EFFECT OF SALINITY ON SURVIVAL, GROWTH AND BREEDING OF THE SHRIMP

MACROBRACHIUM IDELLA (HILGENDORF)

By

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THESIS

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TO MY PARENTS

DECLARATION

I hereby declare that this thesis entitled "EFFECT OF SALINITY ON SURVIVAL, GROWTH AND BREEDING OF THE SHRIMP MACROBRACHIUM IDELLA (HILGENDORF)" 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, GROWTH AND BREEDING OF THE SHRIMP MACROBRACHIUM IDELLA (HILGENDORF)" is a record of research work done independently by Sri. Ignatius. C.A. 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

Among the increasing number of aquatic animals that have been coming to the fore as candidate species for aquaculture, the freshwater prawns are perhaps the most recent (Jhingran, 1982). At present commercial production, by way of farming in confined areas, is practised to a lesser extent and there is immense scope for development of freshwater prawn culture, by resorting to scientific farming of already established species and also by adding more new species.

The genus Macrobrachium includes the most important cultivable freshwater prawns. Though, there are more than 100 species belonging to this genus, the aquacultural potential of only very few species, such as Macrobrachium rosenbergii, M. americanum, M. malcolmsonii, M. carcinus, M. acanthurus and M. tenellum has been investigated. There are so many other medium sized species, the aquaculture of which may be biologically and commercially feasible. Macrobrachium idella is one such species, inhabiting the estuaries and rivers of Kerala, offering a fishery of some magnitude in the central and south Kerala.

Salinity is the most important environmental factor of brackishwater species and such freshwater species, like M. idella undertaking migration to brackishwater areas, critically influencing the organisms in their distribution, survival, growth, breeding and larval development. Although some works have been undertaken to understand the life history and bionomics of M. idella, studies to understand the influence of salinity, if any, on survival, growth, breeding and larval development are very few.

For successful farming of any organism, seed supply is a major prerequisite. Collection of seed from natural waters alone may not be sufficient, and in such cases, seed production in hatcheries is inevitable. This necessitates the knowledge of optimum conditions required for larval development, to undertake large scale seed production.

The main problem for the development of commercial culture of M. idella is the lack of adequate information on its growth rate under different stocking densities, production systems etc. Today the technology of freshwater prawn culture has been revolutionized much; but how far this could be made applicable in the case of M. idella is to be found out. In pond rearing, the survival and growth of cultivated species are generally influenced by various biotic and abiotic factors. Among the various abiotic factors salinity is the key factor determining the survival, growth and production. Once the environmental optima are found out, it is possible to rear the organism in the preferred environment by applying the modern management techniques and thus maximise the production.

It is in this context, the present study has been taken up with the major objective of elucidating the salinity tolerance and preference for growth as well as breeding of M. idella. A brief review of literature on distribution, growth, food and feeding, breeding biology, larval development, culture and the effect of salinity on survival, growth, breeding and larval development of prawns of the family Palaemonidae, is given followed by results and discussion of the experiments conducted to find out the effect of salinity on survival, growth and reproduction of M. idella.

II. REVIEW OF LITERATURE

II REVIEW OF LITERATURE

2.1 Distribution

The prawns of the genus Macrobrachium are found in a variety of brackish and freshwater habitats, many having a wide geographical distribution (Holthuis, 1952a). The largest and commercially the most important species Macrobrachium rosenbergii is widely distributed in the Indo-Pacific region (Cowles, 1914). The next largest species viz. M. americanum has got a restricted distribution, being present in the Pacific coast of America between Mexico and Peru (Holthuis, 1952b). M. carcinus and M. acanthurus, which are next in importance, are known to occur in the Atlantic coast of America (Holthuis, 1952b). M. malcolmsonii (Patwardhan, 1958) and M. birmanicum choprai. (Tiwari, 1947), the two equally important freshwater prawns, growing to almost the same size as that of M. americanum and M. carcinus, are distributed in the Indian sub-continent.

Among the medium sized species, M. rude is distributed in East Africa, Madagascar, India and Sri Lanka (Henderson and Matthai, 1910). Another medium sized species, M. equidens has its distribution from Madagascar to Southern China (Holthuis, 1950) and in India this species is present only in Kerala (Kurian and Sebastian, 1986).

