EFFECT OF SALINITY ON SURVIVAL AND GROWTH OF MACROBRACHIUM ROSENBERGII (De Man)

Ву

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THESIS

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MASTER OF FISHERIES SCIENCE

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DEPARTMENT OF AQUACULTURE COLLEGE OF FISHERIES PANANGAD - COCHIN

To MY PARENTS, BROTHERS AND SISTERS

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DECLARATION

I hereby declare that this thesis entitled "EFFECT OF SALINITY ON SURVIVAL AND GROWTH OF <u>MACROBRACHIUM ROSENBERGII</u> (DE MAN)" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled "EFFECT OF SALINITY ON SURVIVAL AND GROWTH OF <u>MACROBRACHIUM ROSENBERGII</u> (DE MAN)" is a record of research work done independently by Sri. Venugopalan. I.K. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to him.

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I. INTRODUCTION

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I. INTRODUCTION

Crustaceans form one of the most valuable food resources of the world by virtue of their importance as an esteemed delicacy and as an item of export. Of late, the harvest of commercial prawns suffers great seasonal variability and has failed to keep pace with the ever increasing demands of the domestic and export markets. In order to minimize the reliance on capture fisheries and also to have an year round supply of prawns several attempts are being made to culture both freshwater and marine forms in captivity. Intensive efforts are underway in many parts of the world to work out cost-effective technologies suited to the areas as well as the species under consideration.

Lately, the culture of freshwater prawns as a means of increasing food production is gaining importance in many countries. Among the freshwater cultivable prawns the genus <u>Macrobrachium</u> is by far the most important. Of the well over 125 species of <u>Macrobrachium</u>, only a few species like <u>Macrobrachium rosenbergii</u>, <u>M. acanthurus</u>, <u>M. carcinus</u>, <u>M. ohione</u>, <u>M. lar</u>, <u>M. americanum</u>, <u>M. malcolmsonii</u>, <u>M. asperlus</u>, <u>M. lanceifrons</u>, <u>M. vollenhoveni</u>, <u>M. rude</u> and <u>M. tenellum</u> are of importance from the culture point of view.

As in the case with many other crustacean resources, <u>Macrobrachium</u> fishery is also faced with problems like over fishing during the spawning season, deterioration of its natural habitats and spawning grounds due to pollution and the obstruction during spawning migration, owing to the construction of water exclusion bunds and modern irrigation systems. Since the deteriorating conditions prevailing in the natural habitat of the prawn are not easily resolvable, commercial production of seed in hatcheries and rearing them to marketable size in ponds have become the only alternatives. Of the different species of <u>Macrobrachium</u>, the giant prawn, <u>M. rosenbergli</u> is considered as the most potential species for aquaculture owing to its fast growth rate, hardiness and very high consumer preference. As a result of the special attention and interest, the culture of this species has become a commercial reality in many countries today (Ling 1969 a; Uno and Soo 1969; Singholka 1978; Aquacop, 1979 b).

Farming of <u>M. rosenbergii</u> has gained importance on a global basis, after the successful attempts in rearing the larvae of this species (Ling 1962 & 1964; Ling and Costello, 1976) and the development of mass culture techniques (Fujimura and Okamota 1972; Fujimura 1974; Aquacop, 1977a). Within a short span of time, the technique of larval rearing underwent considerable refinement and improvement in several countries.

Formulation of improved aquaculture practices calls for the elucidation of the environmental requirements for culture of the target species. The increasing interest in the culture of M. rosenbergii has stimulated a series of investigations on its environmental requirements. Among the abiotic factors, salinity assumes greater significance, as this influences the survival, growth, distribution and food intake of many organisms. Many species of palaemonid prawns are known to inhabit in a wide variety of environmental salinities. The giant freshwater prawn M. rosenbergii is also reported to adapt to a wide range of salinities and in order to take up controlled cultivation of this prawn, it is necessary to know its maximum growth, survival and production potentialities under different ecological conditions. Data on both optimal, acceptable and stress ranges of environmental conditions for successful rearing of this species is needed and once the ideal conditions for growth are found out, it is possible to control the environment by various management techniques in order to achieve maximum production.

A knowledge of the survival and growth under different salinities within the tolerance range of this euryhaline species, will be of help in utilizing vast stretches of low saline areas for its culture. This is of particular significance especially in developing countries like India having vast areas of saline swamps.

The present investigation is undertaken with a view to elucidate the optimum salinity conditions for the successful culture of this commercially important prawn species as the information available so far in this regard, on this species is not adequate.

The effect of salinity on the survival of M. rosenbergii had been determined by conducting short-term tolerance studies by subjecting them under varying salinities from 0 - 35 ppt over a period of 120 hrs. To find out the optimum range of salinity for best growth, the juveniles of M. rosenbergii were cultured in different ranges of salinities from 0 - 10 ppt in cement cisterns for a period of 3 months. A field study was also conducted in one fresh and two brackishwater ponds of varying salinities to get further confirmation of the results obtained in the laboratory, by culturing the prawns at a stocking density of 10,000/ha for a period of 100 days. The oxygen consumption and ammonia excretion of prawns reared at different salinity levels were studied and 0: N ratios estimated since they are likely to give an index of the optimum salinity levels for growth.

11. REVIEW OF LITERATURE

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II. REVIEW OF LITERATURE

Salinity is reported to have profound influence on survival, growth, distribution and food intake of a number of fresh water organisms including <u>M.rosenbergii</u>. This species is widely distributed in fresh and brackish water environments and is presently the focus of attention as a suitable species for culture. The available information on this important prawn, especially on distribution, biology, growth survival and production in various natural and culture systems, environmental requirements for culture and the possible effects of salinity on its growth, survival, oxygen consumption, ammonia excretion and oxygen : nitrogen ratios are reviewed.

2.1 Distribution

<u>M. rosenbergii</u> commonly known as the giant freshwater prawn is widely distributed in the Indo-Pacific region (Ling, 1962, 1969a; Holthuis and Rosa, 1965; Johnson, 1967; George, 1969). It occurs year round in most of the rivers especially the lower reaches which are under tidal influence extending upto about 200 Km from the coast (Ling, 1969a). Djajadiredja and Sachlan (1956) indicated this species as economically important in the Indonesian Islands of Sumatra, Java, Borneo, Celebes and the Lesser Sunda Islands. Ahmad (1957) mentioned its occurrence in estuaries in Bangladesh and McVey (1975) reported this species from the Palau Islands. Johnson (1968) and Longhurst (1970) reported the existence of a commercial fishery of this species in Malaysia and Indonesia.

This species has been transplanted to different parts of the world like Hawaii, Africa, the Caribbean, Central and South America, Israel, Japan, Mauritius, Tahiti, Taiwan and the United Kingdom for research and culture purposes (New and Singholka, 1982; Sandifer and Smith, 1985).

In Indian waters it is reported to occur along both the coasts, with the distribution on the West Coast from Indus Delta to Malabar coast and in the east coast in deltaic Bengal (George, 1969). It supports a more or less good fishery in the backwaters and Pampa river system of Kerala (Raman, 1967) and the Hooghly estuarine system in West Bengal (Rao, 1967). Jones (1967) indicated a regular fishery for the species in the Bombay area, Kerala and the Northern half of east coast and in other areas of the Indian coasts, the fishery is reported to be occasional.

2.2 Size and Growth

Among the freshwater prawns, <u>M. rosenbergii</u> grows to the largest size. The maximum length of the species according to Patwardhan (1937) is 3 feet (90cm) from tip of the telson to tip of extended second leg. The maximum size recorded for the species is 320mm for male and 250mm for female (Holthuis, 1980). Raman (1967) has also recorded a maximum size of 320mm in the case of males. But, Jayachandran and Joseph (1982) reported the capture of a male and a female measuring 326mm and 283mm respectively from the Valapattanam river.

Rajyalakshmi (1962) has investigated the age and growth of this species occuring in the Hooghly estuary and found that sexual dimorphism in growth is exhibited by this species with the males attaining lengths of 107 and 149mm at the end of first and second years of life while females 82.5, 103.5 and 168.5mm at the end of first, second and third years respectively. According to Rao (1967) of the immature individuals having a total length above 30mm, males moult 6 times a year while females moult only 5 times indicating better growth for males. Ling (1969a) on the other hand found the growth rates of young males and females to be the same, and after reaching a length of about 18cm and a weight of about 60g, the growth rate of females decreases, and there is little growth beyond 22cm in length and 120g in weight, but the males keep on growing to about 200g each. However, Sandifer and Smith (1985) found that, while females and males grow at the same rate upto a mean size of about 17g, slowing of the growth rate in the female becomes apparent at sizes above 25g or so. <u>M. rosenbergii</u> is reported to be omnivorous in its feeding habit (John, 1957; Johnson, 1967; Raman, 1967; Rao, 1967; George, 1969; Ling, 1969a). Examination of stomach contents in the field indicates that this species ingests a wide variety of food items, of both plant and animal origin, as well as detritus, thus functioning as a primary consumer, a secondary consumer and a detritivore in its natural environment (John, 1957; Rao, 1967). According to Ling (1969a) common items of food include aquatic worms, aquatic insects and insect larvae, small molluscs and crustaceans, flesh and offal of fish and other animals, grains, seeds, nuts, frults, algae and tender leaves and stem of aquatic plants. John (1957) observed that the prawn's diet depends on its environment. She found that the stomach contents of prawns collected from canals, rivers and lakes consisted largely of organic detritus, crustacean appendages, vegetable matter and molluscan fragments. Quantitative and qualitative analysis of digestive enzymes have confirmed the omnivorous nature of <u>M. rosenbergii</u> (Lee <u>et al.</u>, 1980).

The cannibalistic nature of this species is reported by many workers (Rao, 1965; Ling, 1969a; Wickins, 1972; Forster and Beard, 1974; Segal and Roe, 1975; Peebles, 1977) and Rao (1965) had noted them eating their own moult and dead eggs.

2.4 Breeding Biology

2.4.1 Breeding Season.

The breeding season of <u>M. rosenbergii</u> is different in various river systems. Rajyalakshmi (1961) and Rao (1967) found this species to have a restricted spawning season in the Hooghly estuary, extending from December - July with peak spawning during March - May. Raman (1967) reported the spawning period of <u>M. rosenbergii</u> in Kerala to be from August - December with a peak in October - November. Rao (1967) considers the rise in temperature and salinity during the season as the physiological drive for attaining maturity and undertaking spawning migration. This species is reported to have almost year round breeding in Balgoda lake in Srilanka, with two peak spawning seasons - a major one from May - July and a minor one from November - January (Jinadasa, 1985).

2.4.2 Breeding Migration.

<u>M. rosenbergii</u> performs an interesting spawning migration from its original fresh water habitat to estuarine regions and spawns in areas where salinity fluctuates between 5 and 20 ppt. (George, 1969). After metamorphosis, the young juveniles remain in brackish water areas for 1 or 2 weeks and on attaining a size of 2-3cm, migrate slowly upstream to their fresh water habitats. After 2 months, they become fast swimmers and even move against swift currents or cross rapids by crawling and will climb over bunds or dams 2-3 metre high, through the water dripping over the dam, (Ling, 1969a). Ibrahim (1962) reported similar movement of the juveniles of <u>M.malcolmsonii</u> over the first anicut in the Godavari and their successful negotiation of the barrier to reach the upper region.

Due to the differences in the spawning periods in the Hooghly estuary and Kerala backwaters, the migration in and out of the estuarine areas occur at different times of the year in these two places. While the migration of adults into backwaters in Kerala takes place at a time when the salinity is on the decrease, in the Hooghly the migration occurs when the salinity is on the increase in the winter and summer seasons. In Balgoda lake in Srilanka, the migration of adults to the lake occurs when the monsoonal rains lower both the salinity and temperature of the lake (Jinadasa, 1985). An increase in salinity in the backwaters coincides with the return migration of adults into the rivers in Kerala while in the Hooghly this occurs during the monsoon months when the salinity is on the decrease (Raman, 1967; Rao, 1967). Raman (1967) found the juveniles and large males to be quite at home at the river mouths when the salinity is nearly 18 ppt indicating that salinity alone is not the inducing factor for their return migration to the riverine habitats. Possibly, temperature may also be influential in effecting these movements. According to Raman (1964) during the summer months the juveniles are seen concentrating in the deeper areas of the Pampa river near Pulikizh and with the onset of the monsoon they come down the rivers and enter the backwaters. According to John (1957) the optimum temperature for normal activity of the prawn is $29-34^{\circ}$ C and at the peak summer months they are probably moving up the rivers, and remaining in deeper slushy basins of the river systems.

2.4.3 Sex-Ratio.

In the Pampa river system when the fishery starts in May or June, the males far outnumber the females. From August onwards females predominate and this condition continues upto September - October (Raman, 1967). In November males once again become numerous. In the Hooghly estuarine system, differential proportion of sexes during different periods of the year are reported (Rao, 1967). Males are predominent in February and again in September, while females are more dominant from April to June and again from September to January than males. During the peak of spawning season, March to May only berried females are found in the spawning grounds (Rao, 1967). The absence of mature males during the period in this region according to Rao (1967) may be due to the non-migration of mature males into this region from their fresh water habitats during the spawning season. Percentage of immature males and females also show similar trend of differential migration, males appearing in the fishery early in January while females enter later during March - April months (Rao, 1967).

2.4.4 Mating and Spawning.

The mating behaviour of <u>M</u>. rosenbergii was studied by Ling, (1962, 1969a); Rao, (1965) and Chow <u>et al.</u> (1982). According to Ling (1962) soon after the

pre-mating moult the female prawn secretes a substance which strongly attracts the males. Release of sex pheromones by the females after the parturlal or premating moult is also reported in the freshwater prawn, <u>M. kistnensis</u> (Sarojini <u>et al.</u>, 1984) and such a possibility has been suspected in the case of <u>M. idella</u> (Shyama, 1987). Rao (1965) observed the spawning behaviour of the species in the laboratory and opined that the courting behaviour is initiated only when a female which had just completed pre-spawning moult is available in the victnity. The male deposits the sperm in a gelatinous mass on the ventral median thoracic region of the female. Extrusion of eggs takes place several hours after mating and generally within 24 hours after the pre-mating moult (Rao, 1965; Ling, 1969a; Sandifer and Smith, 1979; Chow <u>et al.</u>, 1982). Unmated females would also release the eggs within 24 hours of pre-mating moult but these eggs would drop-off within 2-3 days (Ling, 1969a).

The females carry the eggs in the brood pouch formed of the first four pairs of pleopods. Vigorous aeration is provided by the female prawn with the movements of her pleopods throughout the incubation period (Ling, 1969a).

2.4.5 Fecundity.

The fecundity of the species from different regions has been studied by many workers and it is reported to be about 1,00,000 - 1,60,000 (Chacko, 1955), 7000 -1,11,400 (Rajyalakshmi, 1961), 60,000 - 1,00,000 (Ling, 1964) 1,39,000 - 5,03,000 (Raman, 1967) and 82,000 - 1,70,000 (Jinadasa, 1985)Rao,R.M.1986) reported that the fecundity ranges from 40,000 - 1,50,000 depending upon the size of the female, but an average female of 200mm can lay about 70,000 eggs, the fecundity being more in the wild than in the pond reared brood stock.

2.4.6 Incubation Period.

The incubation period is reported to be about 20-21 days by John (1957) about 19-20 days by George (1969) and 15-24 days by Rao (1986). According to Ling (1969a) the incubation period lasts for about 19 days at a temperature of $26-28^{\circ}$ C.

2.5 Larval Development

The larval development of the species from Malaysian waters was described by Ling and Merican (1961) and Ling (1969a). Ling (1962) has described 12 larval stages for the species but in a later study he grouped them into 8 stages (Ling, 1969a) and Uno and Soo (1969) described 11 stages for the species. The occurance of many stages in the larval life has been reported in several other species of <u>Macrobrachium</u>. Thus there are 10 larval stages for <u>M. acanthurus</u> (Choudhury, 1970, 1971c) and <u>M. idella</u> (Pillai and Mohamad, 1973), 12 for <u>M. carcinus</u> (Choudhury, 1971a) and 16 for <u>M. malcolmsonii</u> (Kevalramani <u>et al.</u>, 1971).

All larval stages of <u>M</u>. <u>rosenbergii</u> are active swimmers and are planktonic in habit (Ling, 1969a). They swim at a slightly oblique angle with tail first and ventral side upwards. According to Ling (1969a) all larval stages require brackish water of salinity 12-18 ppt for optimum growth and survival and larvae hatched in fresh water will die-off within 4-5 days unless removed to brackish water. In nature, larvae may hatch in both bracksth and fresh water but those that hatch in fresh water must be carried down the river to the estuaries within 4-5 days; otherwise they do not survive (Ling, 1969a).

2.6 Growth, Survival and Production in various culture systems

The giant fresh water prawn M. rosenbergii can grow very rapidly under optimum conditions. Owing to the fast growth rate and other desirable culture characteristics, it has become the focus of attention as a potential prawn species for culture. For rearing prawns to market size, different techniques like monoculture, polyculture and culture in rice fields are in vogue in many countries. Though they are farmed mainly in fresh water areas, they can also grow well in low saline brackish water areas (Ling and Costello, 1976; Popper and Davidson, 1982; Smith <u>et al.</u>, 1982a). Several workers have reported varying degrees of growth, survival and production by culturing the prawn under mono and polyculture systems in earthern ponds.

2.6.1 Monoculture.

Many studies have been conducted to evaluate the effect of varying stocking densities on growth, survival and production of M. rosenbergii. Smith et al. (1976) stocked earthern ponds with laboratory reared prawns having a mean size of 0.006-2.6 g at densities ranging from 29,400 - 1,66,600/ha area and obtained a survival rate of 81.2%. While studying the effect of stocking density on growth and survival of M. rosenbergii in ponds, Willis and Berrigan (1977) stocked earthern ponds with juveniles at a rate of $5/m^2$ and post-larvae at densities of 5,10 and $20/m^2$. They found juveniles (0.76 - 0.792g) to grow to a mean weight of 43.256g whereas post-larvae (0.04-0.063 g) to 29.749g within 24 weeks. The survival ranged from 38-92% with maximum equivalent production of 2278.61 Kg/ha in ponds stocked with $20/m^2$ of surface area.

Ong and Pang (1982) stocked <u>M. rosenbergii</u> juveniles of size 1.5cm - 2.0cm in length at a stocking density of $5-10/m^2$ and within 6 months the prawns were

found to attain an average size of 11.7cm and 57g. At lower stocking densities of 4.3 and 6.5/m² Smith et al. (1982b) obtained an average production of 573 kg/ha (range 155-900 kg/ha) with a survival rate of 44.2% (range 13.4 -67.9%) within a period of 125 - 173 days. The effect of three stocking strategies on the production of prawn, M. rosenbergii was examined in earthern ponds by Smith et al. (1981) and Smith and Sandifer (1982). In both these studies, the stocking of post-larvae resulted in lower production levels and less valuable prawns as compared to stocking a mixture of post-larvae + juveniles or juveniles only at varying stocking densities. Smith et al. (1983) further studied the biological and economic effect of stocking larger juveniles, (1 and 2 g in size) for seasonal production of prawns and found that when 1 and 2 g prawns were stocked in ponds at a density of 43,100/ha, final prawn size increased by 13 and 25% respectively and total production increased by 111 and 208 kg/ha. At a stocking density 64,600/ha final prawn size increased by 17 and 27% respectively and production increased by 183 and 299 kg/ha. Many workers reported greater prawn yields at higher stocking densities but mean size of the harvested prawns decreased as density increased. (Sandifer and Smith, 1975; Smith et al. 1976, 1978 and 1981; Willis and Berrigan, 1977; Brody et al., 1980; Cohen et al., 1983b; Subrahmanyam, 1984).

Willis and Berrigan (1978) studied the effects of feeding, fertilization and selective harvest on pond culture of <u>M. rosenbergii</u>. Post-larval <u>M. rosenbergii</u> were stocked in 12 numbers of 0.025ha earthern ponds at a density of $10/m^2$ and the results showed that ponds selectively harvested had highest mean production equivalent to 1203kg/ha and highest mean survival 79.9%. Selective harvest increased production by 28%. Comparative growth studies by Stahl (1979) with prawns stocked at the rate of $18/m^2$ revealed that prawns raised on a diet which included both natural foods and commercial pellets exhibited signi-

ficantly higher growth than the prawns raised solely of natural foods or of commercial pellets.

According to many, the major constraints to successful <u>Macrobrachium</u> culture are the high cost of food required during the grow-out period (Shang and Fujimura, 1977; Roberts and Bauer, 1978; Stahl, 1979), cannibalism and territorial aggression (Fujimura, 1974) and the loss in yields resulting from the wide size variations in the cultured prawns (Ra'anan and Cohen, 1983, 1984a; Peebles, 1979b; Sandifer and Smith, 1985). According to Peebles (1978) mortality estimates for <u>M. rosenbergli</u> grown from post-larvae to adult range from 20-50% and he (1979a) has attributed cannibalism and territorial aggression to be due to competition for space and food.

Sandifer and Smith (1975) found that <u>M</u>. <u>rosenbergii</u> post-larvel populations exhibit a positively skewed size distribution with 15-20% of the individuals ('jumpers') showing a much higher growth rate compared with the rest of the population. Selective removal of 'jumpers' are reported to enhance the growth of the remaining population (Ra'anan, 1980) which suggests a suppressive effect of the 'jumpers' on the remainder of the population (Sandifer and Smith, 1975). According to Ra'anan and Cohen (1984b) juveniles of <u>M</u>. <u>rosenbergli</u> grown in groups were characterised by the development of two separate size classes which were not there among individuals reared in isolation. Although the biological basis for the appearance of two size classes is not definitely known (Ra'anan and Cohen 1984a), experimental evidence suggests that the size variation in <u>M</u>. <u>rosenbergli</u> is more attributable to interaction within the prawn group than to genetic differences between individual prawns (Ra'anan and Cohen, 1984b).

Stocking separate large juveniles as opposed to stocking a whole mixed population containing individuals exhibiting a wide size distribution, improved yields (1367 and 1127 Kg/ha respectively) and the percentage of large market size prawns (61 and 48% respectively) (Ra'anan and Cohen, 1983). Large and uniform sized animals can be produced by periodic selective harvesting (Fujimura, 1974; Malecha, 1983) and this is shown to be effective in year round production (Goodwin and Hanson, 1975; Hanson and Goodwin, 1977). Roberts and Bauer (1978) reported a production increase by 28% as a result of selective harvesting.

