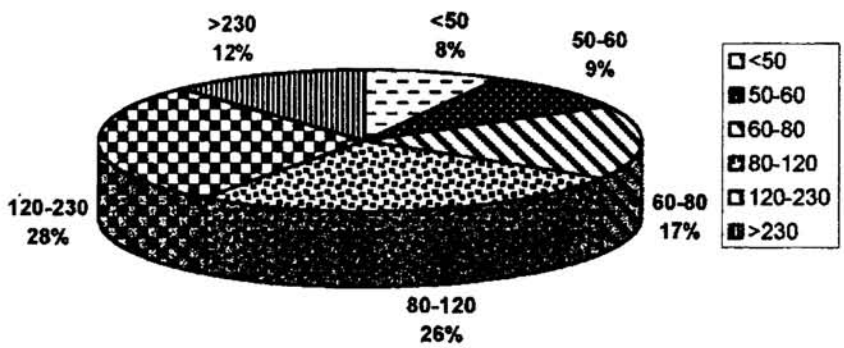


Fig 11.5 Market yield structure of harvested population of Treatment 3 (Modified cull harvest)



Chapter 12.

Techno-economic feasibility of farming of *Macrobrachium rosenbergii*

1. Introduction

The success in developing techniques for both the mass postlarvae production and grow-out system of *Macrobrachium rosenbergii* has accelerated freshwater prawn culture in many parts of the world. However, the sustenance of aquaculture is usually determined by the income it generates, which in turn is dependent on factors that are both internal and external to this sector (Shang, 1990). In freshwater prawn farming, much attention has been paid on bio-technical aspects to increase the net yield from a unit area by resorting to various pre and post stocking management practices (Karplus *et al.*, 1986a, 1987; Smith *et al.*, 1981; Siddiqui *et al.*, 1997; Kurup *et al.*, 2000), however, importance of aquaculture economics is often neglected. The level of production in Kuttanad is mainly dependant on stocking rate, survival rate and growth rate, which are in turn affected by (1) bio-technical factors such as fertilization and feeding, mono or polyculture, different stocking and harvesting strategies, etc; (2) environmental factors such as water quality, diseases and predators, and (3) physical facilities available such as suitability of the site and its construction, soil condition and equipments used. Hence, the cost of production relates to the level of input, the type of culture system, the size of operation, as well as the institutional factors such as costs of credit, marketing, land lease, etc. Therefore,

an increase in yield, reduction in costs and increases in price are the major means of increasing profit. Although prawn farming in tropical areas, such as, Hawaii, has been shown to be profitable by Shang and Fujimura (1977), such a study is lacking in a tropical wetland ecosystem like Kuttanad. Robert and Bauer (1978) discussed the economics of *M. rosenbergii* production for grow-outs in South Carolina. Since the potentiality of prawn farming in these low-lying areas are immense, a study in this direction was found highly warranted.

The differential growth pattern evinced among the male population is one of the major bottlenecks confronting the profitability in the farming of this species. The different size groups of prawns at the final harvest enabled the exporters to grade them under different weight classes and prices. Hence larger prawns demand better prices, while the undersized prawns are usually low priced or non-marketed. In Kuttanad, at present a frail marketing structure persists that is often prone to variation with season and time. Prawns are marketed 'head-on' either under a six grade system (as shown in previous chapters) or under a two grade system (<50 g and >50 g weight classes). The scampi culture can be made economically viable by resorting to an eco-friendly and cost effective culture technique, by resorting to innovative strategies, which will be helpful in minimising individual heterogeneous growth. In the present study an attempt was made to standardize the most appropriate marketing strategy relevant to Kuttanad grow-outs of *M. rosenbergii*. A detailed micro-level economic feasibility study on prawn farming in Kuttanad was also attempted by incorporating the pre and post management practices being followed in this wetland ecosystem.

2. Materials and Methods

In order to bring out the role of definite marketing strategy in the economic feasibility of farming *M. rosenbergii* in two natural grow-outs of Kuttanad, the final harvested production observed from a polder and a coconut garden channel were economically evaluated under two separate marketing price structures. A micro level economic analysis of the various pre and post stocking management practices being practiced in Kuttanad are described in earlier chapters (Chapter 7 to 11) was also carried out. The cost-return data on 40 polders and 34 coconut garden channels were collected with suitably structured and pretested proforma. Cost-return analysis and production function analysis were carried out to bring out the economic viability of prawn culture.

2.1 **Cost return Analysis**: The present study was based on the methodologies of Shang (1990), which includes estimation of production costs, estimation of revenue and economic analysis.

2.1.1 Production cost: The total cost of production referred in the analysis is the cost of all inputs used in farm production in a given production year. The total cost (TC) comprises of two components total fixed cost (TFC) and total variable cost (TVC), i.e., TC = TFC + TVC, and that the input costs are estimated based on the price level prevailing during 1999-2001.

2.1.2 Total Revenue: The total revenue (TR) or the gross revenue referred in the analysis is the total value of production obtained by a grow-out during the culture period, which includes the price obtained from the marketing of prawns. In all the grow-outs studied a six-grade

market structure was followed and the price offered were that prevailing during 1999.

2.1.3 Economic Analysis: In order to evaluate the economic performance of the different aquaculture systems brought under the perview of the analysis, the following conceptual procedures have been adopted.

(a) Net profit (NR) = Total revenue (TR) – Total cost (TC)

(b) Return to capital and management (RKM) = NR + (Depreciation cost + interest)

(c) Rate of return on capital investment (RK) = $\frac{\text{RKM}}{\text{Initial investment}}$

(d) Ratio of profit to operating cost (RTIOP) = $\frac{\text{NR}}{\text{TC}}$

(e) Payback period (PBACK) = $\frac{\text{Initial capital cost}}{(\text{NR} + \text{depreciation cost})}$

(f) Break even price (BEP) = $\frac{\text{TC}}{\text{Quantity of output produced}}$

(g) Break even production (BEPR) = $\frac{\text{TC}}{\text{Unit price of output}}$

2.2 Production function Analysis

The influence of five major inputs (area of pond, stocking density, feed cost, amount of artificial substrates and pond preparation charges) on the production of freshwater prawn *M. rosenbergii* was worked out by regression analysis. Cobb-Douglas production function was worked out to find the bio-economic relation of various inputs on the output. The production function is algebraically expressed as,

$$Y = A\, X_1^{\beta_1} X_2^{\beta_2} X_3^{\beta_3} X_4^{\beta_4} X_5^{\beta_5}$$

Where Y = the average production in kg (or output), A = a constant and $X_1 \ldots X_5$ = the five input variables:

X_1 = Water area in hectare

X_2 = Stocking density (No./ha)

X_3 = Artificial substrates (No./ ha)

X_4 = Pond preparation charges (Rs./ha)

X_6 = Cost of feed (Rs./ha)

$\beta_1 \ldots \beta_5$ = the elasticity of production inputs $X_1 \ldots X_5$

The coefficient of each variable was tested to find out the level of significance employing the t-test. The mean value for output and economic scale was also worked out.