M. idella, known as "Slender river prawn" (Holthuis, 1980), is also a medium sized species and is widely distributed in East African countries, Madagascar, India, Indonesia and Malayan Archipelago (Henderson and Matthai, 1910). According to Jayachandran (1984),

M. idella is a commercially important palaemonid prawn of Kerala which is caught in large quantities. He described two subspecies of M. idella, viz, M. idella idella and M. idella georgii. Of these, M. idella georgii is available abundantly in rivers such as Meenachil, Manimala, Pamba and Achencoil and in stray numbers in the Pallickal river. M. idella idella, on the other hand, is distributed in large numbers in almost all the rivers of Kerala.

Among the small sized freshwater prawns, the two most important ones are M. lamarrei (Ahmad, 1957) and M. dayanum (Chopra and Tiwari, 1947). They have a restricted distribution, being present only in the Indian sub-continent.

2.2 Size and Growth

Among the different species of Macrobrachium, M. rosenbergii grows to the largest size (Patwardhan, 1958; Raman, 1967; Holthuis, 1980), the maximum size recorded being 326 mm for males and 283 mm for females (Jayachandran and Joseph, 1982). Other species attaining large size are M. americanum, M. carcinus, M. malcolmsonii, M. acanthurus and M. birmanicum choprai; followed by M. rude, M. idella, M. equidens, M. scabriculum, M. lamarrei and M. dayanum. The maximum size recorded in the case of M. americanum is 250 mm for males and 193 mm for females (Holthuis, 1952b). M. carcinus which also is comparatively larger, attains a maximum size of 233 mm in the case of males and 170 mm in the case of females (Holthuis, 1952b). Among the Indian prawns, the males of M. malcolmsonii are known to reach a maximum size of 231 mm, and

females 200 mm (Holthuis, 1980). The maximum size recorded for M. rude is 117 mm (Henderson and Matthai, 1910), it being 111 mm for M. idella (Henderson and Matthai, 1910), and 98 mm for M. equidens (Holthuis, 1950), whereas it is only 69 mm for M. lamarrei (Holthuis, 1980).

In most of the species of Macrobrachium, such as M. rosenbergii (Smith et al., 1976), M. malcolmsonii (Ibrahim, 1962), M. idella (Jayachandran, 1984), M. rudis (Jones, 1967) and M. dayanum (Koshy and Tiwari, 1975), males are larger than females. But in the case of M. lamarrei, females are larger than males (Koshy and Tiwari, 1975).

Henderson and Matthai (1910) observed that in many, if not in all species, two forms of males are met with, viz. normal males usually of considerable size, with larger chelipeds specially developed, and the males of the second type ("male feminises") generally smaller, but sometimes attaining the same size as normal males, in which the chelipeds resemble those of females. This difference in the development of secondary sex characters among males of M. idae has been reported to be linked with the extent of development of the androgenic gland (Thampy and John, 1973). Jayachandran and Joseph (1988) studied the growth pattern of M. idella and M. scabriculum. They found that the males of M. scabriculum exhibit isometric growth; but the females of M. scabriculum and both sexes of M. idella do not exhibit isometric growth. Growth of females of M. rosenbergii was found to be reduced during egg development, since a proportion of the available energy is used for the development of oocytes (Wickins and Beard, 1974).

Variations in size and growth rate of individuals, soon after metamorphosis of the larvae into post-larvae, had been noted in M. rosenbergii (Wickins, 1972b; Forster and Beard, 1974). Although the average growth is inversely related to density, the wide variation in size is believed to be induced by intrinsic rather than environmental factors (Sandifer and Smith, 1975; Ra'anan, 1982). But Ra'anan and Cohen (1984a&b) concluded that variations in size are due to interactions within the prawn group than it is by genetic difference in growth potential of individual prawns.

Lewis et al. (1966) found that the growth rates of tank reared specimens and of a natural population of M. carcinus were almost identical. Growth and survival of M. rosenbergii in tanks show an inverse relationship with population density (Sandifer and Smith, 1975) and directly with the presence of substrate (Smith and Sandifer 1975). More growth has been noted while horizontal substrates had been used than vertical substrates (Smith and Sandifer, 1975).