Effect of different culture systems on growth, survival and production of <u>M. rosenbergii</u> was studied by Menasveta and Piyatiratitivokul (1982). A large earthern pond, nylon cages submerged in an irrigation channel and a long ditch located in an orchard were stocked with juvenile prawns at a density of 5 numbers/m². The results indicated a significant difference in growth obtained in these three systems. The prawns reared in the cages, exhibited the poorest growth but the highest survival (52.5%). Survival of prawns reared in the earthern pond and the long ditch were 48% and 35.2% respectively. The production in the three systems were 2625 Kg/ha/yr, 1725 Kg/ha/yr and 925 Kg/ha/yr in the pond, cages and ditch respectively.

The production of <u>M</u>. <u>rosenbergli</u> monosex populations was examined under intensive growth conditions in cages (Sagi <u>et al.</u>, 1986). While an all male population yielded 473 g/m², all female and a mixed population produced 248g/m² respectively during a grow-out period of 150 days. A comparison of growth performances of the females when reared alone and when grown together with males had shown that the biomass of the female in the all – female population was significantly higher than that of the mixed population. It has been stated that females are strongly affected by the presence of males, resulting in growth inhibition, while males are hardly influenced by the presence of females.

The feasibility of culture of <u>M</u>. <u>rosenbergii</u> in reservoirs and lakes were studied by Panicker and Kadri (1978) and Limpadanai and Tansakul (1980).

stocked juvenile <u>M. rosenbergii</u> of three sizes in a reservoir using net pens of two sizes with varying stocking densities. While smaller prawns of size 2.45cm suffered total mortality, prawns of 3.94cm length at stocking, grew faster than 11.24cm prawns, matured in 9 months and showed a survival rate of 22%.

Though there are many studies on the culture of M. rosenbergli in different culture systems, intensive grow-out of this prawn has received relatively little Most of the intensive rearing experiments deal with nursery rearing attention. of the species (Sandifer and Smith 1977, 1978; Kneale and Wang, 1979; Smith and Sandifer, 1979; Smith et al. 1983) or its culture in indoor laboratory tanks (Wickins, 1972; Forster and Beard, 1974; Sandifer and Smith, 1975, 1978; Mancebo 1978). Such intensive indoor culture systems are reported to be highly expensive for pranw production (McSweeny, 1977) and hence intensive grow-out of Macrobrachium in outdoor systems is gaining importance in recent years. Eble (1979) stocked, ponds equipped with hanging net habitats receiving heated water from a power plant at a stocking density of $36 - 54/m^2$ of prawns having size 0.7 - 1.0 g and within a period of 113 - 132 days, a gross production of 3,416 - 5,835 kg/ha was obtained: the survival ranged between 58 - 62 %. Ιn a semi-intensive grow-out trial in out door concrete tanks, Sandifer et al. (1982) achieved a gross production equivalent to 4,700 kg/ha.

Since increased density per surface area causes a decrease in weight of the prawns, addition of net substrates into the water column with a view to increase the surface area was tried by many workers (Smith and Sandifer, 1975; Ra'anan <u>et al.</u>, 1984). Wickins (1972) employed vertical sheets of plastic mesh in the rearing tanks and his projected yields ranged from $227g/m^2$ tank floor (density 87 prawns/m² tank bottom area) to $685g/m^2$ tank floor area. Smith and Sandifer (1975)

studied the effect of artificial substrates on increasing production of tank-reared <u>M. rosenbergii</u> in two separate experiments. In the first experiment after 4 weeks, average prawn mortality was 4 times greater in tanks without substrates than in tanks fitted with five - tiered horizontal substrate units. Ra'anan <u>et al.</u>, (1984) found that the yields of marketable prawns were 24% higher in ponds with net substrates and the overall survival also increased by 10%.

2.6.2 Polyculture.

<u>M. rosenbergii</u> being a benthophagic omnivore is an excellent candidate for polyculture (Cohen <u>et al.</u>, 1983a). According to these authors, the two major problems in prawn monoculture, viz, the occurrence of undesired unicellular or multicellular algal blooms and the low average weight and the wide size distribution of prawns stocked at high densities can be solved using polyculture with fish. Different combinations of fish were tried with freshwater prawns in many countries. Mullet was cultured along with freshwater prawns in Columbia (Martinez - Silva <u>et al.</u>, 1977). Joseph (1978) demonstrated the commercial feasibility of polyculture of <u>M. rosenbergii</u> with carps like <u>Labeo rohita</u>, <u>L. fimbriatus</u>, <u>Cirrhinus</u> <u>mrigala</u>, <u>Catla catla</u> and <u>Cyprinus carpio</u>. Malecha <u>et al.</u> (1981a), studied the feasibility of raising the fresh water prawn <u>M. rosenbergii</u> without supplemental feeding in a polyculture system consisting of chinese carps and common carp wherein, the production of prawn was 322.31kg/ha for 135 days and that of fish, 2516 kg/ha in 175 days.

The feasibility of growing the giant prawn, <u>M. rosenbergii</u> along with channel cat fish, <u>Ictalurus punctatus</u> was evaluated by many workers (Huner <u>et al.</u>, 1980a&b; Miltner <u>et al.</u>, 1983; Pavel <u>et al.</u>, 1985; Heinen <u>et al.</u>, 1987). Huner <u>et al.</u> (1980a) stocked post-larval prawns at 25,000/ha with cat fish fry at 5000/ha and obtained a mean prawn survival, harvest weight and yield of 58.3%, 29.0g and 423 Kg/ha respectively. Miltner et al. (1983) found that the size or stocking density of cat fish has no effect on the survival of the prawns. Tilapia nilotica and M. rosenbergii were stocked separately and in combination in 0.1 ha fertilized earthern ponds by Guerrero and Guerrero (1977) and obtained an increase in yield of T. nilotica by 21% in ponds stocked with 5 Kg/ha adult shrimps over that of ponds with T. nilotica only. Presence of shrimps also increased the total yield of polyculture ponds by 22% compared to that of monoculture ponds with T. nilotica alone. Behrends et al.(1985) studied the feasibility of culture of M. rosenbergii along with Tilapia spp., channel catfish, F-1 hybrid of the Chinese carps (female Aristichthys nobilis x male Hypophthalmichthys molitrix) and grass carp and obtained average net production ranging from 3,150 - 3,180 Kg/ha within 124 - 150 days when the prawns, tilapia, grass carp, channel catfish and hybrid carps were stocked at 20,000/ha, 2500/ha, 120/ha, 7500/ha and 160/ha respectively. In a recent study, Rouse et al.(1987) found that stocking size of tilapia had no effect on prawn growth, but prawn survival was adversely affected by tilapia fry.

In a study to evaluate the effect of culturing grass carp, silver carp, big head carp and viviparous top minnow, <u>Gambusia</u> sp. on the production of M. <u>rosenbergli</u>, Tinsutapanich <u>et al.</u>(1982) got total yields of prawn ranging from 1000-1587.5 Kg/ha with a survival rate of 56-64% in rearing periods of 6-8 months and 20 days. Cohen <u>et al.</u>(1983a) reported that stocking of prawns at a low desnity of about 5000/ha in polyculture with fin fishes resulted in 90% of the prawns growing to market size (> 45g) within 130 days with a survival rate of 43-96% and yields of 96-312 Kg/ha. Results of polyculture of fresh water prawns with common carps, tilapias and various chinese carps at varied prawn stocking rates showed that the growth and survival of fish and prawns are independent, with the prawns influenced only by their own stocking density and were not influenced by the species of fish co-stocked with them (Wohlfarth <u>et al.</u>, 1985). In polyculture experiments using the glant freshwater prawn, M. rosenbergii and Indian major carps like <u>Catla catla</u>, <u>Labeo rohita</u>, <u>Cirrhinus mrigala</u> and the Chinese carps <u>Hypophthalmichthys molitrix</u> and <u>Gtenopharyngodon idella</u> a fish harvest of 1000 - 1750 Kg/ha and a prawn harvest of 50-165 Kg/ha/9 months and 15 days were reported (Chondar, 1987). Iqlas Ahamed <u>et al.</u>(1987) while studying the compatibility of <u>M. rosenbergii</u> for polyculture with major carps found that the growth and survival of prawns were the best in tanks under polyculture with carps, when compared to monocultures. Perry and Tarver (1987) conducted an experiment to compare the production of prawn, <u>M. rosenbergii</u> in monoculture and <u>polyculture</u> with golden shiner, <u>Notemigonus crysoleucas</u> and obtained productions of 640 Kg/ha and 629 Kg/ha of prawns in polyculture and monoculture respectively.

The population structure and weight distribution of <u>M</u>. rosenbergii raised in earthern ponds in polyculture with fish were studied by Karplus <u>et al.</u>(1986a). Prawns were stocked at 1, 2, 3 or $4/m^2$ and found that females were more abundant than males in all the treatments. They obtained a survival of 85% independent of prawn stocking density. Yields increased and mean prawn size decreased with prawn density. In another study, Karplus <u>et al.</u>(1986b) examined the effect of size grading of juvenile <u>M</u>. rosenbergii prior to stocking on their population structure and production in polyculture. Juvenile prawns were size graded into an upper (one - third of the population) and a lower (two - thirds) fraction prior to stocking at a density of $2/m^2$ into earthern ponds for a grow-out period of 105 days. The effects of grading on morphotype differentiation, population structure and yield characteristic were analysed and the results showed that the relative position of juvenile prawns within the population is more important than the absolute size in determining further growth rate and yield.

2.6.3 Culture in Brackish water Ponds.

The feasibility of use of brackish water for the cultivation of M. rosenbergii

had been indicated by many. According to Ling and Costello (1976), M.rosenbergii grows well in brackish water of a salinity upto about 10 ppt. The growth, survival and production of M. rosenbergli in brackish water ponds were studied by Perry and Tarver (1981), Chatry and Huner (1982), Popper and Davidson (1982) and In replicated stocking densities of $2.5/m^2$, $4.9/m^2$ and Smith et al.(1982a). $7.4/m^2$ in brackish water ponds, production equivalent to 408 Kg/ha, 619 Kg/ha and 510 Kg/ha respectively was obtained within a period of 140 days by Perry and Tarver (1981). Popper and Davidson (1982) stocked prawns along with mollies (Poecilia reticulata) in a pond in which the salinity fluctuated between 12 to 25 ppt with an average salinity of 16 ppt. According to them the prawns attained an average weight of 8g in 60 days and 26g in 240 days. The culture potential of M. rosenbergii in brackish water ponds was further investigated by Smith et al.(1982a) and found mean salinities of 5.7, 7.2, 10.0, 13.0 and 15.3 ppt in ponds as giving equally good growth and survival rates as in the fresh water Rajyalakshmi and Maheswardu (1986) have studied the possibility of ponds. culturing M .rosenbergii in brackish water ponds along with the tiger prawn, Penaeus monodon. They found M. rosenbergii to grow to 56mm and 2.5g within a period of 30 days, while P.monodon had grown to 80mm and 4.5g within the same' period.

2.7 Effect of Salinity on Survival and Growth

Palaemonid prawns are known to have wide salinity tolerances and preferences. While some species are restricted to the narrow salinity ranges, others are known to tolerate salinities ranging from fresh to hyper saline water (Castille and Lawrence, 1981). The larval, post-larval and adult stages of <u>M. rosenbergii</u> are reported to be euryhaline to a considerable degree. In nature, the prawns commonly spawn in brackish water and the juveniles make their way upstream to fresh water habitats. Ling and Merican (1961) reported the regular occurrence of <u>M. rosenbergii</u> in brackish waters in Malaysia. Raman (1967) has also reported the capture of adults in salinities upto 18ppt. The spawning of the species is reported to occur in areas where the salinites fluctuate between $5_{and 20}$ ppt(George, 1969). According to Rao (1969) the extreme limits of salinity tolerance for the species in Hooghly estuary is 0-16 ppt. Gravid females of <u>M. rosenbergii</u> are reported to occur in the upper reaches of Mandovi and Zuari estuaries during the monsoon period (June-September) when the salinity fluctuates between 0 and 20 ppt <u>(Nair et al. 1977)</u>.

The salinity ranges for rearing larval stages of <u>M</u>. rosenbergii have been worked out by Ling (1962, 1969a & b). Larvae hatched in 3-6 ppt brackish water could tolerate immediate transfer to salinities ranging between zero and 21 ppt without serious losses (Fujimura, 1966). In larval rearing experiments, many workers have used salinities within the range of 11-18 ppt (Fujimura, 1966; Ling, 1969b; Fujimura and Okamoto, 1972; Meeran and Sebastian, 1976; Dugan <u>et al.</u>, 1975; Aquacop, 1977a; Adisukresno <u>et al.</u>, 1982; Aniello and Singh, 1982; Chineah, 1982; Lee, 1982; Suharto <u>et al.</u>, 1982). Wickins (1972) however found 26 ppt salinity to be more suitable in the initial one-half of the rearing period and suggested a gradual increase of salinity from 2-26 ppt in mass larval rearing.

According to Read (1986) most species of <u>Macrobrachium</u> are dependent on brackish water for complete larval development and the tolerance of the larvae to salinity varies from species to species to a considerable degree. Lewis and Ward (1965) obtained best survival of the larvae of <u>M. carcinus</u> in a salinity of 21 ppt but Choudhury (1971a & b) found proper development of the larval stages at 14-17 ppt. Choudhury (1970; 1971c) observed that though the adults of <u>M. acanthurus</u> live and breed in fresh water, brackish water of salinity range 15-20 ppt is essential for survival and moulting of the larvae of this species. Uno (1971) found the most favourable salinities for metamorphosis to post-larvae in <u>M. nipponense</u> to be between 8 and 13 ppt. Though Wong (1987) could rear the larvae of this species in a range of salinity between zero and 15 ppt, maximum survival and metamorphosis to post-larvae was reported to be between 7.5 and 12.5 ppt. Guest and Durocher (1979) reported metamorphosis of the larvae of <u>M. amazonicum</u> in salinities between 1 and 15 ppt but they failed to do so in fresh water. According to McNamara <u>et al.</u> (1983), the percentage survival of <u>M. amazonicum</u> larvae after 6 days in fresh water and sea water was 17% and 10% respectively but survival at intermediate salinities of 7-28 ppt was 100%. Kewalramani (1973) established the necessity of salinity in the larval life history of <u>M. malcolmsonii</u>. The larvae of <u>M. hothuisi</u> were reported to die in salinities above or below 14-17 ppt (Moreira <u>et al.</u>, 1979). Lee and Fielder (1981/82) found that the larvae of <u>M. australiense</u> do not require any salinity for metamorphosis to post-larvae and salinities above 20 ppt were found to inhibit metamorphosis.

Lakshmi, et al. (1982) reared all the ten zoeal stages of M. idella in fresh water and in different salinities of 5, 10, 15, 20, 30 and 35 ppt. The normal development of the larvae was recorded in the salinity range of 10-25 ppt but the optimal survival was at 15 ppt. Pillai (1982) obtained maximum survival of the larvae of M. equidens at a salinity range of 20-25 ppt. On the basis of salinity tolerance exhibited by the larval stages, Read (1986) has divided them into three categories, (i) Euryhaline larvae that survive in both low (> 7 ppt) and high (35 ppt) Saline water; M. petersii, M. acanthurus and M. olfersii being included in this category; (ii) stenohaline larvae that survive in low saline water but cannot tolerate high salinity (> 28 ppt); M. rosenbergii, M. carcinus, M. holthuisi and M. amazonicum belonging to this group, and (iii) Stenohaline larvae that survive in fresh water, but cannot tolerate salinities greater than 20 ppt, example being M. australiense. The tolerance of post-larval <u>M</u>. <u>rosenbergii</u> to gradual and rapid increases in salinity was studied by Sandifer <u>et al.</u>(1975) who could demonstrate that in both abrupt and gradual increases of salinity, mortalities occur at levels ≥ 30 ppt. Acclimation substantially increased the survival time at 35 ppt. Silverthorn and Reese (1978) studied the cold tolerance at three salinities of post-larval <u>M. rosenbergii</u>. When post-larvae of <u>M. rosenbergii</u> acclimated to 27, 22 or 16^oC and 5, 8 or 14 ppt were given thermal shock to 16^oC or 13/16^oC for one week, the survival was found to be significantly better in animals acclimated to 22^oC or below. In this case salinity had no significant influence on survival. Singh (1980) showed that the salinity tolerance of adults and post-larvae of <u>M. rosenbergii</u> is much the same in slow acclimation experiments.

According to Kinne (1971) growth is restricted to significantly narrower salinity ranges than is survival in most euryhaline invertebrates. Though there are a fairly large number of studies on the growth of <u>M</u>. rosenbergii in fresh water, studies on the growth of this species in different salinity conditions are only very few. Wickins (1972) working with 9 day old post-larvae acclimated at a salinity of 15 ppt for one week found that they grew rapidly when transferred to 2ppt salinity, compared to those kept in the original 15 ppt salinity. Other laboratory trials also indicated that juvenile prawns grow more rapidly in fresh or slightly brackish water (\leq 5 ppt) when compared to more brackish water upto 15 ppt Goodwin and Hanson, 1975; Sandifer <u>et al.</u> Ms).

Perdue and Nakamura (1976) found that <u>M</u>. <u>rosenbergii</u> juveniles of the size of 3.39 cm when reared in salinities of 0 ppt, 2 ppt, 8.5 ppt and 15 ppt over a seven week period had given a better percentage increase in weight and mean growth rate in fresh water and 2 ppt, but there was little difference statistically between 2 ppt and fresh water. Nair <u>et al.</u> (1977) while studying the effect of salinity on growth and survival of the larvae of <u>M</u>. rosenbergii found 15 ppt to be the optimum salinity at which maximum growth and survival occurred. In a laboratory trial Popper and Davidson (1982) reared juvenile <u>M. rosenbergli</u> in 30 L aquaria in salinities ranging from zero to 25 ppt and found that <u>M. rosenbergli</u> grows best in salinities of 10 to 15 ppt. Barring this study by Popper and Davidson (1982), in all other experiments maximal growth of <u>M. rosenbergli</u> was obtained in fresh water or slightly saline water.

2.8 Water Quality Requirements for Culture

Elucidation of water quality requirements is important for the successful cultivation of any organism. These requirements include optimum levels of temperature, salinity, dissolved oxygen, pH, hardness and alkalinity, an absence of nitrogenous excretory wastes and other pollutants at lethal levels.

2.8.1 <u>Temperature</u>.

There are no specific studies relating to the effect of pond temperature on the survival and growth of post-larval, juvenile and adult <u>M.rosenbergii</u>. The occurrence of this species is reported from habitats of about $25^{\circ}C$ in Malaysia (Johnson, 1967 and from 27 to $34^{\circ}C$ in India (John, 1957; Rao, 1967). According to Fujimura (1974) growth of this species is reduced at temperatures below $22 - 23^{\circ}C$. Several laboratory studies conducted reveal the optimum temperature for larval rearing to be around $28 - 30^{\circ}C$ (Uno <u>et al.,1975;</u> Farmanfarmaian and Moore, 1978; Crowell and Nakamura, 1980; Crowell, 1981).

Many workers have reported the lethal extremes of temperature for acclimated juveniles and adult prawns to be about $13^{\circ}C$ and $38^{\circ}C$. However, continued exposure at temperatures of less than $18^{\circ}C$ or greater than $33^{\circ}C$ leads to increased mortality of prawns (Uno <u>et al.</u>, 1975; Armstrong, 1978; Farmanfarmaian and Moore, 1978). Despite the tolerance to a broad temperature ranges of the species Uno <u>et al.</u> 1975 found a reduction in growth, activity and survival at temperatures outside the range of 22 to $33^{\circ}C$. A study conducted by Silverthorn and Reese (1978) revealed that permanent stunting of growth does not occur as a result of prolonged exposure to low temperature. These workers studied the growth of post-larvae which had been maintained at 16° C or below for 3 weeks and later returned to 27° C for one month and found that the growth was not significantly different from that of the 27° C controls.

Sarver <u>et al.</u> (1982) observed the mortality of 50% of newly settled postlarvae when exposed to water of 19° C. According to New and Singholka (1982) temperatures below 14° C or above 35° C are lethal to freshwater prawns and they suggested 29 - 31° C as the optimum for their cultivation. More or less similar observation was made by Sandifer and Smith (1985) who reported the optimum temperature to be about 28 - 30° C and according to them prawns could do well in the range of 26 - 31° C.

2.8.2 Salinity.

The larval stages of <u>M</u>. <u>rosenbergii</u> require brackish water for development and survival. The range of salinity for larval rearing vary from 8 - 17 ppt, although Sick and Beaty (1974) reared them in salinities as low as 6 ppt and Wickins (1972) in salinities as high as 26 ppt for some stages. However, Sandifer <u>et al.</u> (1977) reported the optimal salinity range for the larval rearing of this species to be within the range of 12 - 16 ppt.

Several studies have been directed towards the production possibility of <u>M. rosenbergii</u> in brackish water environments (Sandifer and Smith, 1974; Perdue and Nakamura, 1976; Perry and Tarver, 1981; Chatry and Huner, 1982; Popper and Davidson, 1982; Smith <u>et al.</u>, 1982a). In a salinity of 2 - 5 ppt, small juveniles have been observed to grow a little faster than in fresh water but at 15 ppt salinity the growth is comparatively slow (Sandifer and Smith, 1974; Perdue and Nakamura, 1976). Ling and Costello (1976) recommended salinities upto 10 ppt as suitable for the culture of <u>M. rosenbergii</u>. Popper and Davidson (1982) reported growth of <u>M. rosenbergii</u> in salinity levels of approximately 10 - 15 ppt. The above studies

except the one by Popper and Davidson (1982) reveal that although the prawns could tolerate salinities higher than their isosmotic point of 17 - 18 ppt (Sandifer <u>et al.</u>, 1975; Singh, 1977, 1980), optimum growth conditions seem to be at fresh or slightly brackish water.

2.8.3 Dissolved Oxygen.

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Management of dissolved oxygen levels is of paramount importance in prawn culture. Many studies conducted on the oxygen consumption of <u>M</u>. rosenbergii, report that oxygen consumption is an allometric (log) function of body weight (lwai, 1978; Stephenson and Knight, 1980 a & b). Sharp (1976) determined that for a prawn having a dry weight of 0.2 g the concentration at which oxygen becomes limiting is 2.1 ppm at 23° C, 2.9 ppm at 28° C and 4.7 ppm at 33° C. According to Rao, J.B(1986) and Subrahmanyam (1986) the optimum dissolved oxygen level for larval rearing is 4 - 6.5 ppm. Generally larger prawns require more oxygen than smaller ones and thus the larger animals are more susceptible to low oxygen concentrations (Sandifer and Smith, 1985). Smith <u>et al.</u> (1982a) reported the mean size of prawns killed by overnight oxygen depletion in a pond to be twice of that of the survivors.