The externalities like environmental factor, social and biological risk factors etc, were assumed to be uniform in all the culture systems, as their effects could not be quantified.

3. Results

The economic evaluation of production in a polder and coconut garden channel under two marketing strategies is shown in Table 12.1. The present exercise was based on the price structure offered by the private company under six-grade system as follows: <50g = Rs.80/-, 50-60 g = Rs. 120/-, 60-80 g = Rs. 160/-, 80-120 g = Rs.200/-, 120-230 g = Rs. 260/- and >230 g = Rs. 320/-, while that for the two grade system was <50 g = Rs. 130/- and >50 g = Rs. 240/-. Results of economic analysis revealed that the margin of profit incurred in the

polder increased from Rs. 16,085/ha under six-grade to Rs. 23,878 under 2-grade system. Contrary to this, in coconut garden channels, six-grade system was found twice as profitable than two-grade system.

Cost and returns per ha from monoculture of *M. rosenbergii* under five different stocking densities is given in Table 12.2. Results showed that with an increase in stocking density of 15,000/ha to 60,000/ha, a corresponding increase in the net production was also visible. However, a direct relationship between the net production and profit gained from each polder could not be established. Maximum returns in profit were registered in polder 3 (Rs. 41,751/-) that was stocked @ 35,000/ha, but with an increase in the net production in polders thereafter, a corresponding decrease in profit margin was observed. Better results for pay back period (4.3 yrs) and break-even price (Rs. 144.6) were registered from polder stocked @ 35,000/ha. Results on the optimization of appropriate stocking density through economic evaluation for obtaining maximum income in four coconut garden channels under different stocking density is shown in Table 12.3. Results on stocking density and corresponding increase in net production were complementary to the observations as seen in polders. Net production increased from Rs. 25,032/- in channel1 (5000/ha) to 60,120/- in channel 4 (25,000/ha). Although the net return increased from channel 1 to 3, no further increase in profit was recorded with a subsequent increase in net production in channel 4. Among the four channels studied, channel stocked @ 15,000/ha gave better results in respect to faster pay back period (3.6 yrs). A stocking density of 5000/ha for channels was found to be non profitable in the present study.

A nutshell on the economic evaluation of three different nursery management systems for the polders in Kuttanad is shown in Table 12.4. Maximum yield (732.4 kg) and corresponding income (Rs. 1,61,128/-) were seen in polders following a two-phase nursery system. In the present study, farming in the polder without a nursery phase was found non-profitable. Although considerable revenue was spent as part of total operational cost for the two polders following either a single-phase or two-phase nursery system marginal profit were not adversely affected. This was mainly due to the high yield registered in these polders (453.1 and 732.4 kg respectively). Analysis of the productivity of major inputs among the three polders gave a clear upper hand for two-phase nursery system. Lesser pay back period (3.8 yrs) and break-even price (Rs. 153.7/-) and higher break even production (512 kg) seen among the two-phase nursery system which implies its importance in increasing the net revenue from *M. rosenbergii* farming in Kuttanad.

The role of artificial substrates in improving the net yield and income from polders were *M. rosenbergii* culture is been carried out has been economically evaluated and presented in Table 12.5. Results show better performance in production and profits in polders having additional shelters in the form of earthen pipes and tiers of net. Results of economic analysis also showed that among the three polders higher values for net yield and revenue were recorded in polders having net tiers as additional substrates (605. 5 kg and Rs. 20, 468/- respectively). Polder devoid of additional substrate was found to be economically non-profitable. Micro-level analysis showed that least pay back

period (6.5 yrs) and break-even price (Rs.186.2/-) were recorded from polder that incorporated tiers of net as additional substrate.

Results of economic analysis on different adaptive trials conducted in the coconut garden channels of Kuttanad incorporating batch graded, size graded and modified batch graded post larvae is shown in Table 12.6. Better results for net returns were recorded in channels stocked with second hatched and early settled post larvae (Rs. 5085/- and Rs. 9018/- respectively). The production cost among the seven channels varied between Rs. 15, 965/- (First hatched batch) and Rs. 22,230/- (Control channel). Although the operational expense for the first hatched post-larval groups was least among the seven channels studied, the output cost (net revenue) did not exceed the input cost (operational expenditure). Hence in this channel the farming was found to be non-profitable. Higher margins of profit encountered in second hatched and first settled batches can be attributed to the better yield registered in these channels (92.5 kg and 103.8 kg respectively). Similarly, the results on productivity analysis of the major inputs showed lower payback period (3.5 and 2.1 yrs respectively) and break-even price (Rs. 205/- and Rs. 173.1/- respectively) for second hatched and first settled post-larval batches. Hence stocking of these sets of post larvae could correspondingly improve the net yield and income from *M. rosenbergii* farming.

Cost and return analysis per hectare of *M. rosenbergii* farming incorporating three different harvesting strategies is shown in Table 12.7. The low yield observed in polder following one-time harvest in the present study was due to the lower survival rate seen at the end of the culture period (refer chapter

11). The low yield manifested non-profitability from these polders. On the contrary, in polders were partial harvesting through draining and cast netting and modified cull harvesting through seining were carried out, the net revenue generated were high and were recorded to the tune of Rs. 30673/- and Rs. 37,816/- respectively. Net yield of the two polders following cull-harvesting strategies was rather uniform. Results of the economic analysis on the productivity of major inputs showed that between the two polders following partial harvesting, lower break-even price (Rs. 186.6/-) and higher break-even production (437 kg) were observed in polders following cull harvesting through seining.