The growth of M. rosenbergii in various systems has been investigated by many workers. It could be seen that the rate of growth varies related to the stocking density, intensity of the culture operation and also to the survival rate obtained. Willis and Berrigan (1977) reported a growth rate of 0.26g/day (43.25 g/168 days at a stocking density of $5/m^2$). Ong and Pang (1982) obtained a growth rate of 0.32 g/day (11.7 cm and 57 g/180 days at a stocking density of $5\text{-}10/m^2$). Ling (1969b) recorded a growth rate of 0.62 mm/day for this species. In intensive culture system with net

substrates, at a high stocking density of 36-54/m^2, Eble (1979) obtained a growth rate of 0.14 g/day. In laboratory tank culture, Venugopalan (1988) obtained a growth rate of 5.53 mg/day.

In the case of M. americanum, a growth rate of 0.40 mm/day was obtained by Arana (1974) in a tank rearing experiment. The growth rate recorded for M. malcolmsonii in pond culture was 0.14 g/day (Rao et al., 1986). In tank culture of M. acanthurus, Dugan et al. (1975) recorded a growth rate of 0.23 mm/day. On the other hand, in the pond culture of this species Dobkin (1973) obtained a growth rate of 0.35 mm/day. The growth rate recorded for M. carcinus is very low, being only 0.09 mm/day (Dobkin, 1973).

Environmental factors such as temperature, light and salinity are known to influence the growth of palaemonid prawns. A range of 22-33^{o}C was found to be the optimum temperature for growth in the case of M. rosenbergii (Uno et al., 1975), whereas the best growth was obtained at a narrow temperature range of 29-31^{o}C (New and Singholka, 1982). In juveniles of Palaemon serratus, Richard (1978) observed a depressing effect of low temperatures on growth. However, the older animals were not affected by such lowering of temperature. In older animals higher temperatures were found to affect the growth. Biddle et al. (1978) studied the effect of light intensity and indicated that somewhat lower survival but better growth was obtained in the case of prawns reared under decreased light intensity. Various studies demonstrated that the growth rate of M. rosenbergii is maximum at a slight salinity level of 2 ppt (Perdue and Nakamura, 1976; Venugopalan, 1988).

Growth in crustaceans is very much linked to moulting, which is the most prominent process dominating the life of crustaceans; metabolism, reproduction and behaviour are affected both directly and indirectly by the periodic shedding of the exoskeleton (Passano, 1960). According to Kurata (1962), the connection between ecdysis and growth is by no means simple, and very little is known about mechanisms by which the growth at moulting is controlled. However, more recent works have demonstrated a strong connection between various environmental factors such as temperature and salinity, and weight increment per moult (Hartnoll, 1978; Hartnoll and Dalley, 1981). According to Howe (1981), there exists a partial moulting synchrony in groups of M. rosenbergii and a water borne stimulus perhaps released by moulting animals is responsible for this. Lee and Fielder (1983) also, observed an increase in growth in M. australiense in groups than when they were held individually. The intermoult period of M. australiense was found to be increasing with age (Lee and Fielder, 1983). Ra'anan (1983) reported that in M. rosenbergii weight increment during moulting is strongly influenced by the animal size ranking within the original population. In M. lanchesteri, with increase in size, the weight increment per moult decreases (Ponnuchamy et al., 1984). According to Shyama (1987), the intermoult period of adult M. idella is 18 $\pm$ 2.65 days, and the animal during moulting is inactive, soft and prone to predation and cannibalism.

Susceptibility of newly moulted prawns to attack from their companions has been stressed by many workers. Cannibalism has been reported to be the most important cause of mortality in captive prawns (Reeve, 1969a; Meixner, 1969; Forster, 1970; Smith and Sandifer, 1975;

Schulte, 1976). According to Adelung (1971), "Cannibalism could be regarded as an intraspecific mechanism to regulate the population density". It has been reported that in overcrowded tanks, prawns and crabs such as Leander squilla (Schulte, 1976) and Carcinus maenas (Adelung, 1971) are able to delay the next moulting especially when larger specimens of the same species are present; thereby escaping from cannibalism. Cannibalism has been reduced effectively by increasing the effective bottom area and hiding places (Reeve, 1969a; Forster, 1970; Wickins, 1972a; Schulte, 1976; Peebles, 1979a).