According to many authors <u>M</u>. rosenbergii is well adapted to hypoxia but it is better to maintain the oxygen levels above the critical minima for minimizing the stress (Smith <u>et al.</u>, 1976, 1978, 1981). The lowest oxygen level at which the animal can be reared without stress is between 25 and 30% saturation or about 2.25 to 2.75 ppm in the range of 25 to 30° C. Spotts (1982) reported levels of 3 ppm as the minimum acceptable dissolved oxygen concentration. The lowest tolerance level of the prawn at 24 - 28° C is 1 ppm and levels of 3 - 5 ppm are considered to be critical. Hence it is advisable to maintain a dissolved oxygen concentration between 6 and 8 ppm in the prawn ponds.

2.8.4 <u>pH</u>.

To date no specific studies were undertaken with the objective of establishing a relation between water pH, growth and survival of M. <u>rosenbergil</u>. Natividad(1982) reported the occurrence of M. <u>rosenbergil</u> in some philippine river systems in which the pH fluctuated between 3.0 and 9.0. Extremely high pH levels are reported to lead to the formation of calcium carbonate precipitates on the prawns (Cripps and Nakamura, 1979). Some workers have observed the mortality of upto 80% of the post-larvae at a pH level of 9.5 (Sarver <u>et al.</u>, 1979, 1982; Malecha <u>et al.</u>, 1980). High pH value of 10.5 due to plankton blooms was found to cause mass mortality of prawns (Aquacop, 1979a). However, Sandifer and Smith (1985) reported the culture of prawns in South Carolina in ponds in which the pH ranged from 6.0 to 10.5 with no apparent adverse effects, but have suggested that for better performance pH should not be allowed to remain below 6.5 or above 9.0 for a longer duration.

2.8.5 Hardness and Alkalinity.

Prawns require minerals like Calcium and Magnesium for the formation of new exoskeleton and for some other biological purposes. Lynne and Lutz (1982) noted that in <u>M. rosenbergii</u> in addition to the internal sources of calcium, additional uptake of calcium from fresh water may be necessary for complete calcification of the exoskeleton.

Sick and Beaty (1974) observed mass mortality of the early larvae when reared in an artificial sea water medium prepared with well water having total hardness ($CaCo_3$) levels of 50 - 100 ppm. However these authors could observe normal survival and development of the larvae when reared in a medium made with distilled water.

Many workers have reported the growth and survival of <u>M</u>. <u>rosenbergii</u> juveniles in varying levels of hardness. Smith <u>et al.</u> (1976) observed good growth and survival of the prawns at very low hardness levels of 5 - 7 ppm in South Carolina but they noticed a softening of the exoskeleton at these levels. A laboratory study conducted with post-larval M. rosenbergii for a period of 28 days by Heinen (1977) showed no differences in survival and growth at hardness levels of 10 - 310 ppm. Contrary to these results, within the range of 65 - 500 ppm, Cripps and Nakamura (1979) obtained decreasing growth rate with increasing hardness levels. This study also noted a difference in moulting pattern, with the intermoult duration increasing significantly among the prawns grown in 500 ppm medium. Slow growth, encrustation of epibionts such as Bryozoa and Epistylis and precipitation of Calcium carbonate on the prawns had been observed in a commercial prawn farm in which the water had Ca⁺⁺ concentrations of 305 to 638 ppm (Cripps and Nakamura, 1979). New and Singholka (1982) recommended a total hardness (as CaCo₂) level within the range of 40 - 150 ppm as the optimum condition for culture of M. rosenbergii. According to Malecha (1983) for better results prawn farm water should have calcium levels below 100 ppm. According to Sandifer and Smith (1985) waters with hardness levels of 300 ppm or greater should be avoided for the cultivation of M. rosenbergii.

Alkalinity levels above 180 ppm have been reported to cause mass mortality of prawns grown in ponds in South Carolina (Sandifer and Smith, 1985). Total CaCo₃ alkalinity at levels below 180 ppm is reported to have very little effect on prawn growth in ponds.

2.8.6 <u>Nitrogenous Excretory Wastes</u>,

Metabolic wastes are reported to have profound influence on growth and survival of <u>M. rosenbergii</u> in various culture systems. This is particularly true with intensive culture systems in which the stocking densities are comparatively higher. Armstrong <u>et al.</u> (1976) reported nitrite nitrogen (NO₂ - N) at levels as low as 1.8 ppm to retard growth in <u>M. rosenbergii</u> larvae and an incipient LC_{50} value of 3 ppm NO₂ - N. The same workers observed that at 10 ppm NH₃ (8.23 ppm TAN) level no inhibition of growth of larval <u>M. rosenbergii</u> occurred (Armstrong <u>et al.</u>, 1978). The pH and to a lesser extent temperature, determine the proportion of highly toxic un-ionized ammonia present in the aquatic medium. For <u>M.rosenbergii</u> larvae, values for 24 hr and 144 hr $LC_{50's}$ show that as pH increases, the tolerance of larvae to total ammonia levels decreases because of the increasing concentration of unionized ammonia. Armstrong <u>et al.</u> (1978) found reduced growth of the larvae at a total ammonia level of 32 ppm at pH 6.83 and 7.6.

In a study evaluating the tolerances of different species of prawns to recirculated water Wickins (1976b) observed similar tolerances to ammonia of both <u>M. rosenbergii</u> and penaeid shrimps but the former species exhibits less tolerance to nitrite and nitrate when compared to the latter. Acute tolerance of juveniles to ammonia is generally similar to that of the larvae but the growth rate of juveniles exposed to chronic ammonia levels of only 0.16 ppm or greater for 6 weeks was approximately one-third less than controls. According to New and Singholka (1982) the hatchery intake water for <u>M. rosenbergii</u> larval rearing should not have levels of nitrite and nitrate higher than 0.1 ppm (NO₂ - N) and 20 ppm (NO₃ - N) respectively.

2.8.7 Other Toxic Materials.

The water supply to a fresh water prawn farm must be free from pollution with agricultural pesticides, industrial effluents or heavy metals. Though very little is known about the tolerances of prawn to these materials, it is always stressed to choose sites without any such problems. While studying the toxicity of mercury for the larval stages of <u>M.rosenbergii</u> Piyan <u>et al.</u>(1985) found that the first stage larvae had the lowest threshold lethal concentration(TCL) of 0.041ppm of Hg and the post-larvae had a TLC of 0.325ppm Hg. They could observe an abrupt

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increase in the TLC of Hg after the larvae had reached stage 5. According to New and Singholka (1982), iron and magnesium levels should be very low for successful hatchery rearing of <u>Macrobrachium</u> larvae. Although Ferdinando and Manawadu (1982) reported successful larval rearing using well water containing iron of 15 - 20 ppm, high iron levels seem to be detrimental to both hatchery and growout operations.

2.9 Effect of Salinity on Oxygen Consumption, Ammonia Excretion and Oxygen : Nitrogen Ratios

2.9.1 Oxygen Consumption.

Determination of metabolic rates done by measuring oxygen consumption is of prime importance in quantifying the energy expenditure of an animal. The studies conducted to date on the relationship between salinity and metabolism in Macrobrachium spp. have been restricted to selected ontogenetic stages and as such information on metabolic responses in relations to salinity changes for each stage is lacking barring the study by McNamara et al.(1986) in which the effect of salinity on the respiratory metabolism of selected ontogenetic stages like zoeae, post-larvae and adults of the freshwater shrimp Macrobrachium olfersii has been examined. Other investigations carried out are restricted to the first zoeae of M.acanthurus M.amazonicum M.carcinus M.heterochirus M.holthuisi and M.olfersii (Moreira et al., 1980, 1982; McNamara et al., 1983) the laboratory cultured larvae of M. rosenbergii (Stephenson and Knight, 1980a), the post-larvae and juveniles of M. rosenbergii (Nelson et al., 1977a; Stephenson and Knight, 1980a & b; Stern et al., 1984) and of adults of M. acanthurus, M. heterochirus, M. olfersii and M. potiuna (Moreira et al., 1983).

The iso-osmotic point in <u>M. rosenbergli</u> is reported to be at about 17.0-18 ppt (Sandifer <u>et al.</u>, 1975; Singh, 1977, 1980) and at this salinity range a lowering of metabolic rate is normally expected as the energy expended in osmoregulation

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should be lowest at an animal's iso-osmotic point (Potts, 1954; Panikkar, 1968). However, Nelson <u>et al.</u> (1977a) did not find a decrease in metabolic rate associated with that salinity level in this species. They could observe a change in oxygen consumption rate when salinity increased above the iso-osmotic level. Many workers view the increased matabolic rate in different penaeid and palaemonid prawns at salinities differing from the iso-osmotic point, as an indication of the increased energy cost due to osmoregulation (Beadle, 1931; Lofts, 1956; Rao, 1958; Kutty <u>et al.</u>, 1971). A few workers have expressed doubts regarding the validity of this interpretation, who found in many crustaceans including <u>M. rosenbergii</u> a decrease in metabolic rate in supra/sub normal salinities (Dimock and Groves, 1975; Simmons and Knight, 1975; Stephenson and Knight, 1980b; McNamara <u>et al.</u>, 1986; Moreira and McNamara, 1982) or no response to salinity (Elfringhan, 1965; McFarland and Pickens, 1965; McLusky, 1969; Jones, 1974).

According to Nelson <u>et al.</u> (1977a) there is a decrease in oxygen consumption in <u>M. rosenbergii</u> with increasing salinity, past a critical point which in turn is observed to decrease with increasing temperature. Stephenson and Knight (1980b) found no significant change in oxygen consumption in relation to salinity, except in animals of 40 - 49.99 mg dry weight. For the larger post-larvae and the juveniles they observed similar pattern of response to salinity, with the oxygen consumption rates declining in increased salinities from fresh water to 28 ppt. The higher average oxygen consumption rate of <u>M. rosenbergii</u> acclimated in 24 ppt when compared to lower salinity levels may be indicative of a high metabolic rate of this animal at that salinity level (Stern <u>et al.</u>, 1984).

Working with various species of <u>Macrobrachium</u>, Moreira <u>et al.</u> (1982) could observe varying metabolic responses to salinity and on the basis of this they have divided the larval stages of various species into two groups with <u>M. acanthurus</u> and <u>M. olfersii</u> showing an increase in metabolic rate in concentrated or diluted salinities and <u>M. heterochirus</u> and <u>M. carcinus</u> larvae with a tendency to decrease the metabolic rate in both high and/or low salinities. While studying the effect of salinity on the respiratory metabolism in the first zoea and adult female <u>M.olfersii</u> McNamara <u>et al.</u> (1982) found the oxygen consumption rates in the larvae to be the minimum in 14 ppt increasing sharply in 0 ppt and slightly in 35 ppt. Adult oxygen consumption rates showed a maximum value in 21 ppt with the rates declining sharply in 28 and 1 ppt. Moreira <u>et al.</u> (1980) studied the respiratory metabolism of the first zoeal stage of <u>M. holthuisi</u> in relation to salinity and temperature and found it to be significantly influenced by salinity, temperature and their interaction with the temperature exhibiting a more pronounced effect.

2.9.2 Ammonia Excretion.

Ammonia excretion in <u>Macrobrachium</u> spp. has been studied by Wickins(1976a), Nelson <u>et al.</u> 1977b & c), Anatharaman <u>et al.</u> (1981, 1982) and Stern and Cohen (1982). Wickins (1976a) found the nitrogen excretion rate of <u>M. rosenbergii</u> of size range 2 - 27 g to be between 0.85 - 0.25 mg N/g/day. The influence of diets on the ammonia production of juvenile <u>M. rosenbergii</u> was studied by Nelson <u>et al.</u> (1977b), who reported no difference between the diets tested but were related to the body weight of the organism. In a subsequent study, Nelson <u>et al.</u> (1977c) found that irrespective of the diet, the majority of the nitrogen assimilated was excreted. Nelson <u>et al.</u> (1979) investigated the ammonia excretion of a benthic estuarine shrimp <u>Crangon franciscorum</u> and found the rates to be 7 times higher in fed shrimps than in starved ones. Anantharaman <u>et al.</u> (1982) found that the rate of ammonia excretion in <u>M. lanchesteri</u> (de Man) is not related to the feeding rate.

Diurnal variations in ammonia excretion was noted in <u>M. lanchesteri</u> by Anantharaman <u>et al.</u> (1981) who found that during the day maximum ammonia excretion (4.139 μ g/g prawn) occured between 10.00 and 14.00 hrs, while during the night maximum excretion (2.811 μ g/g prawn) was observed between 18.00 and 22.00 hrs.

The rate of ammonia excretion during the different stages in the moult cycle was investigated by Stern and Cohen (1982) and they reported an increase in ammonia excretion from 17.5 μ g NH₃ - N/g/hr during the intermoult to 39.3 μ g NH₃ - N/g/hr during the premoult to moult stages.

Environmental salinity is reported to affect the nitrogen excretion in euryhaline crustaceans and Armstrong <u>et al.</u> (1981) linked this increase or decrease in response to salinity, to the activity of the Na⁺/NH⁺₄ exchange pump in the gills. Sharma (1966) reported an increase in nitrogen excretion in the fresh water cray fish, <u>Orconectes rusticus</u> after subjecting it to an increase in salinity but in <u>M. rosenbergil</u>, Armstrong <u>et al.</u> (1981) found a decline in ammonia excretion rate in response to an increase in salinity. Contrary to the results of Armstrong <u>et al.</u> (1981), Stern <u>et al.</u> (1984) observed an increase in ammonia excretion in this species in higher salinities.

2.9.3 Oxygen : Nitrogen Ratios.

Oxygen to Nitrogen ratios have been used as an index of the energy substrate utilization by many workers (Conover and Corner, 1968; Bayne and Scallad, 1977; Capuzzo and Lancaster, 1979; Regnault, 1981). Working with <u>Homarus americanus</u>, Capuzzo and Lancaster (1979) found a reduction in O : N ratio from 26.7 - 22.1 as representing an increase in protein catabolism when compared to lipid or carbohydrate metabolism. According to Regnault (1981) an O : N ratio of 27 in <u>Crangon crangon</u> is indicative of mostly lipid catabolism. He further noted a reduction in O : N ratio in this species when subjected to continued exposure to stress conditions. Seasonal variations in O : N ratios in <u>Palaemonetes varians</u> have been indicated by Snow and Williams (1971) who could observe values of 6.1 : 1 in winter and 34.2 : 1 in summer.

Clifford and Brick (1983) obtained an O:N ratio of 22.1: 1 in <u>M.rosenbergil</u>, which according to them is indicative of the fact that energy metabolism in this s¢pecies is dominated by the oxidation of carbohydrate with the lipids and protein forming secondary and tertiary substrates. Stern <u>et al.</u> (1984) obtained O:N ratios ranging from 12 - 23 in <u>M. rosenbergil</u> in media having different salinities. The prawns adapted to the dilute media showed the highest O : N ratio and they could observe a change in energy substrate utilization as a result of a change in salinity in M. rosenbergii.

III. MATERIALS AND METHODS

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3.1 Experimental Animals

The juveniles of <u>Macrobrachium rosenbergil</u> required for the experiments were obtained from the hatchery at the Fisheries College campus, Panangad. The post-larvae intended for the study were gradually acclimated to freshwater soon after the metamorphosis from a salinity of 12-14ppt used for larval rearing. Post-larvae maintained in fresh water for 20 days were used for studying the effect of salinity on survival. Animals within the size range of 2.4-3.5 cm and 106-219 mg were used for the experiments.

For the growth studies 30 day old juveniles were used (Fig. 1). In the initial stages of rearing, the juveniles were fed <u>ad libitum</u> on chopped prawn flesh (Metapenaeids) but later switched over to pelleted feed formulated by Sherief (1987) for prawns. For the field study, the post-larvae were maintained in a hapa fixed in a fresh water pond till stocking in the respective ponds. For all the studies healthy juveniles were selected , blotted with a dry towel and weighed in a chemical balance.

3.2 Troughs, Cisterns and Ponds

For the study to evaluate the effect of salinity on the survival of <u>M</u>. <u>ros</u><u>enbergii</u>, circular glass troughs of 7 L capacity were used. For the growth experiment in the laboratory, circular cement cisterns of 500 L capacity filled with 200 L water of the required salinity were used.

For the field trial, three earthern ponds, one fresh water (112 Sq.m) and two brackish water (100 Sq.m. each) were used. In the fresh water pond, the water level had gone down in the summer months (February and March) and in the brackish water ponds there used to be fluctuations in salinity depending on precipitation levels and lateral seepage from the adjacent ponds and backwater.

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3.3 Respirometers

Oxygen consumption and ammonia excretion of individual prawns were measured with respirometer shown in Fig. 2. The respirometer chamber consists of a flat bottom flask having 320 ml capacity provided with an inlet, an outlet and an opening at the top for letting in air while drawing the samples, all of which are connected to latex tubings.

3.4 Preparation of Saline Media

The different salinity levels required for the study were prepared by mixing sea water collected from Cochin bar mouth area with tap water. The amount of sea water needed for making the selected salinity levels were calculated using the formula,

 $V = \frac{\text{Required salinity}}{\text{Salinity of sea water}} X 1000, \text{ where V is the volume of sea water}$ to be diluted with fresh water to make upto 1 L of solution of the needed salinity.

3.5 Preparation of the Feed

The feed used in the experiment was formulated using ingredients like clam meat powder (40%), ground nut oil cake (25%), rice bran (25%) and tapioca powder (8%). These ingredients were made into a paste using the required amount of water (1.25 L per Kg) and was cooked for 30 minutes in an autoclave at ambient pressure. After cooling, 2% supplementary vitamin mixture was added, mixed, pelletized and dried at 60° C overnight in a hot air oven. The proximate composition of the feed is given in Table 1.

3.6 Experimental Procedure

3.6.1 Study to Evaluate the Effect of Salinity on Survival.

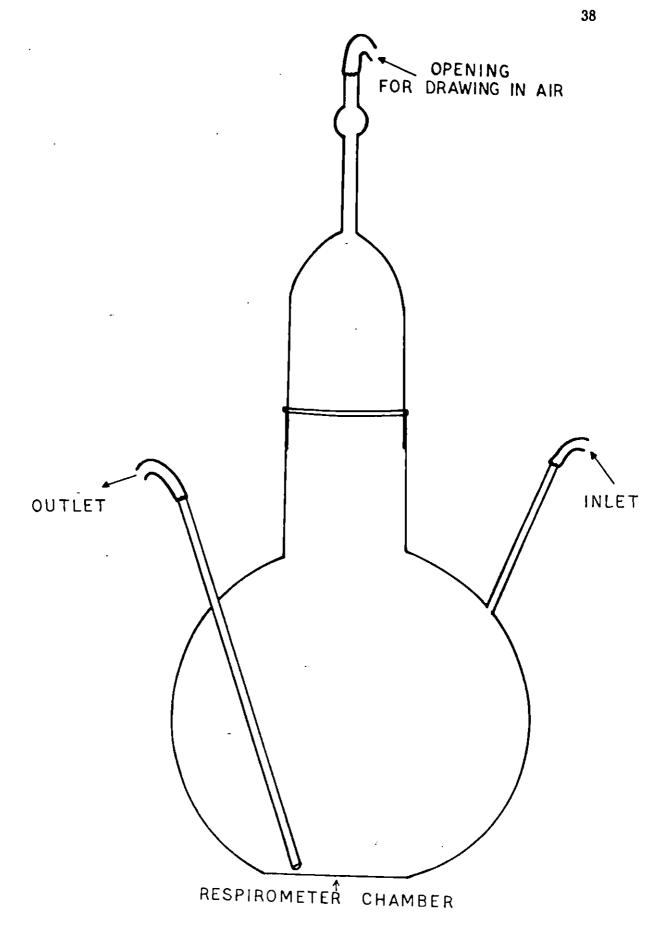
The effect of salinity on survival was studied by effecting sudden transfer

Table 1. Proximate (composition	of	the	feed
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n
5.58
42.13
3.09
9.75



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Fig. 2. Respirometer

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Elapsed	Initial										
time (hrs)	salinity (ppt)	5	10	15	20	25	26.5	28.5	30	35	
2		1.0	1_0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	
12		2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	
24		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
48		5.0	10.0	10,0	10.0	10.0	10.0	10.0	· 10_0	10 0	
72		5.0	10.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	
)4	0	5.0	10.0	15.0	20.0	20.0	20.0	20.0	20.0	20.0	
44	ι.	5.0	10.0	15.0	20.0	25.0	25.0	25.0	25.0	25.0	
56		5.0	10.0	15.0	20.0	25.0	26.5	26.5	26.5	26.5	
72		5.0	10.0	15.0	20-0	25.0 °	26.5	28.5	28.5	28.5	
92		5.0	10.0	15 . 0	20.0	25.0	26.5	28.5	30.0	30.0	
52		5.0	10-0	15.0	20.0	25.0	26.5	28.5	30.0	35.0	

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Table 2. Schedule of acclimation to the test salinity levels

and also after gradual acclimation to the test salinity levels. Salinity levels of 5, 10, 15, 20, 25, 26.5, 28.5, 30 and 35ppt were used in this study. Animals maintained at 0 ppt served as the control. Ten numbers of juvenile prawns were introduced in each trough, holding 5 L of the test medium and each level was replicated twice. Gentle aeration was provided in all the troughs during the course of the experiment. The mortality was assessed at closer intervals for the first 12 hours and then at 12 hourly intervals upto 120 hrs. The animals that did not respond to prodding were considered dead. In the gradual acclimation experiment, the animals were acclimated to the test salinity levels as per the schedule given in Table 2. The animals were not fed during the course of the study in both the cases.

3.6.2 Study to Evaluate the Effect of Salinity on Growth.

3.6.2.1 Laboratory Experiment in Cement Cisterns.

The growth and survival of <u>M</u>. <u>roseabergii</u> juveniles from fresh water to a salinity of 10 ppt with a regular increment of 2 ppt from treatment to treatment were studied, for three months, this range of salinity being fixed on the basis of a preliminary study which had given indications of this being the favoured range of salinity for growth. All the treatments were replicated twice. Ten numbers of the juveniles were weighed after blotting ; in a chemical balance and released to the tanks after slow acclimation. After stocking, the prawns were fed <u>ad libitum</u> on the formulated feed and the unutilized feed pellets and the faecal matter were removed, the very next day using a rubber tubing. Twenty percentage of water was exchanged from each tank on a weekly basis. Water samples were drawn for analysis once in a week. Sampling of the prawns was made on a fortnightly basis.

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3.6.2.2 Field Trial.

Before stocking, the ponds (1 fresh water and 2 brackish water) were dewatered and mahua oil cake (<u>Bassia latifolia</u>) applied at a rate of 250 ppm. All the ponds were limed at a rate of 500 Kg/ha on the next day and raw cowdung was applied at a rate of 2000 Kg/ha. After 1 week of manuring with cowdung, single super phosphate and urea were applied at a rate of 200 Kg/ha and 100 Kg/ha respectively.