The results of the production function analysis are given in Table 12.8. The Cobb-Douglas production function was,

$$Y = 0.005415\ X_1^{0.27}\ X_2^{0.95}\ X_3^{0.54}\ X_4^{0.11}\ X_5^{0.38}$$

An examination of the elasticity coefficients revealed that three variables viz., stocking density, number of artificial substrates and cost of feed were statistically significant ($P<0.05$). The effect of area of the grow-out and cost for pond preparation on yield was not significant. The R^2 was 0.84 suggesting that the selected five variables in regression indicated a good fit and explained up to 84% of the variation in the production.

4. Discussion

Commercial aquaculture production either for domestic consumption or for exports is generally motivated by profit making. Profitability of a farm is a function of costs and returns. Cost of production depends mainly

on culture technologies used and prices of the production inputs, while the returns depend on production levels and market values of the species (Shang, 1986). Detailed analyses on production and marketing of pond-reared freshwater prawn in Kuttanad clearly showed that the economic income is not only related to the yield but also depends on the farming inputs such as cost for seed, feed, operational expense and also on the population structure of final harvested prawns in terms of their sex ratio, mean weight and morphotypic composition. Sandifer *et al.* (1980) and Liao *et al.* (1982) performed economic comparison of stocking and marketing strategies for *M. rosenbergii* farming in South Carolina. In the present study it was seen that a two grade marketing system was more profitable for polders, while for better economic returns a six-grade marketing strategy was beneficial for the coconut garden channels. This may primarily be because of the duration of culture in polders, which extends from 5 to 8 months. During harvesting the resultant mean weight of the prawn fall just above the 50 g mark and since the percentage of larger prawns (>230 g) is considerably reduced and therefore it was found more appropriate to market the prawns under two grade systems. On the other hand, in channels, the period of culture extends to 10 months or year round, which along with low stocking density prevailing in the grow-out enable the prawns to grow to much larger weight groups (>230 g). Hence marketing under a six-grade system was found to generate more income in the channels. Consequently, large-size males, despite their relatively higher head to tail ratio, command a considerably higher price than females (Lin and Boonyaratpalin, 1988).

The essentiality of standardisation of stocking density in the natural grow-outs of Kuttanad as a mean to improve the economic viability of prawn farming has been brought out in the present study. The results showed that as the price of prawns differed with prawn size and form, the economic yield from the farming was not found to have a linear relationship with the harvestable biomass. At higher densities in polders (60,000/ ha) and channels (25,000/ha) the percentage of females and undersized males were high (refer chapter 7). This resulted in reduction in the percentage of marketable prawn in these grow-outs and thus ultimately a decrease in the net profit could be noticed. The occurrence of female in abundance has been observed not only under various stocking densities (Karplus *et al.*, 1986a), but also with various size-graded juveniles (Karplus *et al.*, 1986b). The numerical female dominance in the final harvested population might be explained by the simple reason that females grew more evenly in size and reached marketable size in greater percentage, while males due to their size disparity were polarized into small and large populations (Brody *et al.*, 1980). Another reason for the diminishing returns with an increase in the density was due to the increased input costs on seed and feed as seen in the present study. The increased costs on seed and feed increased the operational cost without reciprocally improving the marketable yield structure.

Importance of incorporation of a nursery phase and inclusion of additional substrates in grow-outs during farming of *M. rosenbergii* to increase the net yield and income in Kuttanad has been well addressed in the present study. The better profit incurred for two-phase nursery system can be attributed to the advantage out of multiple stocking and harvesting. This helps in

maintaining the stocking density at a reasonable size throughout the culture period. Secondly, since the prawns are grown in a phased manner the expenditure for feed and corresponding management practices can be reduced greatly. Such trials on multi-stage rotational stocking and harvesting system for a round the year culture of *M. rosenbergii* was found profitable by Malecha *et al.* (1981b). Liao *et al.* (1982) showed that by incorporation of the nursery system the growing season of *M. rosenbergii* in South Carolina could be extended to about 2-3 months, which in turn increased the profitability from its culture by 38%. The better performance of two-phase nursery system seen in the present study was in accordance to the reports of Liao *et al.* (1982). Similarly, addition of substrates in the form of tiers of net were more profitable than that of earthen pipes since the former method was less labour intensive and required only two men to lay and remove the nets. Secondly, harvesting was easily facilitated by the removal of tiers, whereas more man power were needed to remove the earthen pipes during harvest from the polders.

Various management strategies comparing the economic influence of pre and post stocking management practices to improve the yield and therefore the profit from *M. rosenbergii* farming has been discussed by Sadek *et al.* (1995), Liao and Smith (1982), Avault and Granados (1995), Lacroix *et al.* (1995), Engle (1987), Fitzgerald (1988) and Sastradiwirja (1986). In the present study, among the various batch grading, size grading and modified batch grading techniques examined, second hatched and first settled larval groups by virtue of their faster growth (refer chapter 9) yielded higher revenue. Although, the net yield from channels stocked with laggards and those maintained as control channels were

high, due to the predominance of under sized males and females the mean weight got reduced, which in turn brought lesser returns. Similarly, even though the mean weight of jumpers were high in the present study their contribution in enhancing the net revenue was hampered due to low survival rates recorded from this group.

The practice of intermediate, partial harvest in Kuttanad was found to be a definitely sound management strategy for two reasons. Firstly, the females that dominated the population and matured relatively early at a small size (30-40 g) could be selectively removed in the early harvest. Selling of berried prawns was also a major source of income to the farmer. Secondly the intermediate harvesting also removed the large males and allowed the production to shift gradually to males, which commanded a greater price. Due to repeated harvesting the population density within the polder was kept at a minimal level, which in turn reduced the quantity of input as feed. Another advantage of partial harvesting was the increase in the mean weight and survival rate of prawns (refer chapter 11), which ultimately resulted in greater number of larger prawns at the time of the final harvest. Net yield from the two polders following cull harvesting through draining and cast netting and modified cull harvesting through seining did not vary much, but the additional expenditure on labour and fuel for the former made it comparatively less economically enterprising.

The results of the production function analysis showed that three inputs viz., stocking density, number of additional substrate and cost of feed were significant, and that increasing the application of these three inputs could substantially increase production. A similar observation through Cobb-Douglas

type of production function showed farm size, capital and management practices as significant factors affecting production in freshwater prawn farms (Liao, 1996) in Taiwan. Therefore it can reasonably be concluded that economic analysis is essential to evaluate the viability of investment in aquaculture, determine the efficiency of resource allocation, improve existing management practices, evaluate new culture technology, assess market potential and identify areas in which research success would have high potential payoffs. In order to bring out an economically viable and sustainable aquaculture practice of *M. rosenbergii* in Kuttanad, standardisation of cost and quantity of input parameters are very essential. The present study advocates a stocking density of 35,000/ha with a two-phase nursery system along with provision for additional substrates and cull harvesting strategies in the farming of *M. rosenbergii* in Kuttanad.

Table 12.1 Costs and returns per hectare per crop in *M. rosenbergii* farming under two different marketing strategies

Particulars	Polder 2 grades	Poder 6 grades	Channel 2 grades	Channel 6 grades
I. COSTS				
A. Variable Cost (Rs.)				
Pond preparation	11934	11934	2351	2351
Fertiliser	3628	3628	3293	3293
Seed	30928	30928	9750	9750
Feed	23778	23778	8658	8658
Power	4207	4207	1072	1072
Labour	32667	32667	8920	8920
Fuel	3564	3564	1656	1656
Total Variable Costs	**110703**	**110703**	**35700**	**35700**
B. Fixed Costs (Rs.)				
Pond construction (apportioned)	42341	42341	4233	4233
Depreciation (5%)	2377	2377	1236	1236
Salary/ Wages	8750	8750	3340	3340
Interest on Fixed capital (18%)	13393	13393	964	964
Total Fixed Costs	**66855**	**66855**	**9773**	**9773**
C. Total Costs (A+B) (Rs.)	**177558**	**177558**	**45473**	**45473**
II.RETURNS				
Total cost of production (Rs.)	177558	177558	45473	45473
Total Yield (Kg)	758	758	159	159
Gross Return (Rs.)	201436	193643	49525	54023
Net Return (Rs.)	**23878**	**16085**	**4052**	**8550**

Table 12.2 Cost and returns per hectare of *M. rosenbergii* farming under five separate stocking densities in the Polders of Kuttanad

Particulars	Polder 1 (15,000/ha)	Polder 2 (25,000/ha)	Polder 3 (35,000/ha)	Polder 4 (45,000/ha)	Polder 5 (60,000/ha)
I. COSTS					
A. Variable Cost (Rs.)					
Pond preparation	7580	10271	8534	13952	14280
Fertiliser	2040	1825	1851	2673	4382
Feed	9000	10400	14778	36251	48250
Seed	10320	15000	21000	27000	36000
Power	320	800	1200	1500	1800
Labour	2750	4800	10000	15000	15000
Fuel	1000	1000	1200	3500	4200
Total Variable Costs	**33010**	**44096**	**58563**	**99876**	**123912**
B. Fixed Costs (Rs.)					
Pond construction (apportioned)	8500	8500	10500	12480	11760
Depreciation (5%)	2200	2500	4500	6000	6000
Salary/ Wages	1230	1850	4500	4500	6000
Interest on Fixed capital (18%)	1370	1520	2110	2845	2670
Total Fixed Costs	**13300**	**14370**	**21610**	**25825**	**26430**
C. Total Costs (A+B) (Rs.)	**46310**	**58466**	**80173**	**125701**	**150342**
II.RETURNS					
Total cost of production (Rs.)	46310	58466	80173	125701	150342
Total Yield (Kg)	257.3	309.8	554.2	745.3	842
Gross Return (Rs.)	56606	68156	121924	163966	185240
Net Return (Rs.)	**10296**	**9690**	**41751**	**38265**	**34898**
Productivity of major inputs					
Return to Capital (RKM)	13866	13710	48361	47110	43568
Rate of return on capital (RK)	0.1261	0.1097	0.2418	0.1884	0.1556
Ratio of profit to operating cost	0.2223	0.1657	0.5208	0.3044	0.2321
Payback period (yrs)	8.8	10.3	4.3	5.6	6.8
Break-even price (Rs.)	179.98	188.72	144.66	168.66	178.55
Break even production (kg)	211	266	364	571	683

Table 12.3 Cost and returns per hectare of *M. rosenbergii* farming under four separate stocking densities in the coconut garden channels of Kuttanad

Particulars	Channel 1 (5,000/ha)	Channel 2 (10,000/ha)	Channel 3 (15,000/ha)	Channel 4 (25,000/ha)
I. COSTS				
A. Variable Cost (Rs.)				
Pond preparation	3520	3850	3000	6000
Fertiliser	520	620	800	1000
Feed	3500	3500	8500	10500
Seed	3000	6000	9000	15000
Power	1200	1025	1230	2210
Labour	1800	1860	6000	7800
Fuel	850	850	1250	2000
Total Variable Costs	**14390**	**17705**	**29780**	**44510**
B. Fixed Costs (Rs.)				
Pond construction (apportioned)	7500	7500	7500	7500
Depreciation (5%)	375	375	375	375
Salary/ Wages	1500	1500	1500	1500
Interest on Fixed capital (18%)	1773	1773	1773	1773
Total Fixed Costs	**11148**	**11148**	**11148**	**11148**
C. Total Costs (A+B) (Rs.)	**25538**	**28853**	**40928**	**55658**
II.RETURNS				
Total cost of production (Rs.)	25538	28853	40928	55658
Total Yield (Kg)	89.4	115.4	182.8	250.5
Gross Return (Rs.)	25032	32312	47528	60120
Net Return (Rs.)	**-506**	**3459**	**6600**	**4462**
Productivity of major inputs				
Return to Capital (RKM)	1642	5607	8748	6610
Rate of return on capital (RK)	-0.52	15.34	27.90	19.35
Ratio of profit to operating cost	-0.02	0.12	0.16	0.08
Payback period (yrs)	0.0	6.5	3.6	5.2
Break-even price (Rs.)	285.66	250.03	223.89	222.19
Break even production (kg)	91	103	146	199

Table 12.4 Cost and returns per hectare of *M. rosenbergii* farming under different Nursery management systems in the Polders of Kuttanad