After preparation, the ponds were stocked with juveniles, at a density of 10,000/ha. Weekly observations of water quality parameters like pH, temperature, dissolved oxygen and total alkalinity were made, while salinity was monitored twice in a week. Sampling of prawns was made, the first on the 50th day of stocking and the second on the 80th day. Ten animals from each pond were sampled using a cast net and the individual prawns were measured to the nearest gram and mm using a field balance and a measuring scale. After taking measurements, the prawns were released back into the respective ponds. Harvesting was done on the 100th day of stocking. During harvest the ponds were dewatered and prawns harvested using a cast net-Hand picking was also resorted to after complete draining. The prawns were measured individually after sexing.

3.6.2.3 Estimation of Oxygen : Nitrogen Ratios.

The juveniles used for this study were the ones used for the growth study in cement cisterns. Since the juveniles in the experimental cisterns were smaller in size, some larger sized individuals were separately acclimated and used for the study along with those taken from cisterns.

The prawns were starved for 24 hrs. prior to the experimental run. They were acclimated in the respirometer chamber for 1 hr before readings were taken and the chamber was flushed with the respective growth media throughout the period of equilibration. At the beginning of each experiment a water sample was drawn for estimating the dissolved oxygen and then the respirometer was closed. All the respirometers were placed in a water bath and maintained at a temperature $30 \pm 1^{\circ}$ C. After 1 hr a water sample was drawn for oxygen estimation. The difference was considered to be the oxygen consumed by the animal during the period of experiment. Replicate readings were taken in all the cases and were averaged. Between two runs, the water flow was allowed for 30 minutes for reaeration. After completion of the experiment, the prawns were blotted dry with a towel and weighed. The results are expressed in $\mu g O_2$ per hour and the weight specific respiration rates (μg oxygen/ \overline{g} wet weight/hour) were also calculated.

Ammonia excretion was determined using water samples drawn from the respirometer chamber prior to and after each respirometry. The results are expressed as μ g NH₃ - N/g wet weight/hr. All the readings were taken during the dark phase of the photoperiod, in order to assure uniformity of experimental conditions between each run.

Using the oxygen consumption and ammonia excretion rates O: N ratios of individual prawns in all the salinity levels were estimated following the method given by Bayne et al. (1985).

3.7 Soil and Water Analysis

Soil analysis was performed following the methods described below:

Available phosphorous	:	Bray method (Jackson, 1973)
Available potassium	:	Flame photometric method (Jackson,
		1973).
Organic carbon	:	Titrimetric method (Jackson, 1973).

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Soil pH : 1 : 2.5 mixture was made with distilled water and pH was directly measured using ELICO Digital pH meter model LI - 122.

The various water quality parameters were determined following the standard methods detailed below:

Salinity : Mohr - Knudson titrimetric method (Strickland and Parsons, 1972). Standard winklers method (Strickland Dissolved oxygen and Parsons, 1972). Electrometric method using ELICO pН \$ Digital pH meter model LI - 122. Using a mercury bulb thermometer Temperature : with an accuracy of 0.1°C. Acidimetric titration method (APHA Total alkalinity : et al., 1981). Ammonia : Photometric measurement using indophenol method (Grasshoff et al.,

1983).

3.8 Statistical Analysis

For the study to elucidate the effect of salinity on survival, LC₅₀ values were estimated using the probit analysis technique (Finney, 1971). Slope values, standard errors and 95% confidence limits were calculated.

In the second study, the growth data were converted to linear regressions and the slopes (b) compared on a multiple basis using F - test (Zar, 1974). Since the result showed a significant difference, pair wise comparisons were also made using t - test to assess which levels of salinities differed from which, with Linear regressions for the wet weight and oxygen consumption and wet weight and ammonia excretion were worked out for different salinity levels and the slopes of the regressions compared.

In the field trial, the length and weight measurements of individual prawns obtained from each pond were treated as constituting a random sample and these samples were compared using Analysis of variance technique (Snedecor and Cochran, 1967) to assess the influence of salinity on growth. Since the Analysis of variance performed showed significant difference at 1% level, pair-wise comparisons were made using critical difference values.

IV. EXPERIMENTAL RESULTS

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IV EXPERIMENTAL RESULTS

4.1 Effect of Salinity on Survival

Two sets of experiments were conducted to study the effect of salinity on survival of <u>M</u>. <u>rosenbergil</u>. The first set was to find the effect of abrupt transfer from 0 ppt to higher salinity levels upto 35 ppt but in the second set they were gradually acclimated to test salinity levels upto 35 ppt.

The data of the first experiment to study the effect of abrupt transfer are given in Table 3. Such transfer upto 25 ppt salinity is not found to cause any mortality within 120 hr. But transfer to 26.5 ppt showed 40% mortality of the juveniles within this time period. In 28.5 ppt only 45% of the animals died while in 30 ppt the mortality increased to 70%. Animals exposed to 35 ppt were found to die within 96 hr and as can be seen from Table 3, the animals can tolerate upto 25 ppt salinity for a period of 120 hrs without any mortality even when transferred directly from fresh water.

The cumulative percentage mortality of the juvenile prawns gradually acclimated to the test salinity levels are given in Table 4. The data show that the time-lag for the occurrence of first mortality in the different test levels were more in the acclimated individuals. In the abrupt transfer experiment, these times were 24 hr, 24 hr, 24 hr and 6 hr for 26.5, 28.5, 30 and 35 ppt salinity levels respectively, while in the gradual acclimation experiment the respective time intervals were 60 hr, 36 hr, 12 hr and 12 hr. After 120 hr the survival in each salinity level was found to be 100%, 60%, 55%, 30% and 0% for 0-25, 26.5, 28.5, 30 and 35 ppt salinity respectively in abrupt transfer, while in gradual acclimation the respective figures were 100%, 85%, 70% 40% and 10%.

The salinity-response curves for the 72 hr and 120 hr periods for both abrupt transfer and gradual acclimation are shown in Figs. 3 - 6. The average LC_{50} values at 72 hr obtained were 31.62 ppt and 33.3 ppt for abrupt transfer and

Table 3. Cumulative percentage mortality of freshwater acclimated <u>M. rosenbergii</u> juveniles (Size 120-210 mg) transferred abruptly to 5,10,15,20,25,26.5,28.5,30 and 35 ppt for 120 hrs (10 animals/replicate, 0 ppt control)

	0-25 ppt		20	26.5 ppt			28.5 ppt			30 ppt			35 ppt			
HOURS	Rep	Replicates		Rej	Replicates			Replicates			Replicates			Replicates		
	1	2	Mean	1	2	Mean	-	1	2	Mean	1	2	Mean	1	2	Mean
2	0	0	0	0	0	0		0	0	0	D	0	0	0.	0	0
6	0	0	0	0	0	0		0	0	0	0	0	0	20	0	10
12	0	0	0	Ô	0	0	,	0	0	0	0	0	O	30	20	25
24	0	0	0	0	10	5		10	10	10	20	10	15	30	40	35
36	0	O	0	10	20	15		10	10	10	20	20	20	30	40	35
48	0	Ō	0 ·	10	20	15		10	20	15	20	20	20	40	50 .	45
60	0	0	0	10	20	15		[.] 10	30	20	20	30	25	60	90	75
72	0	0	0	10	20	15		10	30	20	20	30	25	80	90	85
84	0	0	D	10	30	20		20	30	25	20	30	25	80	100	90
96	0	0	0	10	30	20		20	30	35	40	30	35	100	100	100
108	0	0	0	20	30	25		40	30	35	70	70	70	100	100	100
120	0	0	0	40	40	40		50	40	45	70	70	70	100	100	100

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Table 4. Cumulative percentage mortality of <u>M. rosenbergii</u> juveniles (size range 106-200mg) acclimated gradually to the test salinity levels of 5,10,15,20,25,26.5, 28.5, 30,35% (10 animals/replicate, 0% control)

	0-25% 26.5%				•	28.5%			30)%.		35%。 <u>Replicates</u>			
HOURS	Rep	Replicates		Rep	Replicates		Replicates			Replicates					
	1	2	Mean	1	2	Mean	. 1	2	Mean	1	2	Mean	1 :	2	Mean
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6.	0	0	0	0	0	0	0	0	· 0	0	0	0	0	0	Ō
12	0	0	0	0	Ō	0	0	0	, O	20	20	20	20	20	20
24	0	0	Ō	, D	0	0	0	0	0	20	20	20	20	20	20
36	0	0	0	0	0	0	10	0	5	20	20	20	40	60	50
48	0	0	0	0	· 0	0	10 [°]	0	5	20	20	20	40	60	50
60	0	0	0	0	10	5	10	0	5	20	20	20	40	60	50
72	0	0	0	0	10	5	20	10	15	20	20	20	60	80	70
84	0	0	0	10	10	10	20	10	15	20	20	20	60	80	70
96	0	0	0	10	10	10	20	20	20	20	40	30	60	80	70
108	0	0	0	20	10	15	20	30	25	40	60	50	60	80	70
120	0	0	0	20	10	15	30	30	30	60	60	60	80	100	90

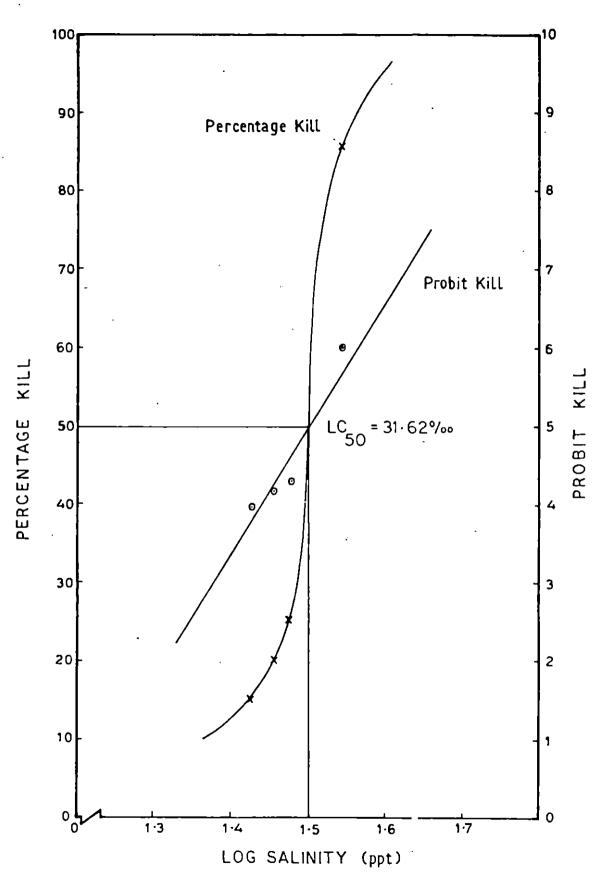
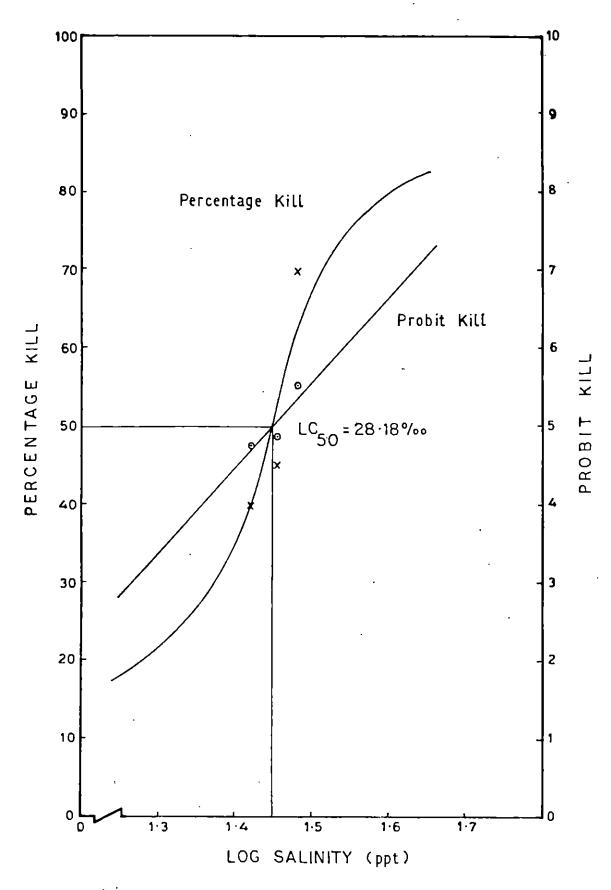


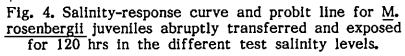
Fig. 3. Salinity-response curve and probit line for abruptly transferred <u>M. rosenbergii</u> juveniles exposed for 72 hrs in the different test salinity levels

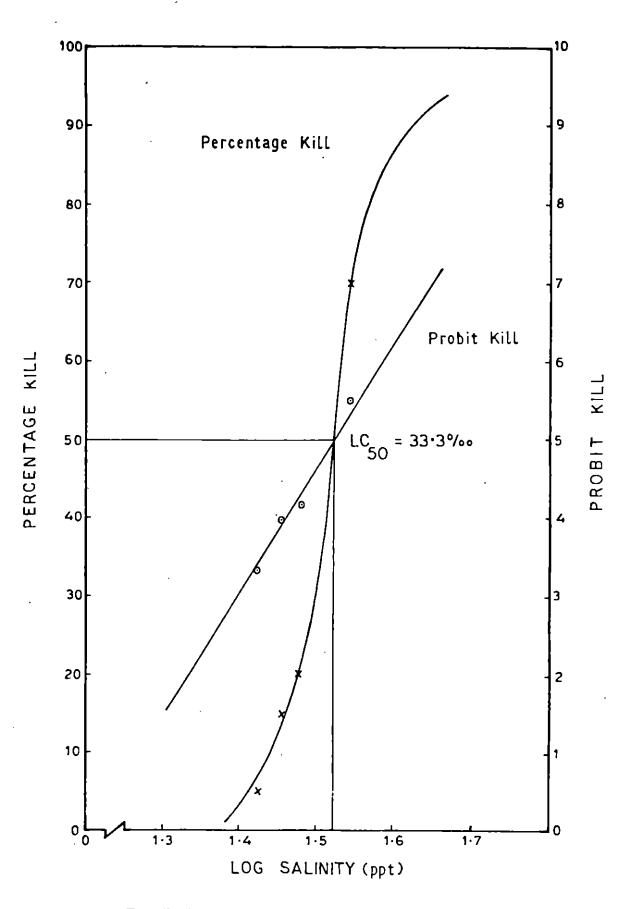
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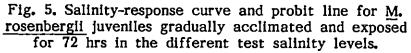


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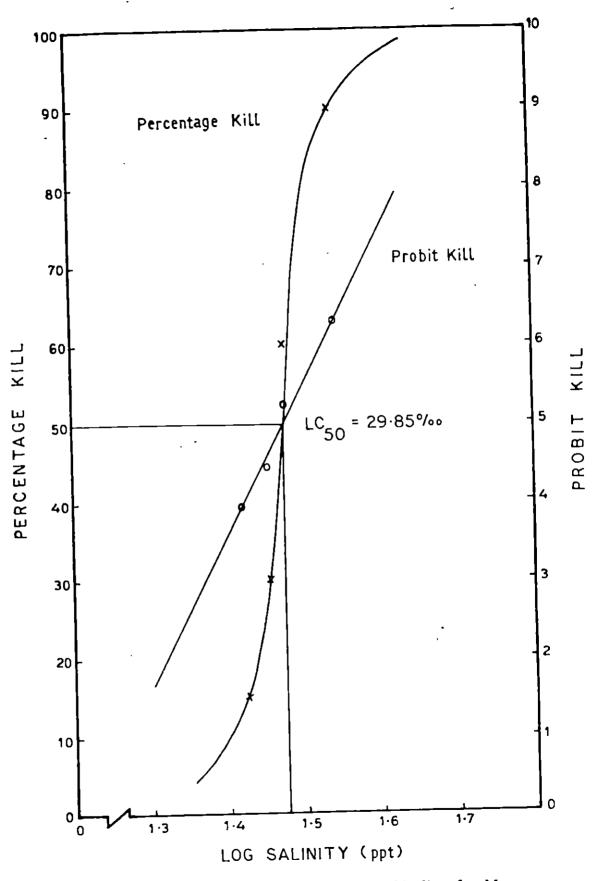


Fig. 6. Salinity-response curve and probit line for <u>M</u>. rosenbergii juveniles gradually acclimated and exposed for 120 hrs in the different test salinity levels.

Table 5. The 72 and 120-hr LC₅₀ values of <u>M. rosenbergii</u> abruptly transferred and gradually acclimated to different test salinity levels together with the corresponding standard error, slope and 95% confidence limits.

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Mode of Transfer	-		Standard	Slope	95% confidence limits				
	Exposure period (hr)	LC ₅₀ (%)			Lower limit	Upper limit			
Abrupt transfer	72	31.62	1.149	15.58	29.45	33.96			
	120	28.18	1.368	11-0	25.62	31.0			
Gradual acclimation	72	33.3	1.367	17.0	30.73	36.1			
	120	29.85	0.80	19-23	28.32	31.47			

gradual acclimation experiments respectively. The 120 hr LC_{50} values for both these cases were found to be 28.18 ppt and 29.85 ppt. The standard errors for the 72 hr LC_{50} values were 1.149 (abrupt) and 1.367 (gradual) and those for 120 hr were 1.368 (abrupt) and 0.80 (gradual). The slope of the probit regression line of abrupt transfer experiment for 72 hr was 15.58 and that for slow acclimation experiment was 17.0. For 120 hr period the corresponding values were 11.0 and 19.23 respectively. The 95% confidence limits for 72 hr LC_{50} values were 29.45 and 33.96 ppt for abrupt transfer and 30.73 and 36.1 ppt for gradual acclimation. The respective figures for the 120 hr LC_{50} values were 25.62 and 31.0 ppt for abrupt transfer and 28.32 and 31.47 for gradual acclimation (Table 5).

4.2 Effect of Salinity on Growth

4.2.1 Laboratory Experiment in Cement Cisterns.

The experiment to evaluate the growth of <u>M</u>. <u>rosenbergii</u> in different salinity levels was conducted in cement cisterns. The growth data of the tank-reared <u>M</u>. <u>rosenbergii</u> is given in Table 6. Growth rate was found to be maximum in 2 ppt (5.53 mg/day) and minimum in 8 ppt (2.49 mg/day). Percentage weight gain was also found to be highest in 2 ppt (206.64%), and the lowest in 8 ppt (68.5%). The fluctuations in the water quality parameters in the experimental cisterns during the course of the study are provided in Table 7.

The growth data were compared using linear regressions computed for different salinity levels. After comparing on a multiple basis, pair-wise comparisons were also done, when found necessary. The regression statistics for the relationship is given in Table 8. Regression lines representing growth in different salinity levels for the rearing period of three months are presented in Figs. 7-12.

Since the comparison of slopes showed significant difference between the treatments at 1% level of significance (Table 9) pair-wise comparisons were made using t-test. As shown in Table 10 there is no significant difference between treatments (p > 0.05) upto 6 ppt indicating that growth is almost uniform upto this

	· · ·	Table 6.	Data showing different sal replicates)	survival a linity level	and growth o s in cement	f <u>M. rosenbergii</u> cisterns (averag	<u>i</u> reared in e values of	
Salinity %.	Number stocked	Total initial weight (g)	Mean Initial weight (g)	Total Final weight (g)	Mean Final weight (g)	Percentage Survival	Growth rate (mg/day)	Percentage weight gain
0	10 `	2.40	0.240	6.52	0.652	100	4.58	171.67
2	10	2.41	0.241	7.39	0.739	100	5.53	206-64
4	10	2.46	0.246	6.79	0-679	100	4_81	176.02
6	10	2.41	0.241	6.41	0-641	100	4.44	165.98
·8	10	3.27	0.327	5.24	0.551	95	2.49	68-50
10	10	2.64	0.264	5.07	0.507	100	2.70	92.05

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Treatments	Ĭ	рН		d oxygen (ppm)	Water te	mperature (^O C)	Total Alkalinity (ppm)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
0‰	8.31	7.77-8.62	5.91	5.60-6.40	27.88	27.0-29.5	55.0	47.5-61.5
2.0‰	8.24	7.88-8.55	5.87	5.15-6.70	27.96	27.0-29.0	42.0	39.0-45.5
4-0%	8,20	8.00-8.40	6-02	5.65-6.25	28.11	27.5-29.0	49.5	45.5-53.0
6.0%	8.22	7.97-8.41	6.00	5.50-6.60	27.96	27.5-29.0	55.0	51 . 5 <mark>-</mark> 58.5
8.0%	8.10	7.65-8.51	5-86	5.20-6.25	27.95	27.0-29.0	50.0	45.0-58.0
10.0‰	7.89	7.52~8.52	5.70	5.30-6.35	28.16	27.5-29.0	59.0	52.5-66.0

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Table 7. Data showing the water quality (summary) of the experiment in cement cisterns

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Table 8. Regression statistics for growth (in wt) of <u>M. rosenbergii</u> reared in different salinity levels against the serial numbers of observations

Salinity ‰ 🕐	Number of observations	Correlation coefficient (r)	Slope	SE
0.0	7	0_97769*	0.071607	0.00688
2.0	· 7	0.99771*	0 08339	0.00253
4.0	7 .	0.99343*	0.0733214	0.00377
6.0	· 7	0 _99 95*	0.066679	0.00093
8.0	7	0.97474*	0.03689	0.00381
10-0	7	0.99599*	0.04193	0.00168

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* Significant at 1% level

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Table 9. Comparison of slopes of the regression lines representing growth of <u>M. rosenbergii</u> in different salinities against serial numbers of observations.