Particulars	No Nursery	Single Phase Nursery	Two-phase Nursery
I. COSTS			
A. Variable Cost (Rs.)			
Pond preparation	7832	10271	8534
Fertiliser	2589	1825	1851
Feed	10450	18830	23778
Seed	12590	24240	36925
Power	1200	800	1200
Labour	9460	4800	10000
Fuel	2000	1000	1200
Total Variable Costs	**46121**	**61766**	**83488**
B. Fixed Costs (Rs.)			
Pond construction (apportioned)	8500	8500	10500
Depreciation (5%)	2200	2500	4500
Salary/ Wages	1230	5200	12000
Interest on Fixed capital (18%)	1370	1520	2110
Total Fixed Costs	**13300**	**17720**	**29110**
C. Total Costs (A+B) (Rs.)	**59421**	**79486**	**112598**
II.RETURNS			
Total cost of production (Rs.)	59421	79486	112598
Total Yield (Kg)	257.3	453.1	732.4
Gross Return (Rs.)	56606	99682	161128
Net Return (Rs.)	**-2815**	**20196**	**48530**
Productivity of major inputs			
Return to Capital (RKM)	755	24216	55140
Rate of return on capital (RK)	0.02	0.20	0.28
Ratio of profit to operating cost	-0.05	0.25	0.43
Payback period (yrs)	0.0	5.3	3.8
Break-even price (Rs.)	230.94	175.43	153.74
Break even production (kg)	270	361	512

Table 12.5 Cost and returns per hectare of *M. rosenbergii* farming with and without the inclusion of artificial substrates in Polders of Kuttanad

Particulars	Without Substrate	Pipes as Substrate	Net tiers as Substrate
I. COSTS			
A. Variable Cost (Rs.)			
Pond preparation	5300	9250	11934
Fertiliser	2344	1973	3628
Feed	8500	20236	34580
Seed	12590	24240	24240
Power	1500	1200	1500
Labour	5000	9600	10500
Fuel	1250	1500	1200
Total Variable Costs	**36484**	**67999**	**87582**
B. Fixed Costs (Rs.)			
Pond construction (apportioned)	5850	9210	10500
Depreciation (5%)	1630	1800	2550
Salary/ Wages	4500	6730	10000
Interest on Fixed capital (18%)	2770	2850	2110
Total Fixed Costs	**14750**	**20590**	**25160**
C. Total Costs (A+B)	**51234**	**88589**	**112742**
II.RETURNS			
Total cost of production (Rs.)	51234	88589	112742
Total Yield (Kg)	205.2	453.1	605.5
Gross Return (Rs.)	45144	99682	133210
Net Return (Rs.)	**-6090**	**11093**	**20468**
Productivity of major inputs			
Return to Capital (RKM)	-1690	15743	25128
Rate of return on capital (RK)	-0.03	0.13	0.17
Ratio of profit to operating cost	-0.12	0.25	0.43
Payback period (yrs)	-13.5	9.3	6.5
Break-even price (Rs.)	249.68	195.52	186.20
Break even production (kg)	233	403	512

Table 12. 6 Cost and returns per hectare of *M. rosenbergii* farming in the channels incorporating different adaptive trials

Particulars	1st hatched batch	2nd hatched batch	Early settled batch	Late settled batch	Jumpers	Laggards	Control
I. COSTS							
A. Variable Cost (Rs.)							
Pond preparation	1200	1200	1200	1200	1200	1500	2800
Fertiliser	100	100	100	100	100	100	100
Feed	2240	2240	2045	2240	1240	2240	2800
Seed	6000	6000	4500	6000	6000	6000	6000
Power	200	200	500	200	250	600	200
Labour	1200	1200	1200	1200	1200	1200	1680
Fuel	800	1500	1800	1800	1500	1800	1700
Total Variable Costs	**11740**	**12440**	**11345**	**12740**	**11490**	**13440**	**15280**
B. Fixed Costs (Rs.)							
Pond construction (apportioned)	1500	2500	2500	2800	2500	2500	2800
Depreciation (5%)	625	625	625	650	625	625	650
Salary/ Wages	1500	2400	2500	2500	2500	2500	2500
Interest on Fixed capital (18%)	600	1000	1000	1000	1000	1000	1000
Total Fixed Costs	**4225**	**6525**	**6625**	**6950**	**6625**	**6625**	**6950**
C. Total Costs (A+B) (Rs.)	**15965**	**18965**	**17970**	**19690**	**18115**	**20065**	**22230**
II.RETURNS							
Total cost of production (Rs.)	15965	18965	17970	19690	18115	20065	22230
Total Yield (Kg)	60.85	92.5	103.8	82.5	63.7	96.8	111.5
Gross Return (Rs.)	15821	24050	26988	21450	19110	21296	24530
Net Return (Rs.)	**-144**	**5085**	**9018**	**1760**	**995**	**1231**	**2300**
Productivity of major inputs							
Return to Capital (RKM)	1081	6710	10643	3410	2620	2856	3950
Rate of return on capital (RK)	0.05	0.34	0.53	0.17	0.13	0.14	0.20
Ratio of profit to operating cost	-0.01	0.25	0.43	0.09	0.25	0.43	0.43
Payback period (yrs)	41.6	3.5	2.1	8.3	12.3	10.8	6.8
Break-even price (Rs.)	262.37	205.03	173.12	238.67	284.38	207.28	199.37
Break even production (kg)	73	86	82	90	82	91	101

Table 12.7 Cost and returns per hectare of *M. rosenbergii* under three different harvesting strategies in Polders of Kuttanad

Particulars	Full Harvest	Cull Harvest	Modified cull harvest
I. COSTS			
A. Variable Cost (Rs.)			
Pond preparation	8500	12000	10500
Fertiliser	1500	2200	2400
Feed	10580	26350	32580
Seed	15000	18500	18500
Power	750	1000	1000
Labour	3500	9500	10500
Fuel	2000	3500	2800
Total Variable Costs	**41830**	**73050**	**78280**
B. Fixed Costs (Rs.)			
Pond construction (apportioned)	5850	5225	5500
Depreciation (5%)	1630	1800	1850
Salary/ Wages	4500	6730	8500
Interest on Fixed capital (18%)	2770	2850	2110
Total Fixed Costs	**14750**	**16605**	**17960**
C. Total Costs (A+B)	**56580**	**89655**	**96240**
II.RETURNS			
Total cost of production (Rs.)	63820	89655	96240
Total Yield (Kg)	185.5	462.8	515.6
Gross Return (Rs.)	48230	120328	134056
Net Return (Rs.)	**-15590**	**30673**	**37816**
Productivity of major inputs			
Return to Capital (RKM)	-11190	35323	41776
Rate of return on capital (RK)	-0.19	0.29	0.28
Ratio of profit to operating cost	-0.24	0.25	0.43
Payback period	-4.3	3.7	3.8
Break-even price	344.04	193.72	186.66
Break even production	290	408	437

Table 12.8 Estimated Cobb-Douglas production function for *M. rosenbergii* production in polders of Kuttanad following a modified extensive system

	INPUTS				
Intercept = 0.005415 **R square = 0.84** **Net production = 616.4 kg/ha**	**Area (ha)**	**Stocking Density (No./ha)**	**Artificial substrate (No/ ha)**	**Cost for Pond preparation (Rs/ ha)**	**Cost of Feed (Rs/ha)**
Production coefficient	0.27	0.95	0.54	0.11	0.38
Standard error	0.62	2.88	2.67	0.49	0.95
t-value	0.54	3.07	2.91	1.06	1.58
Level of significance	NS	P < 0.01	P < 0.01	NS	P < 0.05
Input means	1.2	38,450	3500	9555.5	254

NS = Not significant

Summary

Macrobrachium rosenbergii (de Man), the giant freshwater prawn with trade name Scampi has emerged as the prime candidate species for freshwater aquaculture in different parts of the Indo-pacific regions. A sound knowledge on the biology of this species, its rapid growth and bigger size it could attain, greater disease resistance and good demand in both domestic and export markets have made it the most dear species for culture in the freshwater grow-outs of India. Moreover, with the successful development of a larviculture technology for its seed production in the past few decades, a new wave of enthusiasm among the 'scampi' farmers could be noticed which has paved the way for the massive propagation of its modified extensive monoculture or integrated polyculture farming of this species along with fish species like Catla, Rohu and Mrigal. Kerala is endowed with large freshwater resources and low-lying paddy fields, which have been traditionally utilised for rice cultivation alone. Kuttanad, the rice bowl of Kerala, encompasses an intricate polder system, which has been constructed over the years for agricultural purposes. *M. rosenbergii* being a denizen of the region, gained more attention as a desirable species for the aquaculture in the polders and coconut garden channels of Kuttanad.

A preliminary survey was conducted in the Kuttanad region (Kerala) with a view to collect details of total area brought under farming practices of *M. rosenbergii* and also to bring out its techno-economic feasibility. From the data so obtained, it could be seen that scampi culture is being taken up

as a follow up crop of paddy in a most arbitrary manner and without following any scientific norms and principles. The survey revealed that the culture of *M. rosenbergii* in Kuttanad is basically carried out in three types of grow-outs such as polders, homestead ponds and coconut garden channels. However, the principal biological limiting factor affecting the production of this species in grow-outs appears to be the differential growth evinced by the adult males in the final harvested population. In the farming sector where no concern is shown to minimize this heterogeneous individual growth, a sizeable portion of the prawns in the final harvested population fall below the acceptable marketable size structure. Against this background, the proposed study was undertaken to understand and to unravel the factors underlying the size heterogeneity in *M. rosenbergii* and also to develop an eco-friendly, economically sustainable and scientific culture practice incorporating innovative management strategies to improve the marketable yield and income from scampi farming practiced in Kuttanad.

Hatchery trials to investigate the role of ontogenesis in Heterogeneous Individual Growth (HIG) showed that later hatched larvae had faster growth, metamorphosis and attained the PL stage much faster than "First hatched" batch. Post larvae obtained from "Major hatched" batch were faster, larger and more active when compared to "Minor hatched" batch. Heterogeneity among the differentially settled PL were least for "First settled" group. HIG was evident from the first day of hatching itself, and a definite variation in the rate of metamorphosis, stage progression, growth and survival of larvae could be

discernible during ontogenesis depending up on the differential hatching order and intensity.

Investigations on the intrinsic factors associated with differential somatic growth seen among the male morphotypes of *M.* rosenbergii showed that a significant difference in the percentage composition of protein, total amino acids and RNA/DNA ratios in all the morphotypes could be observed in the muscle tissue. Highest values of protein were recorded in t-SOC and SOC, while SM and OBC registered the lowest. Highest value of total amino acid and free amino acid were recorded in SM, in contrast to the lowest values in t-SOC and WBC. In all the morphotypes studied, the level of acidic amino acids such as Glutamic acid and Aspartic acid were found high. The results of pair wise analysis and ANOVA, which showed significant difference between SM, OC and BC morphotypes in their biochemical composition, indicated that there exists a clear distinction in the biochemical buildup of various male morphotypes of *M. rosenbergii*. The variations in the biochemical composition of male morphotypes can be considered as an index for heterogeneity. In the present study, a perceptible difference in the levels of RNA and DNA was noticed, which may further be responsible for the difference in the protein anabolism. Hence, the difference in protein synthesis seen among various male morphotypes of *M. rosenbergii* can be considered as one of the major intrinsic factors governing heterogeneous individual growth in this species. Investigations on the protein nature through electrophoresis showed that the banding pattern for native PAGE had only slight variation, but a clear difference in SDS-PAGE bands could be observed for all the morphotypes. Percentage of low molecular weight proteins

increased as morphotypes transformed to successive stages. Myofibrillar fraction followed by sarcoplasmic protein fraction contributed maximum percentage of protein fraction in all the morphotypes studied. The amino acid make up of each morphotype is different and hence the protein resulting from the binding of these amino acids also vary in composition and structure giving the muscle tissue a different composition. This could be a major factor whereby the texture of the prawn changes from SM → OBC. The results of protein fractionisation further confirmed these finding. From the results of the present study, it can be concluded that protein differentiation through fractionisation and electrophoretic separation can be taken as benchmarks for further biochemical characterization studies related to intraspecies variations in protein synthesis. Moreover, results of the present study manifest the possibility of genetic involvement in the size disparity among male morphotypes of *M. rosenbergii*.

Electronmicroscopic studies on the hormone producing centres of male morphotypes of *M. rosenbergii* were carried out and the results show that there exists a distinct structural difference in the cytology of tissues analysed. The cells of OC had larger and numerous exogenous vacuoles when compared to BC. Deposition of chromatosomes in chromatophores were more in BC followed by OC. Cell extension in BC was 1.5 μm, which was much lesser in OC (1.1 μm) and SM (0.65 μm). Colouration of cheliped was found to be a cumulative effect of pigment migration to chromatophore as well as the stretching of microtubules. There exist a clear differentiation in the structural and cellular activity of chromatophores in different male morphotypes of *M. rosenbergii*.