· .		x ²	XY	Y ²	Residual sum of squares	d.f
Regression	1	28	2.005	0.150198	0.006626	5
	2	28	2.335	D.195616	0.000894	5
	3	28	2.053	0.15252	0.001991	5
	4	28	1.867	D.12461	0.000121	5
	5	28	1.033	0.04014	0.00203	5
-	6	28	1.174	0.04962	0.000396	5
Pooled regression					0.012058	30
Common regression		168	10.467	0.7127	0.06057	
Com	anico	on of slo		0.06057-0.0120	158)/5	
COm			pc3. i	0.012058/30		
			= 2	24.14*		

* Significant at 1% level

Pairs	d.f.	t-values
Between O and 2 %,	10	1.608 NS
0 and 4	10	0-2182 NS
0 and 6	10	0.7099 NS
0 and 8	10	4.415 S
0 and 10	10	4 . 190 S
2 and 4	10	2.218 NS
2 and 6	10	3.657 S
2 and 8	10	5 .9137 S
2 and 10	10	13.658 S
4 and 6	10	1.7098 NS
4 and 8	10	6.814 S
4 and 10	10	7.602 S
6 and 8	10	7.6227 S
6 and 10	· 10	12.879 S
8 and 10	10	1.2324 NS

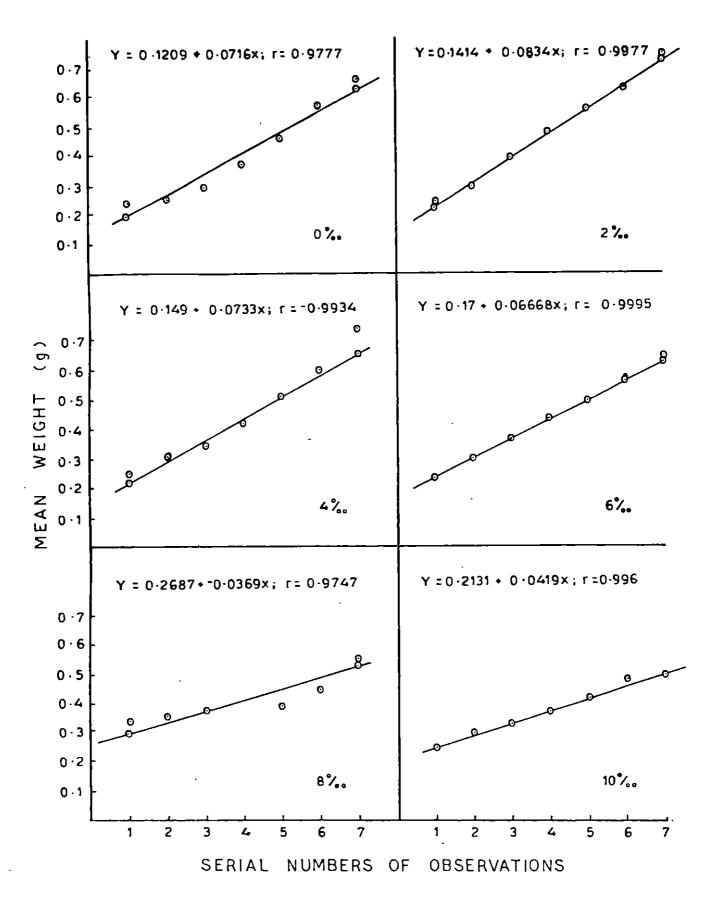
Results of pair-wise comparison of growth of <u>M.</u> rose	<u>1</u> –
bergiireared under different salinity conditions.	

NS Not significant

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S Significant at 1% level



Figs. 7-12. Relation between mean weight and serial number of observations of <u>M. rosenbergii</u> reared under different salinity conditions.

salinity level. However, in the comparison between the regression co-efficients of 2 ppt and 6 ppt a slight difference is noticed. All the treatments upto 6 ppt differed significantly from 8 and 10 ppt and between 8 and 10 ppt there is no significant difference at 1% level.

4.2.2 Field Trial.

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The trial to assess the growth of prawns in the field in fresh as well as in saline conditions was done in one fresh (I) and two brackish water ponds (II & III). The salinity in the brackish water ponds fluctuated between 1.8 ppt and 10.84 ppt and 2.1 ppt & 20.4 ppt with mean salinities of 7.27 ppt and 12.7 ppt respectively for pond No. II and III. The mean weight and length of the prawns with their standard deviations are given in Table 11 and these are plotted against the respective sampling days in Figs. 13-15. The salinity fluctuations in the ponds are also shown in the above Figs. From these, it can be seen that the variations in size in terms of weight are more pronounced than in terms of length. The prawns reached an average length of 11.45 cm and an average weight of 12.15 g in pond No. II no. II and 12.81 g in pond No. II and 9.8 cm and 6.53 g in pond No. III respectively.

At the time of second sampling difficulty was experienced in collecting representative samples from pond No. II. The stocking and production details of the three ponds are given in Table 12. The stocking density was 10,000/ha, number stocked in ponds I, II and III being 112, 100 and 100 respectively. The production data along with their mean salinity levels are plotted in Fig. 16 which show that with an increase in salinity in the pond, there is a decrease in production. Survival was found to be higher in pond No. I (59%) followed by pond No. III (58%) and pond No. II (45%). The mean size at harvest was found to be higher in pond No. II (12.80 g Vs 12.12 g in pond No. I and 6.53 g in pond No. III). The highest production was obtained from pond No. I (fresh water) followed by pond No. II (mean salinity 7.27%) and pond No. III (mean salinity 12.7%). The respective production figures were 79.99 Kg/ha, 57.6 Kg/ha and 37.87 Kg/ha in 100 days.

The harvested prawns (Fig. 17) were subjected to a population analysis and it was found that there was female domination in all the three ponds (Table 13). The male to female ratio was found to be almost similar in the three ponds with the females forming 78.8%, 81.8% and 79.3% of the prawn harvest from pond No.I, II and III respectively. The percentage contribution of females to total production was 64.93, 66.4 and 69.3 in that order. The mean length and weight of the male population was found to be higher in all the three ponds with their mean weights being 20.04 g, 24.25 g and 9.75 g as against the corresponding figures for females of 10.01 g, 10.26 g and 5.67 g respectively in these ponds.

The fluctuations in dissolved oxygen in the ponds are shown in Fig. 18. The minimum dissolved oxygen level was observed in the fresh water pond, where the levels ranged from 1.9 - 5.28 ppm with a mean value of 4.1 ppm (Table 14). In ponds II and III the ranges were 2.92 - 6.8 ppm and 3.23 - 6.1 ppm with mean values of 4.8 and 4.77 ppm. On the day of second sampling mortality of five prawns was observed in the fresh water pond and on the subsequent day the dawn dissolved oxygen level: in this pond was found to be 0.82 ppm. The mean pH values for the ponds were 8.06, 7.18 and 7.33 for ponds I, II and III respectively (Fig. 19). While in pond No. I the total alkalinity values fluctuated from 22 - 62, in pond No. II and III the respective levels ranged from 7.8 - 19 and 10.9 - 19.8 (Fig. 20). The temperature fluctuations in the ponds were found to be almost uniform in all the ponds throughout the culture period (Fig. 21) and ranged from $27 - 32^{\circ}$ C each in pond No. I and II and 27 - 31.5° C in pond No. III.

The results of the soil analysis are shown in Table 15. The available phosphorus in the ponds ranged from 17.6 - 40 Kg/ha, 22 - 42 Kg/ha and 19.8-55 Kg/ha in pond No. I, II and III respectively. The content of available potassium was very



Pond No.1 (Fresh water)		(Brackish w	water)	Pond No.III (Brackish water) (Max. sal.20.4 ppt)		
Length	Weight	Length (cm)	Weight	Length	Weight	
(cm)	(gm)		_ (gm)	(cm)	(gm)	
2.27	0.235	2 . 15	0.226	2.1	0.21	
<u>+</u> 0.4809	<u>+</u> 0.0882	<u>+</u> 0.4743	<u>+</u> 0.083	<u>+</u> 0.258	<u>+</u> 0.0480	
8.72	6.9	8.56	6.2	7 . 52	3.2	
<u>+</u> 1.6752	<u>≁</u> ~4.557	<u>+</u> 1.2563	<u>+</u> 2.740	<u>+</u> 0.6973	<u>+</u> 1.0328	
11.3318	10-4545	11_04	14.1	8.2375	4.75	
<u>+</u> 2.1628	<u>+</u> 5-7795	<u>+</u> 2_2839	<u>+</u> 7.8804	<u>+</u> 1.4889	<u>+</u> 2.7646	
11_4515	12.1212	10.9295	12 . 8068	9 . 8069	6.5345	
<u>+</u> _2_8592	<u>+</u> 8.8279	<u>+</u> 3.0842	<u>+</u> 10.2371	<u>+</u> 1.9259	<u>+</u> 4.6748	
	(Fr Length (cm) 2.27 <u>+</u> 0.4809 8.72 <u>+</u> 1.6752 11.3318 <u>+</u> 2.1628 11.4515	(Fresh water) Length Weight (cm) 0.235 ± 0.4809 ± 0.0882 8.72 6.9 ± 1.6752 ± 0.0882 8.72 ± 0.0882 11.3318 10.4545 ± 2.1628 ± 5.7795 11.4515 12.1212	Length (cm)Weight (gm)Length (cm)2.27 ± 0.4809 0.235 ± 0.0882 2.15 ± 0.4743 8.72 ± 1.6752 6.9 ± 1.6752 8.56 ± 1.2563 11.3318 ± 2.1628 10.4545 ± 5.7795 11.04 ± 2.2839 11.451512.121210.9295	(Fresh water)(Brackish water) (Max. sal. 10.84 ppt)Length (cm)Weight (gm)Length (cm)Weight (gm)2.27 ± 0.4809 0.235 ± 0.0882 2.15 ± 0.4743 0.226 ± 0.0833 8.72 ± 1.6752 6.9 ± 2.740 8.56 ± 1.6752 6.2 ± 2.740 11.3318 ± 2.1628 10.4545 ± 5.7795 11.04 ± 2.2839 14.1 ± 7.8804 11.451512.121210.9295 ± 12.8068	Fond No.1(Fresh water)(Brackish water)(Brackish water)Length (cm)Weight (gm)Length (cm)Weight (gm)Length (cm)Length (gm)Length (cm)2.27 ± 0.4809 0.235 ± 0.0882 2.15 ± 0.4743 0.226 ± 0.083 2.1 ± 0.083 2.1 ± 0.258 8.72 ± 1.6752 6.9 $\pm ^{4-4}.557$ 8.56 ± 1.2563 6.2 ± 2.740 7.52 ± 0.6973 11.3318 ± 2.1628 10.4545 ± 5.7795 11.04 ± 2.2839 14.1 ± 7.8804 8.2375 ± 1.4889 11.451512.121210.9295 ± 12.8068 9.8069 ± 4.0250	

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Table 11. Mean length and weight at stocking, samplings and harvest of <u>M. rosenbergii</u> reared in one freshwater pond and two brackishwater ponds together with standard deviations

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Table 12.	Data showing stocking, harvesting and production details of M. rosen-
	bergii reared in one freshwater pond and two brackish water ponds

Pond details		Stocking data			Production			
	Number of Prawns	Mean size (g)	Density (No./M ²)	Elapsed days	Survival (%)	Mean size (g)	Growth rate/ (g /day)	(Kg/ha/100 days)
Pond No.I (Fresh water)	112	0.235	1	100	59	12.12	0.119	79.99
Pond No.II ¹ (Brackishwater)	100	0.201	1	100	45	12.80	0.126	57.60
Pond No.III ² (Brackishwater)	100	0.226	1	100	58	6.53	0.063	37.87

1. Salinity fluctuated from 1.8 ppt to 10.84 ppt with a mean salinity of 7.27 ppt

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2. Salinity fluctuated from 2.1 ppt to 20.4 ppt with a mean salinity of 12.7 ppt

	Pond No.I (Fresh water pond)		Pond No (Max. Sal.	-II 10.84 ppt)	Pond No.III (Max. Sal. 20.4 ppt)	
	Males	Females	Males	Females	Males	Females
Sex distribution (%)	21.2	78.8	18.2	81-8	20.7	79.3
Mean Weight (g)	20.04	10-01	24.25	10.26	9.75	5.67
Mean Length (cm)	13.89	10.79	13-91	10.27	10.91	9.52
Weight range (g [.])	9-40	1-32	10-42	1-34	2-24	1-14
Length range (cm)	12-16.9	4.5-16	11.2-16.8	4.7-15.5	7.7-14.2	4.9-13
Contribution to total Production (%)	35.07	64.93	33.6	66.4	30.7	69.3

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Table 13. Population analysis and sex-wise production details of <u>M</u>. <u>rosenbergii</u> reared in one freshwater pond and two brackishwater ponds

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		inity ppt)	рН		Dissol	ved Oxygen (ppm)	Water te (⁰ (emperature)	Total al (p	kalinity opm)
Pond	Mean	Range	. <u> </u>	Range	Mean	Range	Mean	Range	Mean	Range
No.I (Freshwater)	0.319	0.18-0.54	8.06	7.1-8.58	4.10	1.9-5.28	29	27-32	41.52	22-62
No.II (Max. Sali- nity upto 10.84 ppt)	7.27	1.8~10.84	7.18	6.81-7.52	4.80	2.92-6.80	28.8	27-32	14.44	7.8-19
No.III (Max. Sali- nity upto 20.4 ppt)	12.74	2.1-20.4	7.33	6.41-8.15	4.77	3.23-6.10	29	27-31.5	15.05	10.9-19.

Table 14. Data showing the water quality (summary) in the experimental ponds

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· · ·	Pond	No.I (Fre	sh water)	Pond	No.II (Brackish	water)	Pond No	.III (B	rackish	water)
<i>.</i> .	·	Sampling	 gs			Sam	plings			Samp	lings	
	1	2	3	4	1	2	3	4	1	2	3	4
Available Phosphorus (kg/ha)	19.8	28.6	17.6	40	31	42	35	22	19.8	55	40	30.8
Available Potassium (kg/ha)	128	55 ·	92	27	37	73	146	55	73	92	110	101
Organic Carbon (%)	0.21	0.39	0.16	0.37	0.81	0.32	0.64	0.37	0.55	0.76	0.37	0.28
рН	6.2	6.2	6-1	5.6	5.7	6.1	5.3	5.4	6.5	6.1	5-8	6.0

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Table 15. Results of soil analysis of the culture ponds

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Table 16. Analysis of variance table comparing the growth of <u>M. rosen</u> <u>bergii</u> reared in one freshwater pond and two brackish water ponds

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Source	Sum of squares	Degrees of freedom	Mean sum of squares	F. ratio
ſreatment	1316.929	2	658-4645	9.928*
Error	10944.07	165	66.327	
fotal	12261.988	- 167		

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* Significant at 1% level

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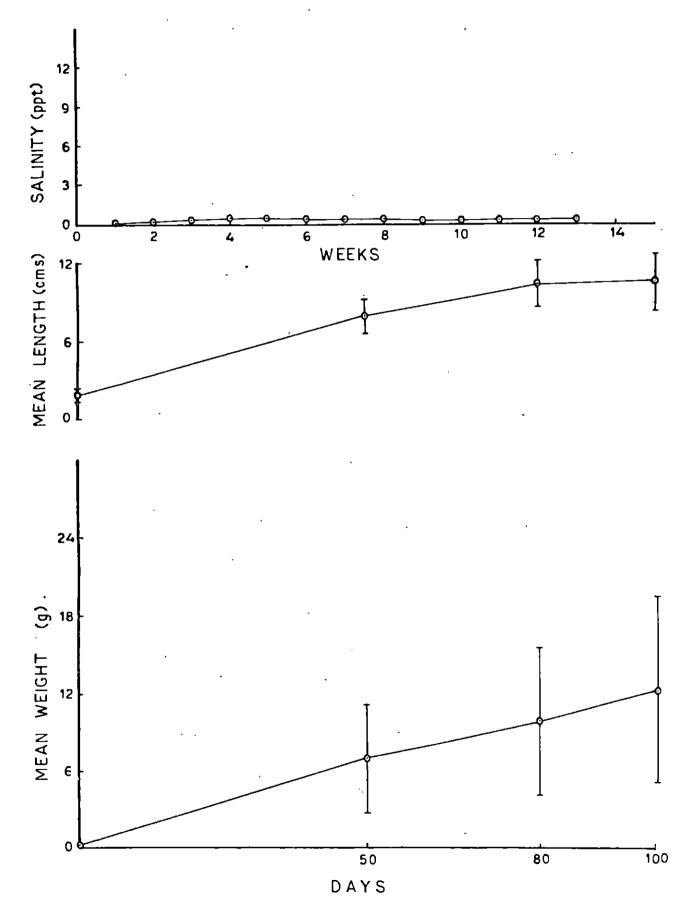
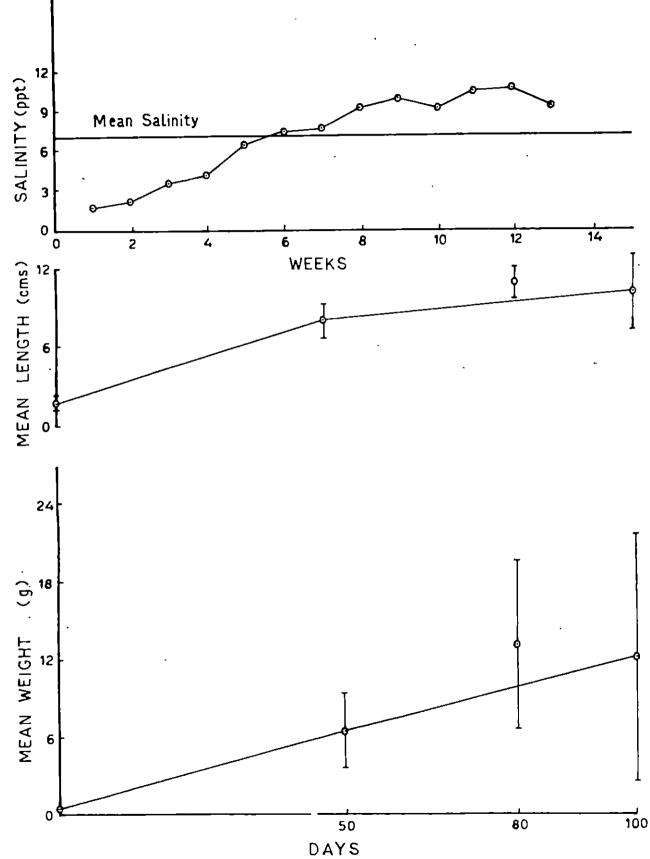


Fig. 13. Graph showing the growth of <u>M</u>. <u>rosenbergii</u> together with the fluctuations in salinity in pond No. I. Vertical lines indicate standard deviations.



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Fig. 14. Graph showing the growth of <u>M. rosenbergii</u> together with the fluctuations in salinity in pond No. II. Vertical lines indicate standard deviations.

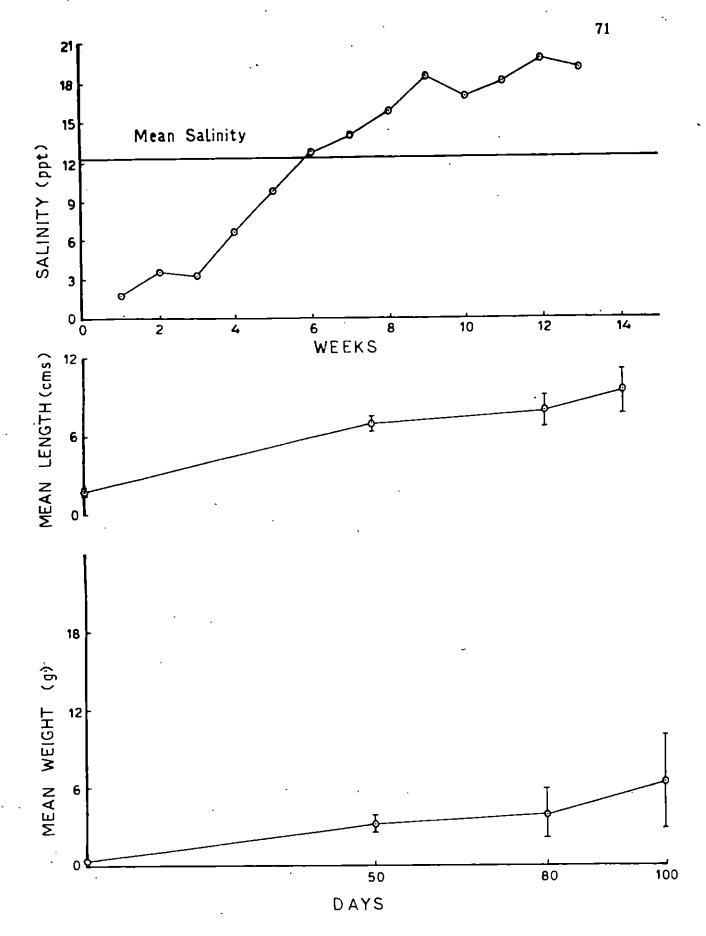


Fig. 15. Graph showing the growth of <u>M. rosenbergii</u> together with the fluctuations in salinity in pond No. III. Vertical lines indicate standard deviations.

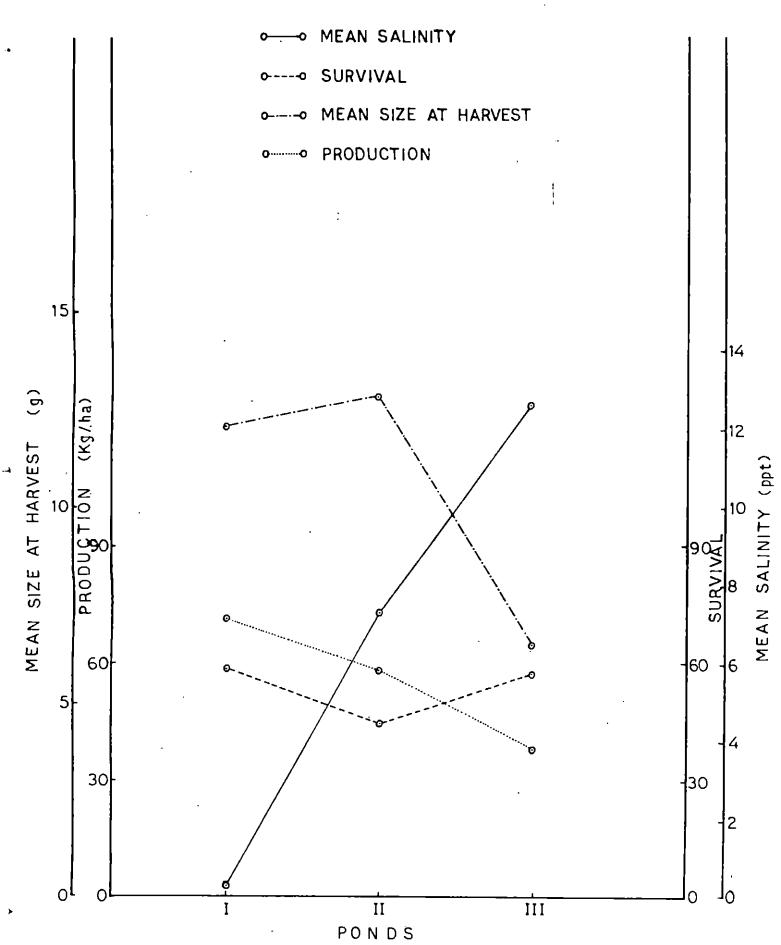
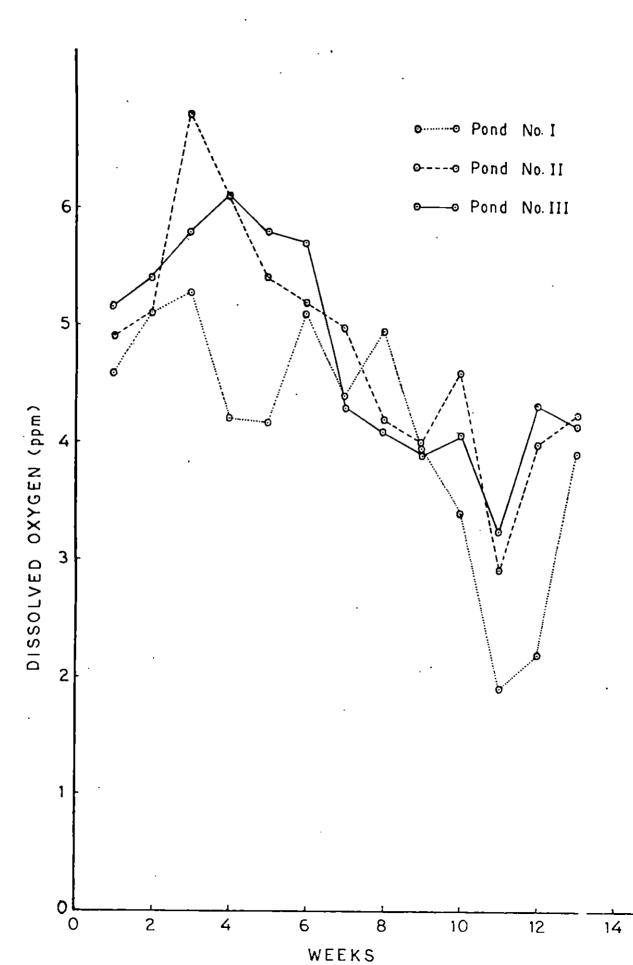


Fig. 16. Graph showing production, survival and mean size at harvest of the prawns reared in the ponds together with their mean salinities.