Commensurating with the morphotypic transformation from SM to OC to BC, a change in cellular activity could also be observed. The pigment migration in *M. rosenbergii* is a unidirectional process, whereby only accumulation and dispersion of pigment granules occur within the chromatophore and the pigments do not move out of the chromatophore. The claw colouration distinct to a particular morphotype can well be attributed to the difference in the pattern of pigment migration seen among the three morphotypes. With progressive transformation of a morphotype to its successive stages, more and more pigments enter the chromatophore and in accordance with this, a change in the shade as well as tint of the cell could be seen. The uniqueness in the intensity of claw coloration in each morphotype is in turn the result of pigment aggregation and corresponding cellular extension. A perceptible involvement of microtubules in the transport of pigment granules to the chromatophore could be discernible. Furthermore, the influence of neuro-secretory centres of the sinus gland in the production of pigment dispersing hormone could also be addressed in the present study.

Allozymes evaluated were Alcohol dehydrogenase (ALDH), Esterase (EST-1), Isocitrate dehydrogenase (IDH), Malate dehydrogenase (MDH), Aldehyde oxidase (AO), Glucose 6-phosphate dehydrogenase (G6PDH), Glucose 6-phosphate isomerase (GPI), Lactate dehydrogenase (LDH), Octanol dehydrogenase (ODH), Phosphoglucomutase (PGM) and Aspartate amino Transferase (AAT-1). Allellic frequency of isozymes of different morphotypes were analysed using the zymogram pattern of homozygotic and heterozygotic bands. The results of Allozyme characterisation studies revealed that a clear

difference in the banding pattern of 5 isozymes could be possible for MDH, G6PDH, AAT-1, EST-1 and PGM. Maximum Allelic variation was observed for AAT-1 and G6PDH and specific allelic loci for OC and BC were recorded from PGM. The result of genotypic differentiation studies showed that the dissimilarity of allelic expression in terminal morphotypes was not significant. Distance of genetic diversity among gene expressing loci was more for SM to BC (0.0055) followed by OC to BC (0.0043) and SM to OC (0.0021). It could, therefore, be concluded that three morphotypes of freshwater prawn *M. rosenbergii* differ both in their morphology and pattern of gene expression. The high value of genetic similarity between them indicates that degree of differentiation within these morphotypes is less. In contrast, the results of genetic distance showed a close relatedness between SM and OC morphotypes, while the BC showed maximum heterozygosity with its counterparts. In all the three morphotypes, 5 separate polymorphic loci were seen. These polymorphic loci occupied specific allelic loci in each morphotype and only the position of these allele differed between morphotypes. The polymorphic loci found during the present study were *MDH**, *G6PDH**, *EST**, *AAT** and *PGM** enzyme systems. The high genetic similarity observed in all the three morphotypes rules out the possibility of a separate species status for each morphotype. However, a noteworthy observation was that there occurs a perceptible variation in the enzyme-banding pattern of the three morphotypes, which in turn, manifests the possibility of genetic involvement for the size heterogeneity seen among the single aged population of adult male morphotypes of *M. rosenbergii*.

Of the 23 primers screened (OPA 1-20; OPAD 4,5 and 17) best 6 primers were selected for the present study. A distinct difference in the banding pattern for 6 primers namely OPA-2, OPA-4, OPA-6, OPA-7, OPA-8 and OPA-9 could be recorded. Maximum dissimilarity for all the morphotypes was recorded in OPA-2 (3.82) followed by OPA-6 (2.96) and OPA-8 (1.43). Results of similarity indices showed that the relatedness in the banding pattern of DNA for SM to OC and less when compared to that of BC. Genetic distance between SM to BC was high (0.0076) followed by OC to BC (0.0045) and SM to OC (0.0018) and this would be useful in proving the transformation pathway of SM→OC→BC. It would, thus, appear that a genetically significant difference at the molecular level exits among the male morphotypes of *M. rosenbergii*, which contributes to its size heterogeneity. Though this difference is perceptibly intrinsic to all morphotypes studied, however their expression is mainly governed by the extrinsic factors. The results of F analysis and genetic similarity index confirm the transformational pathway of this species. The phenotypic expression of the three different morphotypes can be directly corroborated with their genotypic scores. However, the genetic variability studied through DNA amplification techniques in the present study showed significant variations only in the banding pattern of SM. Hence, it could be reasonably be concluded that SM occupies a unique place in the genetic status among the three male morphotypes of *M. rosenbergii*. The genetic diversity within the morphotypes was low and hence assigning of a separate species status to them may not be justifiable. The pattern of banding for SM was diverse and showed significant variation ($P<0.05$). The results emerged from the present study also suggest a

genetic involvement in determining the phenotypic traits. The distinctive growth variations and associated changes in the morphology of freshwater prawn were found related to the differences in the sequenced product of DNA.

Density dependant variation in the population structure could be discernible in the population of *M. rosenbergii* raised in coconut garden channels and polders. Mean weight of prawns was greatly reduced at higher densities. Dominance of undersized SM and WOC was glaring in the final harvested populations in channels and polders stocked under high densities. A stocking density of 15,000/ha for channel and 35,000/ha for polders was found to be good to minimize the size disparity of the harvested population and thus improving the economic returns from the farming of scampi. While working on the marketable yield structure and profit incurred from culture under different densities, it was seen that increase in stocking density beyond 35,000/ha in polders and 15,000/ha in coconut garden channels did not make any difference in the profit due to the dominance of undersized prawns in final harvest. A reduction in stocking density was helpful in increasing the mean weight of prawns, however, it negatively contributes to total yield and thus farming became uneconomic. Based on the present study it can reasonably be asserted that relative proportion of larger OC and BC morphotypes in final population profoundly is profoundly influencing the economic viability of 'scampi' farming. In order to produce higher percentages of these morphotypes, it is highly essential to maintain the stocking density at an optimum level and any addition beyond this will culminate in the reduction of mean weight of prawns. While maintaining an initial stocking density at 3.5/m^2 in polders and 1.5/m^2 in coconut garden channels, a linear

relationship between the economic returns and corresponding profit could be established, which otherwise was not possible in other stocking densities. Therefore, stocking at optimal levels is found as one of the essential prerequisite in the farming of *M. rosenbergii* for ensuring better marketable yield structure and reciprocally the economic returns.