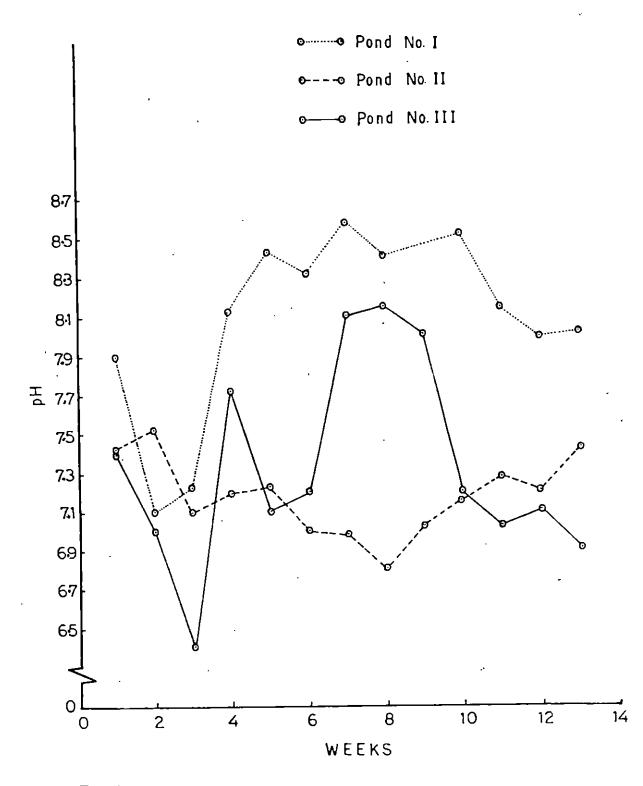


Fig. 19. Graph showing the fluctuations in pH in the culture ponds.

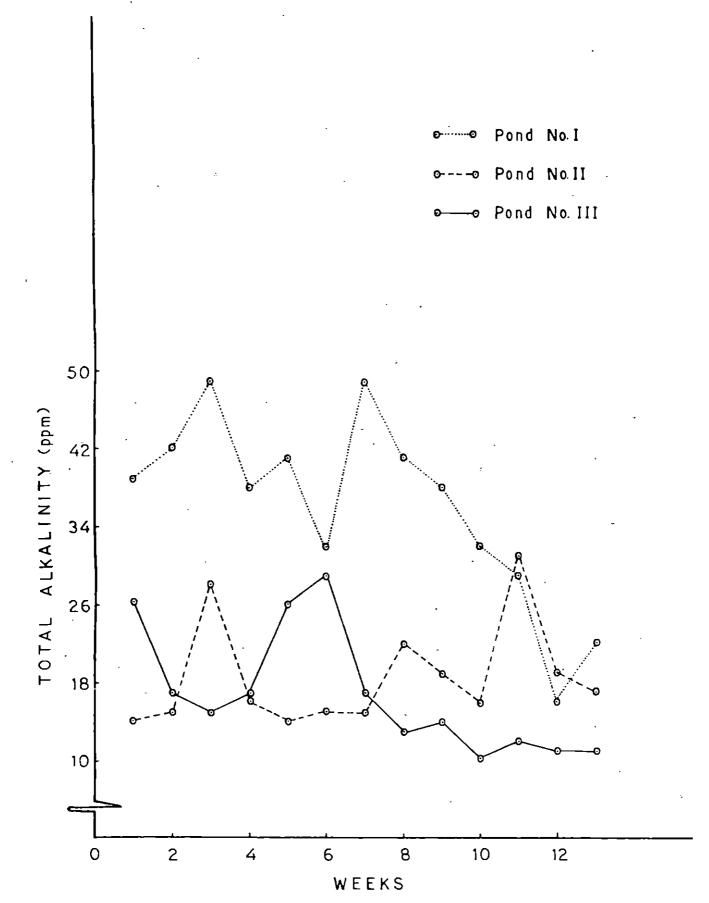


Fig. 20. Graph showing the fluctuations in total alkalinity in the culture ponds.

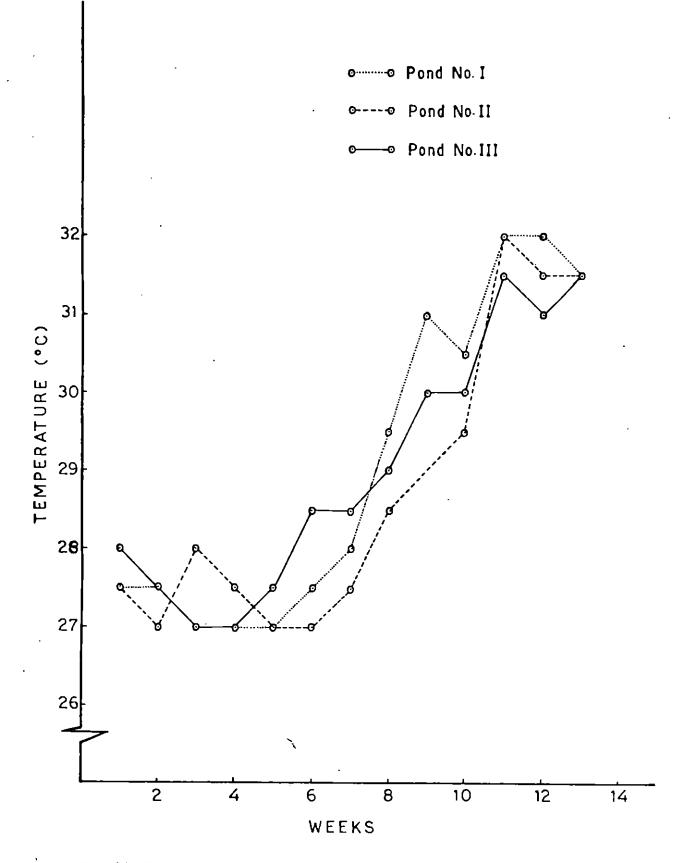


Fig. 21. Graph showing the fluctuations in temperature in the culture ponds.

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high in all the ponds the ranges in pond No. I, II and III being 27 - 128 Kg/ha, 37 - 146 Kg/ha and 73 - 110 Kg/ha. The organic carbon content in the three ponds ranged from 0.16 - 0.39%, 0.32 - 0.81% and 0.28 - 0.76% in pond No. I, II and III respectively. The pH values were found to range from 5.6 - 6.2, 5.3 - 6.1 and 5.8 - 6.5 in pond No. I, II and III respectively, being slightly acidic in all cases.

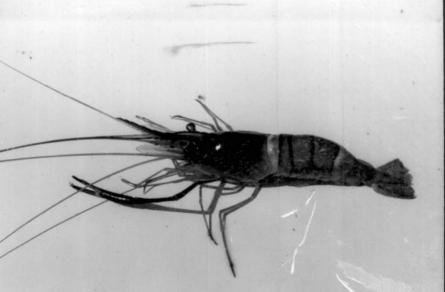
During harvesting in pond No. I, a prawn with an abnormal colouration in the tail region was obtained (Fig. 22). The prawn was found at the surface water near the bund, very weak and reluctant to move away to the interior. The last three segments were characteristically opaque with a brownish tinge, the affected region being the musculature rather than the exoskeleton. It remained alive only for a few hours in the laboratory and before death the opaqueness had spread the entire body. No histopathological study was however conducted to find out the causative agent.

Analysis of variance performed to assess the differences in the influence of salinity on growth in the three ponds showed significant difference at 1% level of significance (Table 16).

Pair-wise comparisons were made using critical difference values (4.1522 at 1% level) to assess which treatment vary from which with respect to growth. Thus it is found that between ponds No. I and II (the difference between the mean weights being 0.68 g) the difference is not significant while between pond No. I and III, and II and III (the difference between the mean weights being 5.59 g and 6.27 g respectively) the differences are found to be significant.

4.2.3 Estimation of Oxygen : Nitrogen Ratios.

Oxygen consumption and ammonia excretion of the individual prawns reared in different salinity levels in cement cisterns were estimated and oxygen to nitrogen ratios computed which serve as an index to find the optimal conditions for growth.



Figs. 23-28 illustrate the relation between oxygen consumption ($\mu g O_2/hr$) and the wet weight of the animal. This relationship is best explained by the equation $Y = ax^b$, which on logarithmic transformation gives the form Log Y = Log a + b Log x, where the symbols are explained as $Y = \mu g$ of Oxygen consumed per hour, x = wet weight of the organims in mg, Log a = the Y intercept of the line and b = the slope. This model was used to compute the regressions of Log Y on Log x at each salinity level. The correlation co-efficients between the logarithms of two variables (r) ranged from 0.9277- 0.9822 for different salinities as shown in Table 17. The slopes of the plots are also provided in the Table together with their standard errors.

The metabolic rate was determined by expressing oxygen consumption on a per gram basis. Metabolic rates were found to be influenced by the weight of the animal as illustrated in the Figs. 29-34. This relationship is explained by the model $y/x = ax^{b-1}$ where $y/x = \mu$ g oxygen consumed per gram per hour and x = the weight of the individual prawn. To determine the constants a and b the data were converted into their logarithms and the linear model Log y/x =Log a + (b-1) Log x was used. The correlation co-efficient for the relationship between the metabolic rates and wet weight ranged from -0.8776 to -0.9520. The oxygen consumption at each salinity level for selected weights were estimated from Similarly the metabolic rates the regression equations and plotted in Fig. 35. determined for such selected weights were also plotted in Fig. 36. From these figures it can be seen that there is an increase in oxygen consumption with weight but when expressed on a per gram basis the rates decrease with weight. The slopes of the regressions between the wet weight and oxygen consumption were compared on a multiple basis using F-test. The b values were not found to be significant at the 5% level of significance (Table 18).

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Salinity (ppt)	Number of animals	Log. oxygen consumption µg/hr on log wet weight (mg) correlation co- efficient (r)	Slope	S.E.	Log µg O ₂ /g/hr on log wet weight (mg) correlation co- efficient (r)	Slope
0	11	0.9443*	0.4584	0.0532	- 0.8776	- 0.5211
2	12	0.9277*	0.5495	0.0699	- 0.8976	- 0.4512
4	13	0.9321*	0.5042	0.059	- 0.9326	- 0.4958
6	12	0.9519*	0.6238	0.0635	- 0.8884	- 0.3886
8	11	0.9822*	0.6277	0.0623	- 0.9520	- 0.3727
10	12	0.9625*	0.5930	0.0528	- 0.9254	- 0.4062

Table 17. Regression statistics for the relationship between oxygen consumption and wet weight of juvenile <u>M. rosenbergij</u> at six levels of salinity

* Significant at 1% level

		x²	XY		۲ ²	Residual sum of squares	d.f.
Regression	1	1.8046	0_8;	2724	0.4252	0.046	9
	2	1.1931	0.6	555	0.4186	0.0584	10
	3	1.5723	0.79	929	0.4601	0.0602	11
	4	1.0262	0.6	401	0.4407	0.0414	10
	5	0.9204	0.5	777	0.3759	0.0133	9
	6	1.1513	0.6	827	0.4369	0.0321	10
Pooled Regression						0-2514	59
Common Regression		7 6679	4-1	761	2.5574	0.2830	
Compari	son o	f slopes:	F =	(0.2	830 - 0.2	2514)/5	
• •	_				0.2514/5	59	
			=	1.48	*		

Table 18. Comparison of slopes of the regression lines of oxygen consumption of <u>M. rosenbergii</u> reared in different salinities against their wet weights

* Not significant at the 5% level

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The linear regressions between the wet weight and ammonia excretion were worked out for different salinity levels under consideration and plotted in Figs. 37-42. The slopes of these regressions were compared on a multiple basis using F-test. The comparison gave a significant result at 5% level, but it was not significant at 1% level (Table 19). The slopes were further compared pair-wise, using t-test and the results did not show significant difference at 5% level except at a few couple of cases (vide Table 20). Since no pattern was evident in these results, the significance was considered at 1% level.

A linear model of the form Log NH_3 - N excretion = Log a + b Log x describes the relationship between ammonia excretion and wet weight where Log a = the y intercept of the line, b = the slope and x = the wet weight of the animal in mg. The r values between the Log ammonia excretion and Log wet weight ranged from 0.9415 to 0.9877 (vide Table 21) all of which were found to be significant at the 1% level of significance. The ammonia excretion rates for selected weights were estiamized for juveniles reared in all the salinity levels and plotted in Fig. 43. The maximum values were observed in 8 ppt which was followed by 10 ppt. There was a reduction in ammonia excretion rates towards the lower salinity levels. From Fig. 43 it can be seen that for smaller individuals the excretion rates were almost uniform in all the salinities but in higher levels difference in these rates were more pronounced.

Weight specific ammonia excretion rates were computed on a per gram basis and these were also found to be influenced by the weight of the animal as shown in Figs. 44-49. This relationship can be explained using the model $z/x = ax^{b-1}$ where $z/x = \mu$ g ammonia excreted/g/hr, x = wet weight of the animal in mg. Log transformation of the data facilitated the use of the linear model Log z/x =Log a + (b-1) Log x for the computation of the constants a and b. The correlation co-efficients for the relationship between weight specific ammonia excretion rates and weight ranged from -0.6169 to -0.9099. Ammonia excretion rates per gram

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wet weight per hour computed for selected weights obtained from the above relationship are plotted in Fig. 50. The excretion rate was observed to decrease in larger individuals.

Oxygen : Nitrogen ratios were estimated for prawns reared in the respective salinity levels and the mean values were found to be 33.51, 31.04, 33.42, 27.65, 27.13 and 27.06 at 0, 2, 4, 6, 8 and 10 ppt respectively. Estimated O : N ratios for selected weights are plotted in Fig. 51 which shows a declining trend towards the higher levels.

		x ²	XY	۲ ²	Residual sum of square	d.f. s
Regression	1	1.8178	1.0471	0.6804	0.0773	10
	2	1-1931	0 8505	0.6268	0.0205	10
	3	1.5723	0.9398	0.5940	0.0322	11
	4	1.0262	0.7529	0.5669	0.0146	10
	5	0.9204	0.7410	0.6542	0.0576	9
	6	1.1513	0.8684	0.6714	0.0164	10
Pooled Regression					0-2186	60
Common Regression		7.6810	5.1996	3.7936	0.2738	
Compariso	n of	slopes: F =	(0.2738 - 0.3	2186)/5		
· · · · · · · · · · · · · · · · ·		• • •	0-2186/	60		
		= :	3.0297*			•

Table 19. Comparison of slopes of the regression lines of Ammonia Excretion of <u>M. rosenbergii</u> reared in different salinities against their wet weights

* Significant at 5% level of significance but not significant at 1% level

<u> </u>			
	Pairs	d.f.	t-values
Between	0 and 2	20	1.6610
	0 and 4	21	0.2759
	0 and 6	20	1 8840
	0 and 8	19	2.1256*
	0 and 10	20	2.1870*
	2 and 4	21	1_8923
	2 and 6	20	0.3684
	2 and 8	19	1.0367
	2 and 10	20	0.7660
	4 and 6	21	0.5901
	4 and 8	20	2.3576*
	4 and 10	21	2.6530*
	6 and 8	19	0.3571
	6 and 10	20	0.3856
	8 and 10	19	0.5824

Table 20.	Results of pair-wise	comparison of ammonia	excretion of
	M. rosenbergii in the	test salinity levels	

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* Significant at 5% level

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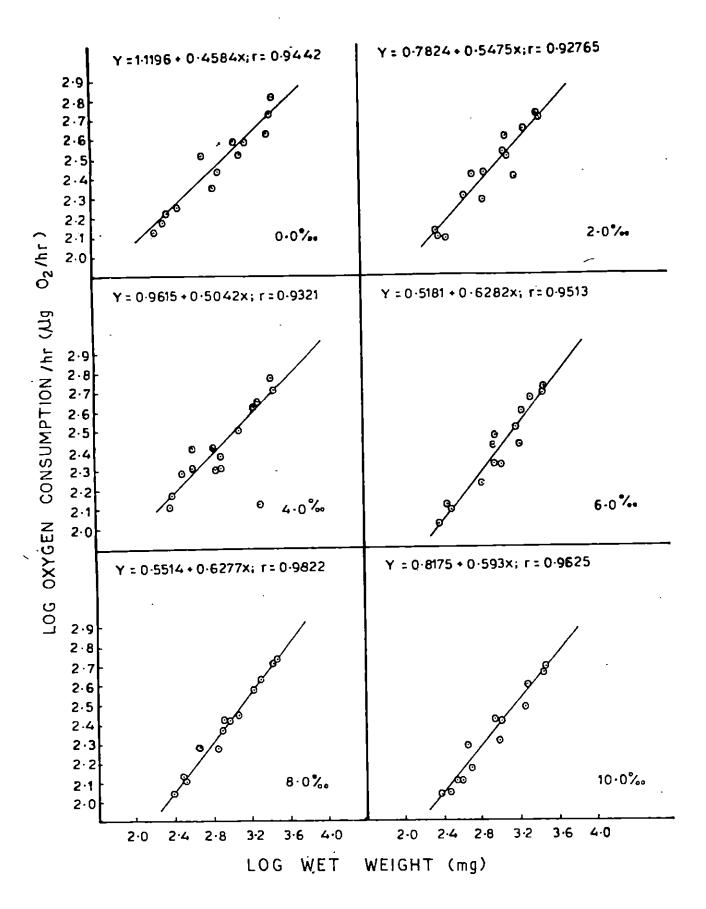
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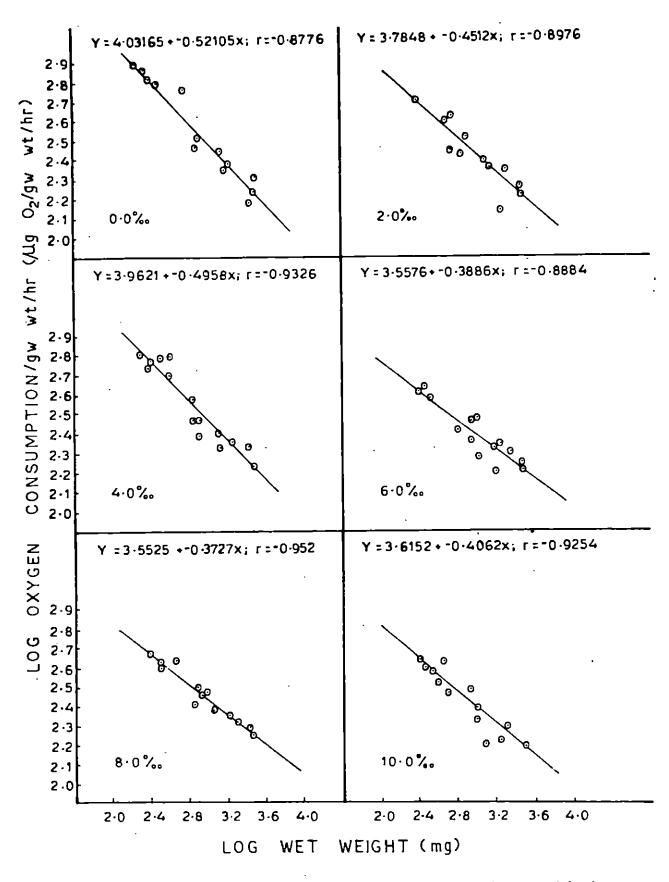
Salinity (ppt)	Number of animals	Log NH ₃ excretion (µg/hr) on log wet weight (mg) correlation coefficient(r)	Slope	S.E.	μg NH ₃ excretion/gm/hr on wet weight (g) corre- lation coefficient (r)	Slope
0	12	0.9415*	0.5760	0.0652	- 0.8989	- 0.4237
2	12	0.9835*	0.7129	0.0415	- 0.9086	- 0.2837
4	13	0 9725*	0.5977	0.0432	- 0.8369	- 0.4774
6	12	0.9870*	0.7337	0.0377	- 0.9099	- 0.2798
8	11	0-9550*	0.8051	0.0834	- 0.6169	- 0.1958
10	12	0.9877*	0.7543	0.0377	- 0.8985	- 0 .24 53

Table 21. Regression statistics for the relationship between Ammonia-N excretion and wet weight of <u>M</u>. <u>rosenbergii</u> juveniles at six levels of salinity

* Significant at 1% level



Figs. 23-28. Relation between oxygen consumption and wet weight (mg) in <u>M. rosenbergii</u> reared under different salinity conditions.



Figs. 29-34. Relation between metabolic rate and wet weight in <u>M. rosenbergii</u> reared under different salinity conditions.

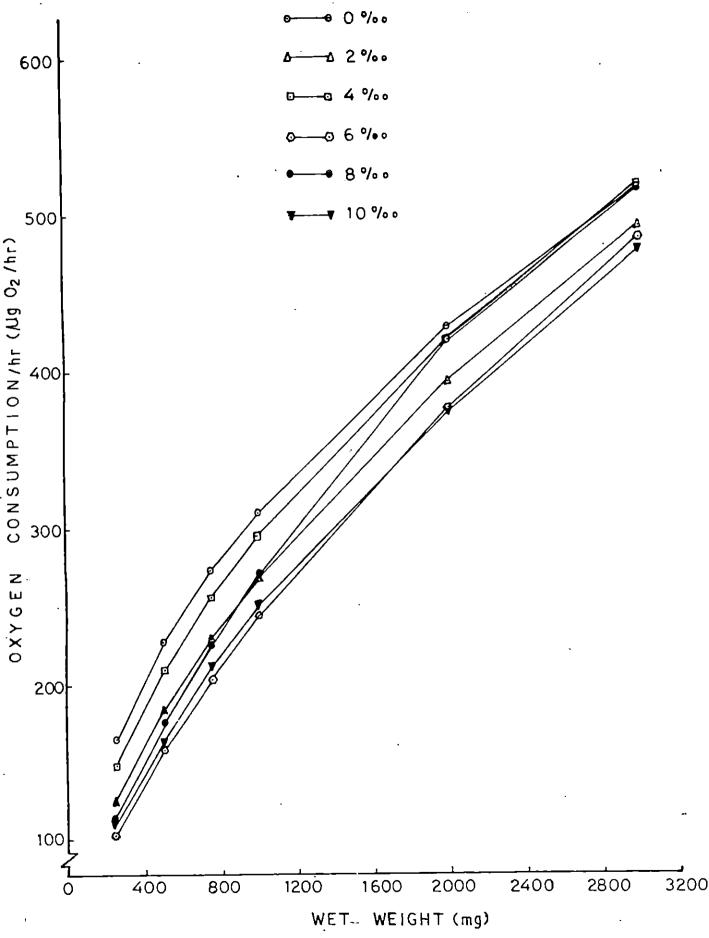


Fig. 35. Graph showing the estimated oxygen consumption rate for selected weights of <u>M</u>. <u>rosenbergii</u>.

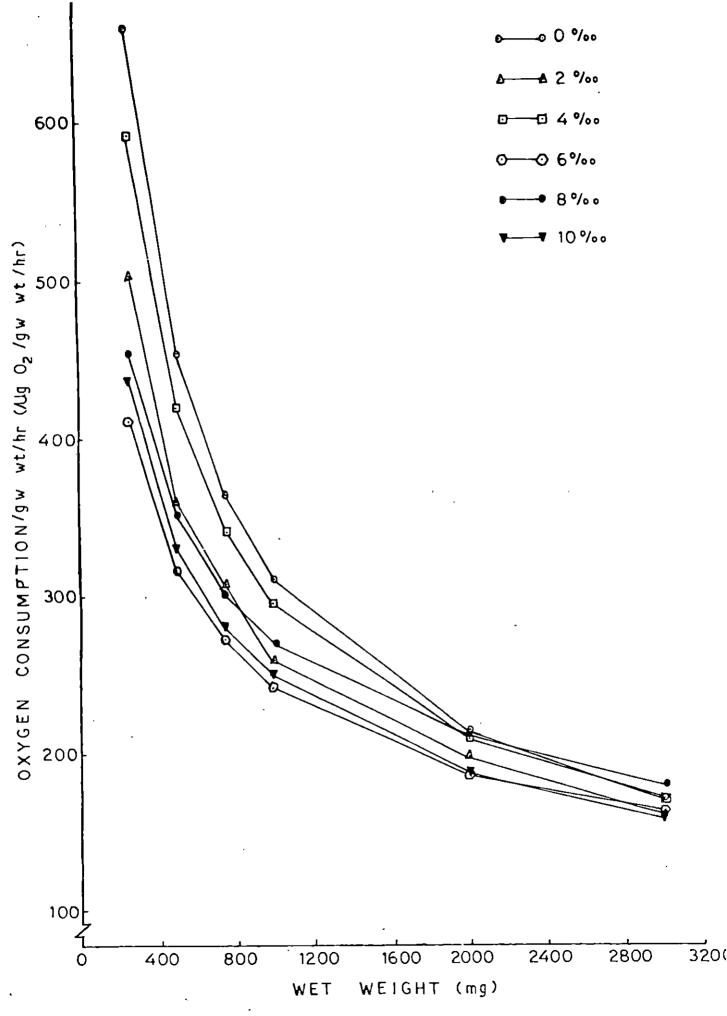
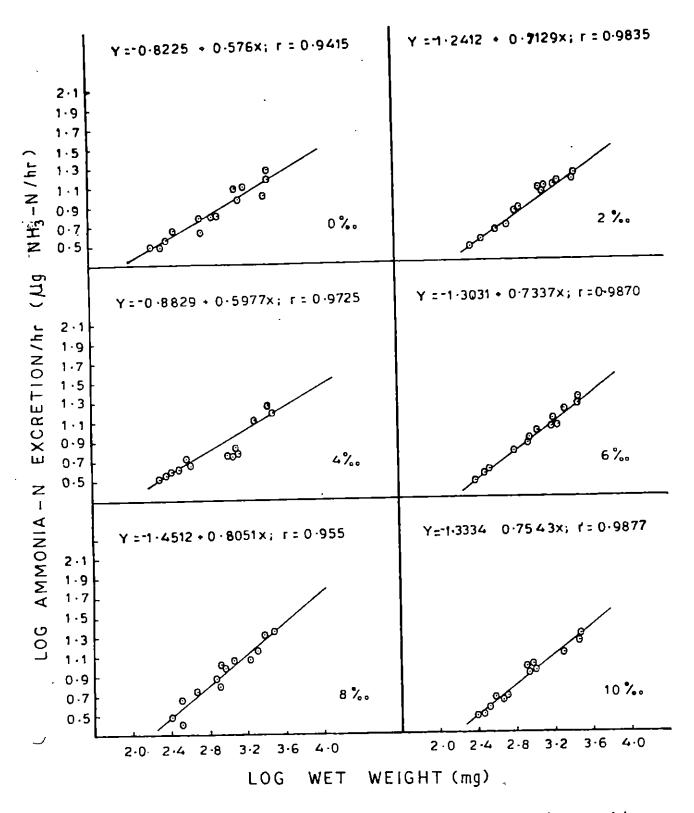


Fig. 36. Graph showing the estimated metabolic rate for selected weights



Figs. 37-42. Relation between ammonia - N excretion and wet weight in <u>M. rosenbergii</u> reared under different salinity conditions.

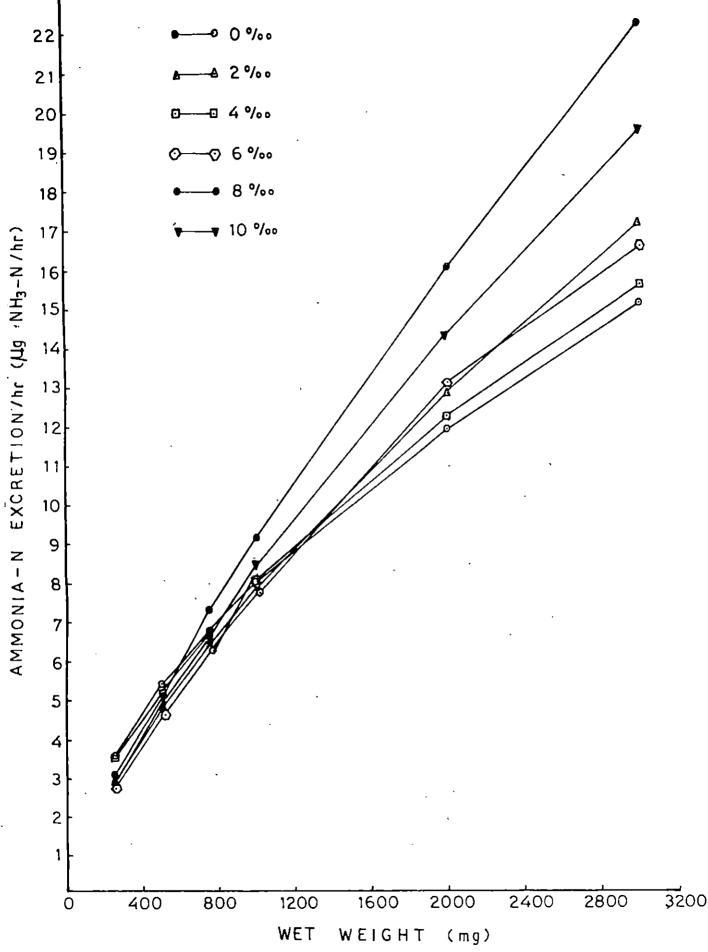
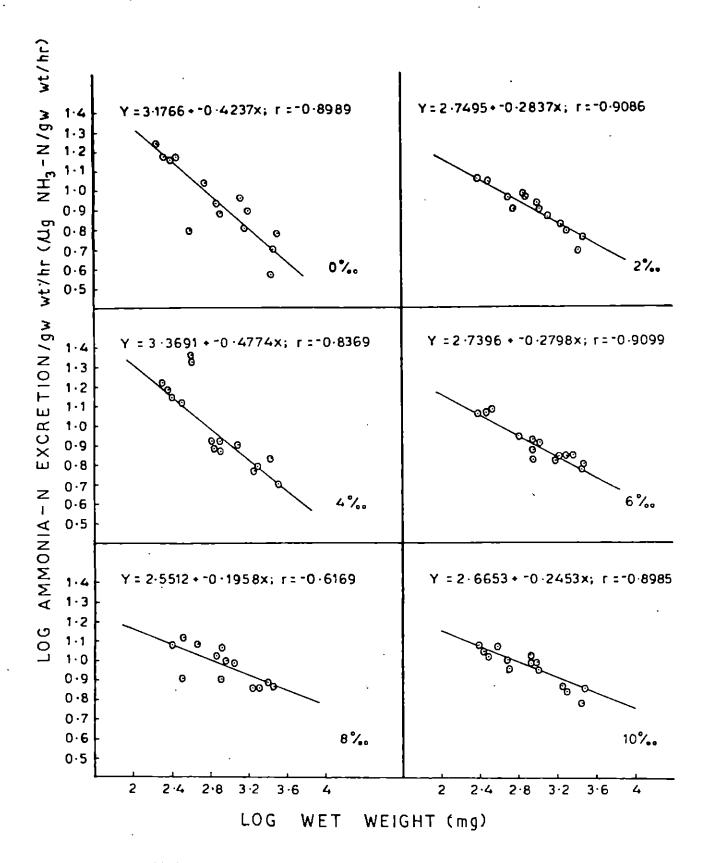


Fig. 43. Graph showing the estimated ammonia - N excretion for selected weights of <u>M</u>. rosenbergli.

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Figs. 44-49. Relation between weight specific ammonia - N excretion and wet weight in <u>M. rosenbergii</u> reared under different salinity conditions.

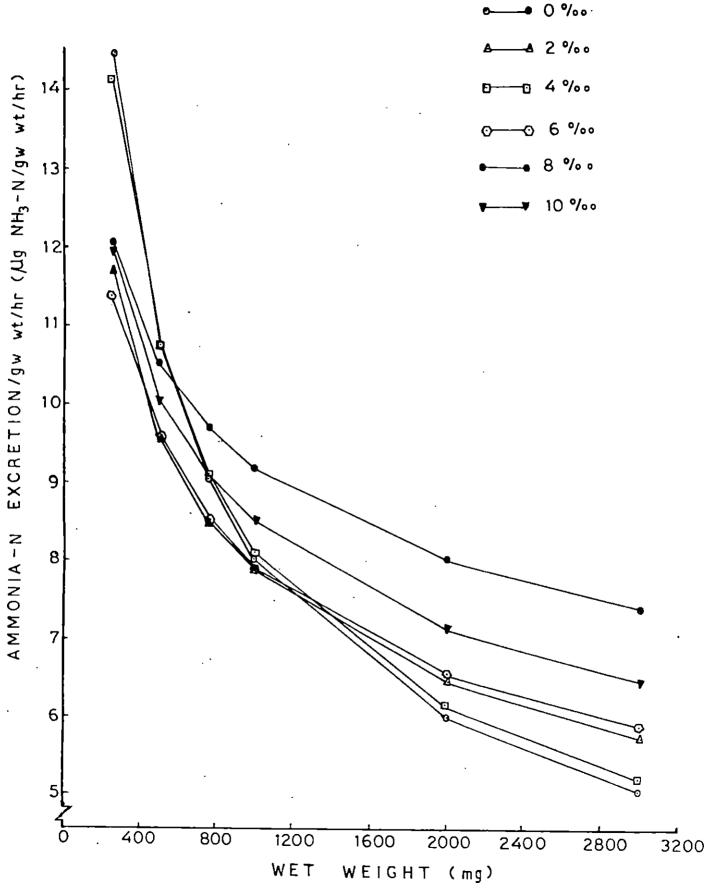


Fig. 50. Graph showing the estimated weight specific ammonia - N excretion for selected weights of <u>M</u>. rosenbergii.

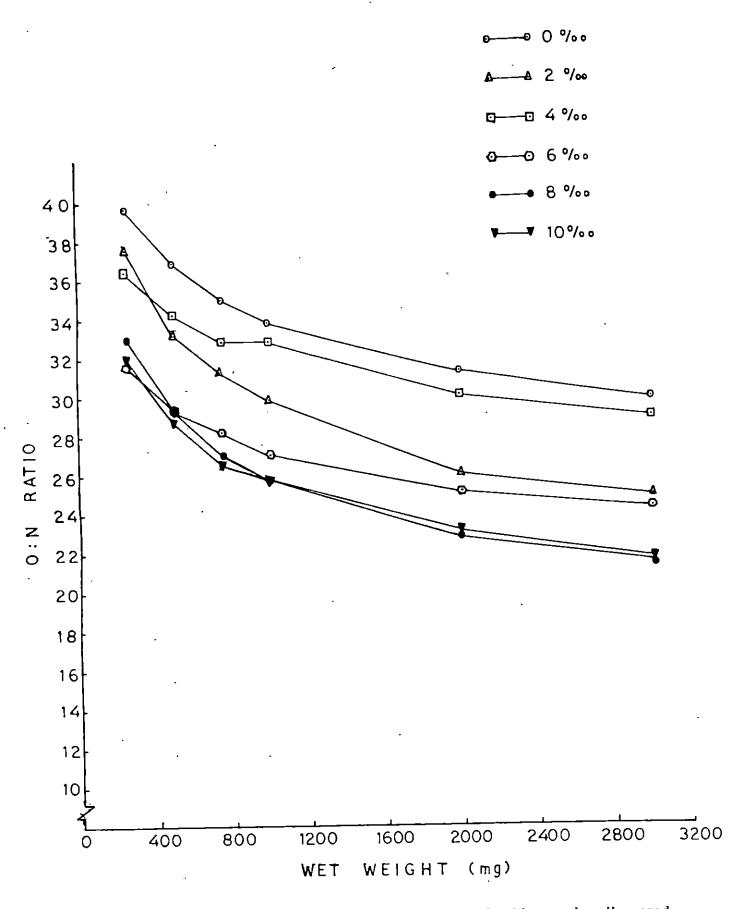


Fig. 51. Graph showing the estimated O: N ratios for <u>M</u>. <u>rosenbergii</u> reared in different salinity levels plotted against their wet weights.

V. DISCUSSION

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V DISCUSSION

5.1 Effect of Salinity on Survival

The results of the present study show that there is no mortality of the juveniles either when transferred abruptly from freshwater or slowly acclimated to different salinities upto 25 ppt within 120 hrs. In the case of abrupt transfer, animals exposed to 35 ppt did not survive beyond 96 hrs, while 30% of them survived when they were acclimated gradually and 10% survived even after 120 hrs indicating that acclimation has some beneficial effects on the survival of <u>M. rosenbergii</u> in higher salinity levels. Laboratory studies on the salinity tolerance of <u>M. rosenbergii</u> by Sandifer <u>et al.</u> (1975) showed post-larval mortalities within a few days at 30 ppt. In both abrupt and gradual increase of salinity, they reported mortalities at levels around 25 ppt and a rapid increase in mortalities at levels \geq 30 ppt. This result is more or less in agreement with the observations of the present study, where the mortality started at a slightly higher salinity level of 26.5 ppt. This probably, may be due to the relatively shorter period of exposure provided in the present study ie, five days as against nine days in the study by Sandifer <u>et al.</u> (1975).

The fact that LC_{50} values were obtained at a lower salinity of 31.62 ppt when abruptly transferred and at a higher salinity of 33.3 ppt when slowly acclimated shows that the prawn could adapt better if the change is effected slowly. Sandifer <u>et al.</u> (1975) also found slow acclimation to increase the survival time of <u>M. rosenbergii</u> post-larvae at 35 ppt salinity. In the present study an increase in survival time was observed in all salinity levels tested when acclimation was done slowly. In the case of post-larvae as well as adults of <u>M. rosenbergii</u>, Singh (1980) found the same range of tolerance when acclimated slowly. The results of the present study show that <u>M. rosenbergii</u> is a highly euryhaline species which can tolerate wider changes in salinity in the external medium. The comparatively high survival rate (58%) obtained in pond No. III wherein the salinity fluctuated between 2.1 - 20.4 ppt shows the euryhaline nature of this prawn. In nature, they are found to occur in regions of wide salinity fluctuations. Raman (1967) has reported the capture of adults of this species in salinities upto 18 ppt. Rao (1969) has observed that it is distributed in areas within the range 0 - 16 ppt salinity in the Hooghly estuary. George (1969) reported the spawning of this species in areas where the salinity fluctuates between 5 and 20 ppt. Nair <u>et al.</u> (1977) observed the occurrence of this species in the upper reaches of Mandovi and Zuari estuaries, where the salinity fluctuates between 0 and 20 ppt.

The prawn, M. rosenbergii is known to tolerate wide fluctuations in salinities by resorting to hyper-hypo osmotic regulation within the tolerance range. Sandifer et al. (1975) found the haemolymph of M. rosenbergii to be hyper osmotic below 18 ppt and hyposmotic above that salinity when exposed to gradually changing salinities. According to the above workers, the post-larvae regulate their blood osmotic concentration within the salinity range of 0-27 ppt with hyper regulation occurring in salinities below 17.5 ppt and hyporegulation in salinities between 17.5 and 27 ppt. In salinities above 27 ppt they found that the regulation ceased and the post-larvae hypoconformed. This osmoregulatory failure at higher salinities may be causing the death of juveniles in salinities above 26.5 ppt as noted in the present study. Some ability to acclimate to higher salinities is indicated by the extended periods a few juveniles were able to tolerate 35 ppt. The effect of acclimation in increasing the survival of the juveniles in higher salinity levels may be due to an increase in the regulatory range of the juveniles. As observed by Panikkar (1968), the highly euryhaline nature of M. rosenbergii may be due to its high powers of osmoregulation and according to Sandifer <u>et al.</u> (1975), this species possesses the ability of both hyperosmotic and hyposmotic regulation over a broad salinity range.

Most species of <u>Macrobrachium</u> are dependent on varying degrees of salinity for complete larval development (Read, 1986) and may exhibit widely differing salinity tolerances and preferences. McNamara <u>et al.</u> (1986) are of the view that the dependence on salinities during the various life cycle stages seems to indicate that they are still evolutionarily in the process of invading fresh water. The observation of Hedgpath (1957) treating fresh water shrimps of the genus <u>Macrobrachium</u> as recent invaders of brackish water biotope seems to be correct.

5.2 Effect of Salinity on Growth

The results of the present study show that the growth of <u>M</u>. rosenbergii is not influenced by salinity upto a level of 6 ppt and at levels beyond that the influence is being felt, the growth rate being slightly lower.

Several other workers have also found better growth of <u>M. rosenbergii</u> in fresh water and water of low salinities in laboratory growth trials (Wickins, 1972; Perdue and Nakamura, 1976; Sandifer <u>et al.</u> Ms; Popper and Davidson, 1982). Wickins(1972) found the post-larvae maintained in 2 ppt to grow to bigger size than those in 15 ppt. Perdue and Nakamura (1976) have also made similar observations with the animals maintained in fresh water and water of salinity 2 ppt showing the greatest percentage increase in weight and mean growth rate when compared to those maintained in 8.5 ppt and 15 ppt. Goodwin and Hanson (1975) and Sandifer <u>et al.</u>Ms} have also reported brackish water having salinity upto 5ppt as suitable for farming <u>M.rosenbergii.</u> All these observations are in agreement with the results of the present study in which better growth is recorded upto 6ppt with the maximum rate obtained in 2ppt (Table 6). Contrary to the observations of the present study and of the above workers (Wickins, 1972;Goodwin and Hanson, 1975; Perdue and Nakamura 1976;Sandifer <u>et al.</u>Ms)Popper and Davidson(1982)observed better growth in salinities ranging from 10-15 ppt in a preliminary laboratory experiment conducted at salinities ranging from 0-25 ppt.

The results of the field trials in the present study, though unreplicated, show that the growth of this species in pond having an average salinity of 7.2 ppt (range 1.8 to 10.84 ppt) is almost equal to that obtained in fresh water, but the growth rate of the prawns recorded in the high salinity pond of average salinity 12.7 ppt (range 2.1-20.4 ppt) is much low. The higher production obtained from the fresh water pond (79.99 Kg/ha/100 days) is due to the high survival rate (59%) obtained in that pond when compared to that (45%) in the pond with medium salinity. The possibility of the salinity fluctuations being the cause for the low survival in this pond can be ruled out because of the fact that in the pond with the greatest salinity fluctuations (pond No. III), the survival (58%) was more or less equal to that of the fresh water pond (59%). Probably, a predator (Therapon jarbua of size 47g) obtained at the time of harvest may be the reason for the killing of a few prawns. The size distribution of the prawns obtained from this pond also supports the above conclusion; the lower sized individuals being comparatively few in this pond at harvest, indicating the possibility of the predator selectively killing the smaller individuals.

The survival obtained in the high saline pond, where the salinity fluctuated between 2.1 and 20.4 ppt (Mean salinity 12.7 ppt) is comparable to that in fresh water indicating that survival of this species has not decreased, in such high salinities showing that <u>M. rosenbergii</u> can easily tolerate such salinities. In brackish water culture trials, Smith <u>et al.</u> (1982a) obtained better survival and growth of <u>M. rosenbergii</u> in ponds with fluctuating salinities as comparable to that obtained in fresh water ponds (Smith <u>et al.</u>, 1976, 1978, Smith and Sandifer, 1982b)

The experiments of culturing M. rosenbergii in brackish water made in

Western Samoa by Popper and Davidson (1982) showed that the prawns could survive and grow in salinities ranging from 12 to 25 ppt. This finding is comparable to the result of the present study wherein the prawns were found to grow, with good survival rate in salinities upto 20.4 ppt, although the growth rate was slightly lower in the higher salinity pond. The study of Popper and Davidson (1982) showed the potential of utilizing brackish water areas for <u>M. rosenbergii</u> culture. In their study, the prawns had grown to an average size of 8 g in 60 days and 26 g in 240 days. The results of the present study as well as the observations of other workers in the field go in favour of the observations of Ling and Costello (1976) that brackish waters upto about 10 ppt are suitable for farming <u>M. rosenbergii</u>.