Better results on mean weight, net production and marketable yield structure were observed in polders following two-phase nursery system. The survival rate showed an increase to 54% in those trials were a two-phase nursery system has been incorporated when compared to mere 26% recorded in 'no-nursery phase' culture. Cost and rate of feed were reduced considerably by 30% due to this fragmented culture technique. The percentage contribution of undersized non-marketable prawns to the final harvested population was less than 10% when compared to the glaringly high values (26%) without a nursery phase. Mean weight and correspondingly the net yield from polders following the two-phase nursery system were significantly high. The advantage of the two-phase nursery system is that the rearing density initially can be kept very high and subsequently can be reduced at one or more levels so that intraspecific growth inhibition and agonistic behavior developed commensurating with the growth of prawns can be significantly reduced. No matter what the initial density is in the diphasic approach, rearing space is more efficiently utilised as rearing density is closely tailored to the prawns' behavioral and physiological response. Further, prawn population size can be assessed each time the animals are harvested and restocked. Since in a wetland ecosystem like Kuttanad, availability of space for post larval rearing do not pose any problem and therefore, adoption

of a two-phase nursery system could be well advocated for an year round farming of *M. rosenbergii*.

The fragile market structure prevailing in Kuttanad is another bottleneck in affecting the successful farming of scampi in this area. The main criteria of the market structure is the size of the prawn and as a result, the farmers are compelled to extend their culture period to another two more months, which adversely affects the total economics. In the present study, higher proportion of larger prawns falling under 120-230 and >230 group were held responsible for the overall production profit in channels stocked with 'jumpers' and 'later hatched' larval groups. Since the survival rates from 'jumpers' were relatively less, its culture was not much economically profitable. On the contrary, the later hatched larval group showed good survival results. Average wet weight and total yield observed in the present study for later hatched larval groups advocate stocking of these groups in channels and polders. Highest survival rate was observed in "Laggards" (24%) while it was least in "Jumpers" (12%). Better mean weight of prawns was recorded from Jumpers (83.11g) followed by first settled (69.23g) and later hatched (67.62g) batches. Predominance of females and undersized SM were seen in Laggards (48% & 12%) and first hatched (50% & 14%) batches. Therefore, stocking of laggards and first hatched larval groups would reduce the net yield from the culture and is not advisable to stock these groups.

Addition of artificial substrate produced linear increase in total production without making any compromise in the average weights. Results of

outdoor tank experiments showed that with an increase of an additional substrate by 30%, the survival rate, mean weight and net production from the tanks showed a significant improvement. Performance of nylon nets erected in the form of tiers gave promising results in terms of net yield and percentage of marketable prawn groups. Besides, these types of substrates are easily replaceable at the time of harvest and therefore can be advocated. Grow-outs provided with 30% additional substrates showed best performance with high survival rate (75.75%), faster stage transformation and high mean weight (8.1 g). In grow-outs, the additional substrates increased the survival rate henceforth by 22%. The substrates facilitated faster morphotypic transformation and thenceforth the preponderance of terminal morphotypes was quite discernable, besides, the mean weight also showed an increase from 45.4 to 68.8g. Retrieval rate from the nursery with netting under 3-tier system was 80% and this explains the favourable condition for the prawns due to the presence of added substrates.

The importance of cull harvesting and modified cull harvesting in improving the marketable yield structure could be established in the present study. The practice of intermediate, partial harvest in Kuttanad was definitely found to be a sound management strategy for two reasons. Firstly, the females that matured relatively early (berried females) could be selectively removed in the early harvest. This would not only provide an additional income to the farmer, but also would reduce the density in the pond and thereby feed cost can be minimized. Secondly, the intermediate harvesting was found useful in recovering large males and thus helpful in resolving the problems of social hierarchy existing among males. The interval between subsequent harvests was

also found very crucial. Since the Blue-clawed males showed an aggressive sexual and territorial behavior that would influence the final production, their removal from the population was highly warranted. Against this, the growth of small-clawed males can be limited to orange-clawed stage since there is a great demand for this morphotypes in export market due to its proportionately greater tail weight. Thus, production of larger OC males would be a viable means for augmenting biomass and economic yield in Kuttanad and elsewhere in the world. Modified cull harvesting system, by using drag net for the selective removal of males and females is more profitable and effective in scampi farming in Kuttanad. Since modified cull harvesting avoids complete draining of the polder, the cost of draining the pond and other labour charges were invariably less, which otherwise negatively influenced the economic returns. Moreover, through selective cull harvesting, farmers could market the specific grade of prawns that fetched them premium prices.

Marketing based on two size grades was found profitable and therefore advantageous for farmers of Kuttanad for polders with culture duration of 6 months. In contrast, a six-grade structure can be recommended for coconut garden channels. Stocking density of 35,000/ha in polders and 10,000/ha in channels were found to be economically sustainable for monoculture of scampi in Kuttanad. The revenue generated from two-phase nursery system was found more profitable than single-phase nursery system. Income from the polders having artificial substrates and shelters were relatively high when compared to those without it. Based on the results generated in the present study, it can be concluded that success of freshwater prawn farming is mainly dependant on the

selection and incorporation of appropriate management strategies. By stocking second hatched or first settled post larvae at a stocking density of 35,000–40,000/ha for polders and 10,000/ha for channels, the production can be increased by 40% in polders and 25% in channels. Along with the incorporation of a two-phase nursery system followed by modified cull harvesting technique the survival rate, mean weight and net production from the grow-outs can be significantly increased. A steady market structure with two-grade is inevitable for making the culture of *M. rosenbergii* economically more viable and sustainable in Kuttanad, which also allows the farmers to curtail their farming operations from existing 8 months to 6 months. This inturn reduces the operational costs left aside for feed and management. In order to bring out an economically viable and sustainable aquaculture practice of *M. rosenbergii* in Kuttanad, standardisation of cost and quantity of input parameters is very essential. Based on the results of this study, it can be concluded that under monoculture system, a stocking density of 35,000/ha by incorporating a two-phase nursery system along with the addition of net as artificial substrates, periodic cull harvesting and adoption of a two grade market system resulted maximum economic returns and therefore recommended for the sustenance of farming of *M. rosenbergii* in polders of Kuttanad (S. India).

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2. Ranjeet. K and Kurup, B. M., 2001. On the successful utilisation of paddy fields for *Macrobrachium rosenbergii* farming in Kuttanad. Paper presented at the National Symposium "Riverine and Reservoir Fisheries- Challenges and Strategies", CIFT, Kochi. Pp, 370-377.

3. Kurup, B. M and Ranjeet. K. 2001. On the successful integration of *Macrobrachium rosenbergii* (de Man) and rice in the polders of a tropical wetland ecosystem. In: Proc. Aquaculture and Environment, (Eds.) N. R. Menon, B. M. Kurup & R. Philip. Pp: 45-59.

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