Population analysis showed that females contributed more biomass than males in all the ponds with the males showing a higher mean size than the females. Smith <u>et al.</u> (1978, 1981 and 1982b) also observed similar population characteristics in their field studies.

In the field trial, large variations in growth among individuals within the same pond was observable. As the animals had grown the size variation increased as is evident from the Figs. 13-15. Similar observations of increasing size differences as the animals had grown to bigger sizes were also made by Sandifer and Smith (1975); Malecha, (1980); Cohen <u>et al.</u>(1981) and Malecha <u>et al.</u> (1981b). This varied growth rate according to Fujimura and Okamoto (1970) and Ra'anan and Cohen (1984b) may be the result of a genetic characteristic of <u>M. rosenbergii</u> or due to the social interaction between the individuals manifested by the so-called "bull effect" exhibited by the larger males.

Many workers hold the view that the maximum growth of an organism should occur at its iso-osmotic point (Canakaratnam, 1959; Ryther and Bardach, 1968) since the energy expended in osmoregulation should be the lowest at an animal's

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iso-osmotic point (Potts, 1954; Panikkar, 1968). The iso-osmotic point of \underline{M} . rosenbergii is reported to be at about 17-18 ppt (Sandifer <u>et al.</u>, 1975, Singh, 1977, 1980). None of the workers could observe maximal growth for this organism at this salinity level (Wickins, 1972; Goodwin and Hanson, 1975; Perdue and Nakamura, 1976; Sandifer and Smith. Ms) indicating that iso-osmotic growth concept is not fully applicable in the case of <u>M</u>. rosenbergii. The only work wherein a comparatively higher growth rate is reported near to the iso-osmotic point is that of Popper and Davidson (1982) in which 10-15 ppt salinity level had given better growth rate. The results of the present study do not favour this conclusion, as better growth is not attained in salinities closer to the iso-osmotic point as the growth rate in the high saline pond having an average salinity of 12.7 ppt (range 2.1 to 20.4) is much lower.

5.3 Water Quality in the Culture Systems

Management of environmental factors such as temperature, pH, alkalinity and dissolved oxygen are of utmost importance in the culture of any aquatic organism. These environmental factors are found to influence the growth and survivial of \underline{M} . rosenbergii greatly.

5.3.1 Temperature.

The temperature of the water in the culture cisterns ranged between 27 and 29.5° C. In the ponds it fluctuated from $27-32^{\circ}$ C during the culture period. As can be seen, they are within the upper and lower temperature tolerance levels of this species which according to Armstrong (1978) are 36° C and 16° C respectively. Continued exposure to temperatures less than 18° C or greater than 33° C⁻is reported to cause increased mortality of the prawns by Uno <u>et al.</u> (1975), Armstrong (1978) and Farmanfarmaian and Moore (1978). Uno <u>et al.</u> (1975) found a reduction in growth, activity and survival at temperatures below 22° C and above 33° C. New and Singholka (1982) found temperatures below 14° C or above 35° C as lethal to freshwater prawns and suggested $29-31^{\circ}$ C as the optimum for their cultivation.

According to Sandifer and Smith (1985) the optimum temperature for growth and survival of this species is $28-30^{\circ}$ C but they can do well within the range of $26-31^{\circ}$ C. The temperature recorded in the present investigation being in the range of $27-29.5^{\circ}$ C in cement cisterns and $27-32^{\circ}$ C in the ponds fall within the range for optimum growth and survival of this prawn species.

5.3.2 pH.

The pH values in the cement cisterns ranged between 7.5 and 8.62. In the ponds it fluctuated between 7.168.58, 6.8167.52 and 6.4168.15 in pond No. I, II and III respectively. According to Hora and Pillay (1962) a feebly alkaline pH of 7.8 is characteristic of a good water suitable for fish culture. Swingle (1947) considers water with pH ranging from 6.5-9 as suitable for fish culture. pH above 9 is considered to be not congenial for M. rosenbergii. According to Cripps and Nakamura (1979) extremely high pH levels lead to the formation of calcium carbonate precipitates on the prawns. Sarver et al. (1979,1982) and Malecha et al.(1980) observed a mortality of 80% of the post-larvae at a pH level of 9.5. Sandifer and Smith (1985) suggested that for better performance of the prawns, the pH should not be allowed to remain below 6.5 or above 9 for a longer period of time. The pH ranges recorded in the present study is well within the tolerance range of the species and optimum conditions of pH were prevailing in the cisterns and ponds during the culture period, except for a drop to 6.41 in pond No. III for a short period during the summer rains which may be owing to the leaching of acid salts from the pond bunds as observed by Mrithunjayan and Thampy (1986).

5.3.3 Total Alkalinity.

The alkalinity levels in the cisterns ranged from 39-66 ppm. In the ponds it ranged from 22-62, 7.8-19 and 10.9-19.8 ppm in pond No. I, II and III respectively. The alkalinity was slightly higher in the cisterns and in the fresh water pond and all the levels obtained are well below the critical values. According to Sandifer and Smith (1985) alkalinity values below 180 ppm have very little effect on the growth of the prawn <u>M. rosenbergii.</u> Mass mortality of this prawn was observed in South Carolina when the alkalinity levels had gone above 180 ppm (Sandifer and Smith, 1985).

5.3.4 Dissolved Oxygen.

Dissolved oxygen is the most critical water quality variable in aquaculture. In the cement cisterns it ranged between 5.15 and 6.7, the levels being well within the tolerance range of M. rosenbergii. In the ponds the dissolved oxygen levels fluctuated between 1.9 and 5.28 ppm in pond No. I (fresh water pond), 2.92 and 6.80 ppm in pond No. II (Salinity range 1.80 and 10.84 ppt) and 3.23 and 6.10 ppm in pond No. III (Salinity range 2.1 and 20.4 ppt), the levels often reaching the critical minimum. According to Spotts (1982) and Subrahmanyam (1987) the minimum acceptable dissolved oxygen concentration in Macrobrachium ponds is 3 ppm. Many authors reported that this species is well adapted to hypoxia, but recommended that for minimizing stress, oxygen levels should be kept above the critical minima (Smith et al., 1976, 1978 and 1981). According to Sandifer and Smith (1985) larger prawns require more oxygen than smaller animals and hence the larger animals are more susceptible to low oxygen concentration. The five individuals which were found dead in the fresh water pond were within the size range of 18-22g and they would have died due to asphyxiation. Similar observations were made by Smith et al.(1982a) who found that the mean size of prawns killed by overnight oxygen depletion in a pond was twice of that of the remaining population in the pond. Thus the fluctuations in dissolved oxygen noted in the present study show that sub-optimal levels of dissolved oxygen were prevailing in the culture ponds, especially in the fresh water pond, when the level of water had gone down steeply in the summer months.

The occurrence of a prawn with an opaque brownish discolouration in the fresh water pond in the present study may be due to this lowering of dissolved oxygen when the level had gone down upto 1.8 ppm. Delves - Broughton and Poupard (1976) reported that the opaque brownish colouration could either be a reaction to the presence of a foreign body in the musculature or a physiological response to the stress. Similar cases as observed in the present study have been reported earlier in <u>M. rosenbergii</u> when they were subjected to environmental stress such as low dissolved oxygen, fluctuations in salinity and temperature, hyperactivity associated with handling and over-crowding (Johnson, 1975, 1977; Delves - Broughton and Poupard (1976).

The occurrence of such pathological conditions had been reported in different ontegenetic stages such as larvae, juveniles and adults of <u>M. rosenbergii</u> (Fujimura and Okamoto, 1972; Aquacop, 1977b; Sindermann, 1977 and Akiyama <u>et al.</u>, 1982). Brock (1983) suggested that the development of opaqueness in the musculature with brownish discolouration due to environmental stress could be tentatively diagnosed as "Spontaneous muscle necrosis" or "Idiopathic muscle necrosis". The sign of disease along with environmental factors recorded in the present case indicate that the animal may possibly be suffering from acute "Spontaneous muscle necrosis"

5.4 Effect of Salinity on Oxygen Consumption, Ammonia Excretion and Oxygen : Nitrogen Ratios

5.4.1 Oxygen Consumption.

The results of the present study show that the oxygen consumption of larger animals was greater than that of smaller animals, but the increase was not proportionate to the increase in body weight. Thus the weight specific oxygen consumption (metabolic rate) expressed as oxygen consumed/g wet wt/unit time of small animals was greater than that of larger ones. The relation between oxygen consumption and wet weight in <u>M. rosenbergii</u> juveniles reared under different sali-

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nity conditions in the present study is in agreement with the pattern described by other workers (Rao, 1958; Subrahmanyan, 1962; Kutty, 1972; Kutt yamma, 1980). Nelson <u>et al.</u> (1977c) and Stephenson and Knight, (1980b) also found significant correlation between the rate of oxygen consumption and dry weight of juvenile <u>M. rosenbergii.</u> According to Prosser (1973) one of the reasons for the decrease in metabolic rate with increasing size is the disproportionate increase of tissues of low metabolic rate (Skeletal material, fats, etc.) during late development.

Salinity as an environmental factor influences the oxygen consumption and thereby the metabolic rates of many aquatic animals. The results of the present study show that within the salinity levels tested, the metabolic rate was unaffected by salinity variations (not significant at 5% level), but displayed a tendency to increase oxygen consumption at lower salinities. Smaller sized specimens showed more pronounced differences in metabolic rates when exposed to varying salinities than larger ones. Similar observations of more pronounced difference in metabolic rates are also reported in <u>Artemia salina</u> (Eliasson, 1953) and in <u>Metapenaeus monoceros</u> (Rao, 1958).

The increase in oxygen consumption towards the lower salinity levels in the present study is indicative of the fact that the metabolic rate of this organism increases towards salinities lower to the iso-osmotic point. This is in agreement with the well known contention that energy expended in osmoregulation should be lowest at an animal's iso-osmotic point (Potts, 1954 and Panikkar, 1968). The transport of ions against concentration gradients necessitated for osmoregulation at lower salinity levels have been shown to make some demand on the metabolic activity of animals (Sutcliffe and Carrick, 1975). Some other workers also have shown that in many crustaceans, respiratory rates will be maximum when the osmotic difference between body fluid and external medium is maximum (Rao,1958;

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King, 1965; Hagerman, 1970; Shumway and Jones, 1981) while the respiratory rate will be minimum when the osmotic difference between the body fluid and the medium is minimum, as the animals will be expending less energy for osmoregulation (Canagaratnam, 1959; Panikkar, 1968; Ryther and Bardach, 1968). A decrease in oxygen consumption in <u>M. rosenbergii</u> in media iso-osmotic with it's body fluid is noted by Nelson <u>et al.</u> (1977a), but Stern <u>et al.</u> (1984) could not observe a decrease in oxygen consumption in this species in salinities close to the iso-osmotic point.

Many workers found the increase in oxygen consumtified caused by osmoregulation to be small or absent (Potts and Parry, 1964; McFarland and Pickens, 1965; McLusky, 1969). According to McFarland and Pickens (1965) the increase in oxygen consumption rate at lower salinities may be due to a combination of increased osmoregulatory work and an increase in locomotory activity. Lange <u>et al.</u> (1972) however, attributed the higher solubility of oxygen coupled with oxygen dependent respiration as mainly responsible for this increase. It is likely that the increased solubility of oxygen coupled with it's increased demand for osmoregulation and locomotion are responsible for the increased rate of oxygen consumption observed in lower salinity levels in the present study

5.4.2 Ammonia Excretion.

The correlation co-efficients (r) for the relation between ammonia excretion and wet weight in <u>M. rosenbergii</u> in the present study range from 0.94147-0.98769 in the test salinity levels indicating a strong correlation between body weight and ammonia excretion in all the test levels. As observed by Nelson <u>et al.</u> (1977b), the correlation is positive when the rate is measured in terms of μ g NH₃/hr and negative when expressed as μ g NH₃/g/hr. The above results indicate that the nitrogen metabolism is more in larger prawns, but the rate of nitrogen metabolism (expressed as μ g NH₃/g wet wt/hr) decreases with increase in body weight. A similar observation has been made by Mangum <u>et al.</u> (1976) in the blue crab, <u>Callinectes</u> sapidus where the rates of ammonia excretion have been reported to be a logarithmic function of body weight regardless of acclimation salinity.

It could be seen that the rate of ammonia excretion is not influenced significantly (1% level of significance) by the salinity within the range studied (0-10 ppt). Weight specific ammonia excretion rates expressed as μ g NH₃/g wet wt/hr for the juvenile prawns is uniform for all the tested salinity levels with very slight reduction in values noted in the case of lower salinity levels.

In the present study, within the test levels salinity has no statistically significant influence on the rate of ammonia excretion. The relationship between the rate of ammonia excretion and wet weight also does not vary between various salinity levels. Although not significantly high, there is some effect for salinity on the ammonia excretion which is more pronounced in larger individuals.

Stern <u>et al.</u> (1984) also found that there is an increase in ammonia excretion with an increase in salinity, the figures being 32.6 μ g/g.f.w/hr at 15 ppt and 49.4 μ g/g.f.w/hr at 24 ppt. Sharma (1966) reported such an increase in nitrogen excretion in the fresh water cray fish <u>Orconectes</u> <u>rusticus</u> when subjected to increasing salinity conditions. Armstrong <u>et al.</u> (1981), however, found a decrease in ammonia excretion rate with an increase in salinity in juveniles of <u>M.rosenbergii</u> and have attributed the reversal of Na⁺/NH₄⁺ counter ion exchange mechanism resulting in a net decrease in ammonia excretion. Stern <u>et al.</u> (1984) have questioned this hypothesis and attributed the cessation in ammonia excretion observed by Armstrong <u>et al.</u> (1981) as a short-term response, a temporary physiological reaction in which blood Na⁺ levels must be controlled immediately in order to balance Na⁺ influx generated by the ion gradient. The fact that 20 hrs after transfer to saline water, the ammonia excretion rates resumed shows that it is only a temporary reaction and not a steady one.

Many workers view the quantity of ammonia excreted as a measure of protein

degradation and loss of nitrogen in excretion represents a reduction in protein accumulation (Cowey and Sargent, 1972; Halver, 1972; Steffens, 1981). The slight increase in ammonia excretion towards the higher salinity levels in the present study may be indicative of increased protein catabolism at these salinity levels and thereby a reduction in growth. The results of growth experiments in the present study also are in favour of this contention, with reduced growth rates in the higher salinity levels of 8 and 10 ppt.

5.4.3 Oxygen : Nitrogen Ratios.

The mean O: N ratios in the present study ranged from 33.51 in 0 ppt to 27.06 in 10 ppt. A decreased O: N ratio in the higher salinity levels may be indicative of an increase in protein catabolism at these salinity levels. The decrease in O : N ratio towards the higher salinity levels observed in this study is in agreement with the observation of Stern et al. (1984) who found higher values of O: N ratios in M. rosenbergii adapted to dilute media. They have noted a change in the substrate being oxidised (from lipid and carbohydrate to proteins) as a consequence of an increase in salinity. Capuzzo and Lancaster (1979) reported a reduction in O: N ratio from 26.7 to 22.1 as representing an increase in protein catabolism when compared to lipid or carbohydrate metabolism in Homarus americanus. Regnault (1981) has reported an O: N ratio of 27 to be indicative of mostly lipid catabolism in Crangon crangon. He could demonstrate a reduction of O: N ratio from 27 to 10 in C. crangon as a consequence of continued exposure to stress conditions. This indicates that in stressed animals mostly proteins are catabolised. Clifford and Brick (1983) found that in M. rosenbergii the O: N ratio to be 22.1: 1 indicating that energy metabolism in this species is dominated by the oxidation of carbohydrate with the lipids and proteins forming secondary and tertiary substrates respectively. The values obtained in the present

study is slightly elevated than that obtained by Clifford and Brick (1983) and this may be due to the differences in the environmental conditions as it is likely that environmental conditions may influence O: N ratio greatly. Thus Snow and Williams (1971) reported seasonal variations in O: N ratios in <u>Palaemonetes varians</u> the ratios being 6.1 : 1 in winter and 34.2 : 1 in summer. Hence it is highly probable that for the same species there are chances of getting conflicting results owing to variations in environmental conditions. The fact that the animals are slightly under stress in the higher salinity levels used in this study, is also confirmed in the growth trial in which maximal growth was observed in the lower salinity levels in the range from 0 to 6 ppt with no significant difference in growth within this range.

VI. SUMMARY

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- The objective of the present study is to evaluate the effect of salinity on survival and growth of the giant freshwater prawn <u>Macrobrachium</u> rosenbergii (De Man).
- 2. Two sets of experiments were conducted to evaluate the effect of salinity on the survival, the first being to find the effect of abrupt transfer from 0 ppt to varying levels of 5 to 35 ppt and the second to find the effect of gradual acclimation from 0 - 35 ppt. In abrupt transfer the juveniles suffered a total mortality within 96 hrs, but in gradual acclimation 10% of the individuals survived even after 120 hrs in 35 ppt. The 72 and 120 hr LC_{50} values were found to be 31.6 ppt and 28.18 ppt for abrupt transfer and 33.3 ppt and 29.85 ppt for gradual acclimation respectively. Abrupt transfer or gradual acclimation to salinities upto 25 ppt was not found to cause any mortality of the juveniles within a period of 120 hrs. Acclimation was found to prolong the survival time in all the test salinities.
- 3. An experiment was conducted to evaluate the effect of salinity on the growth of this prawn, by culturing them in cement cisterns for a period of 90 days in salinities ranging from 0-10 ppt with a regular increment of 2 ppt from 1 salinity level to the other.
- 4. Maximum growth rate (5.53 mg/day) was obtained at 2ppt, but there was no significant difference in growth between levels upto 6 ppt. The minimum growth rate of 2.49 mg/day was obtained in 8 ppt. Salinity levels 8 and 10 ppt differed from all other levels, but between them there was no significant difference.
- 5. A field trial to verify the growth pattern at different levels of salinity was conducted in 3 ponds, one fresh and two brackish water. The salinity ranges of the brackish water ponds used for the field trial were 1.8-10.84 ppt (Mean

7.27 ppt) in pond No. II and 2.1-20.4 ppt (Mean 12.7 ppt) in pond No. III.

- 6. The growth rate of the prawns in the fresh water pond and the brackish water pond with mean salinity 7.27 ppt were found to be almost equal being 0.119 g/day in the fresh and 0.126 g/day in the brackish water pond. But in the high saline pond, the growth rate was found to be low being 0.063 g/day.
- 7. The survival rate was almost equal in the fresh water (59%) and in the high saline pond (58%), suggesting that <u>M. rosenbergii</u> can tolerate and grow in salinities upto 20 ppt although the growth rate is lower in higher salinity levels.
- 8. The data of the water quality parameters of the culture ponds showed that all except dissolved oxygen were within the tolerance range of this species The dissolved oxygen levels in the fresh water pond had gone down to 1.9ppm and in the low saline pond to 2.92 ppm, these levels being below the critical level of 3 ppm.
- 9. A prawn with an opaque brownish colouration on the tail region was obtained from the fresh water pond, where the dissolved oxygen level had gone down. This may probably be affected by "spontaneous muscle necrosis" induced by hypoxia.
- 10. The oxygen consumption of the prawns was found to be influenced by the body weight, the correlation co-efficients being within the range of 0.9277 0.9822 in the different salinity levels. The variations in salinity were not having any significant influence on the oxygen consumption within 0-10 ppt levels, but there was a marginal increase at the lower levels.
- 11. Ammonia excretion was found to be influenced by the body weight, the correlation co-efficients being 0.94152-0.98770 at different salinity levels. When expressed on a per gram basis the rates were found to decrease with an increase in weight. No significant difference in ammonia excretion could be

observed with in the salinity levels tested, but a slight increase towards the higher salinity levels was noted.

12. The mean oxygen : nitrogen ratios were found to range from 33.51 in 0 ppt to 27.06 in 10 ppt. The decreased O : N ratios in the higher salinity levels is an indication of the increased protein catabolism, a fact which has been confirmed from the growth studies wherein higher growth rates were observed in the lower salinity levels.

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ABSTRACT

The effect of salinity on survival and growth of <u>Macrobrachium rosenbergii</u> (De Man) was studied with a view to establish the optimum salinity conditions under which it can be cultured. The effect of salinity on survival was assessed by conducting short-term tolerance studies wherein the effect of abrupt transfer and gradual acclimation to the test salinity levels of 5, 10, 15, 20, 25, 26.5, 28.5, 30 and 35 ppt was separately studied. In both abrupt transfer and gradual acclimation, no mortality was observed upto 25 ppt within a period of 120 hrs. The LC_{50} values for 72 hr and 120 hr were 33.3 ppt and 31.6 ppt for gradual acclimation and 29.85 ppt and 28.18 ppt for abrupt transfer respectively. Acclimation prolonged the survival time of the juveniles in the test salinity levels.

The study on the effect of salinity on growth was conducted in cement cisterns, wherein salinity levels from 0 - 10 ppt with regular increments of 2ppt from treatment to treatment were used. There was no significant difference between treatments upto 6 ppt, but all the levels upto 6 ppt differed from 8 and 10 ppt with respect to growth. A field trial to verify the growth pattern in different salinity levels was conducted in one fresh water pond and two brackish water ponds having different salinity levels. The growth rate of the prawns was almost equal in both the fresh water pond (0.119 g/day) and the pond with lower salinity levels (0.126 g/day), where as it was much lower in the high saline pond (0.063 g/day). The survival rate obtained in the high saline pond (58%) was comparable to fresh water (59%) showing that prawns could survive upto a salinity of 20 ppt, although the growth rate is lower in higher salinity levels.

Studies were conducted to evaluate the effect of salinity on oxygen consumption, ammonia excretion and O : N ratios of <u>M</u>. rosenbergii within the test salinity levels of 0 - 10 ppt. Oxygen consumption and ammonia excretion were found to be strongly influenced by the wet weight of the juveniles, but the influence of salinity on these physiological indices were not significant within the test levels used in this study. However, a trend of an increase in oxygen consumption and decrease in ammonia excretion towards the lower salinity levels was observed. Oxygen to Nitrogen ratios were found to be lower in the higher salinity levels indicating an increase in protein catabolism in such salinity levels.