2.3 Food and Feeding

Palaemonid prawns are known to be omnivorous in feeding habit. This has been observed in Crangon vulgaris (Lloyd and Yonge, 1947), Leander serratus (Forster, 1951), Macrobrachium malcolmsonii (Ibrahim, 1962), M. americanum (Smitherman et al., 1974) M. rosenbergii (John, 1957; Johnson, 1967; Raman, 1967; Rao, 1967; George, 1969; Ling, 1969a; Lee et al., 1980) and M. idella (Jayachandran, 1984). However, M. lanchesteri is known to be an algal feeder (Johnson, 1968).

Panikkar and Menon (1955) stated that the food of prawns consists of along with detritus, mud and sand and plenty of diatoms, particularly when they are abundant in the environment. According to John (1957), the food of prawns depends on its environment.

According to Jayachandran (1984), the common food items of M. idella encountered in the gut are detritus, sand particles, diatoms, filamentous algae, crustacean remains, insect remains, foraminiferans, gastropods, fish

scales etc. Diatoms and detritus were the only food items found among the smallest length group. Detritus was the most common food item of the gut contents of all length groups.

Eating its own cast-off exoskeleton was reported in Palaemon idae (M. idella) by Natraj (1947) and in M. heterochirus by Ching and Velez (1985). Cannibalism was also observed in P. idae (Natraj, 1947) and M. rosenbergii (Rao, 1965; Ling, 1969a; Wickins, 1972b; Forster and Beard, 1974; Segal and Roe, 1975; Peebles, 1977).

2.4 Breeding Biology

2.4.1 Maturity Size.

Many of the palaemonids reach sexual maturity within an year. The size at first maturity of M. rosenbergii has been investigated by many workers. In rivers of West Bengal it takes two years (Rajyalakshmi, 1961 & 1962) and in Kerala one year (Raman, 1967) to attain maturity. Rao (1967) recorded the mean size of 155 mm, as the maturity size in Hooghly estuary. Goorah and Parameswaran (1983) recorded 118 mm and 20 g (5-7 months old), as the smallest size of berried females in ponds at Mauritius. The size at first maturity of M. malcolmsonii is 41 mm according to Ibrahim (1962) and 40-50 mm according to Sankolli and Shenoy (1980). Rajyalakshmi (1980) has reported that the size at first maturity of this species in river Godavari is smaller compared to Hooghly estuary. In M. acanthurus sexual maturity is attained at a size of 40 mm (Berber, 1984). Inyang (1984) recorded a size of 30 mm, as the maturity size of M. felicinum in Africa. The size at first maturity of M. tenellum

in Mexico is 74 mm (Arroya et al., 1982). The size at first maturity of M. idella, according to Jayachandran (1984), is 41 mm. In laboratory culture, Pillai and Mohamed (1973) reported that the newly hatched larvae of this species became mature within 120 days.

2.4.2 Breeding Season.

According to Rajyalakshmi (1961), the appearance of berried females marks the onset of the breeding season, while the time by which majority of prawns appear to have dehised the brood indicates the end of the period.

Dudgeon (1985) stated that restrictions of freshwater caridean breeding periods by low temperatures may be common in subtropic, while perennial breeding is more likely to be typical of tropical regions.

The breeding season in the case of M.rosenbergii varies from region to region and depends on the habitat. In the Hooghly estuary, it extends from December to July, ie. winter and summer months (Rajyalakshmi, 1961 & 1962; Rao, 1967). In Kerala its breeding season is during August - December with a peak in October - November (Raman, 1967), while in the Bolgoda lake of Sri Lanka it has two peak breeding seasons during May-July and December-January (Jinadasa, 1985). M. malcolmsonii in river Godavari has a prolonged breeding season extending from April to November according to Ibrahim (1962), with two spawning peaks, one in June and again during August-October and according to Rajyalakshmi (1974), May-October with peak in July-September.

According to Natraj (1947), the breeding season of P. idae in Travancore area is a prolonged one, beginning from September and extending to the end of February. This species, in Vellayani lake, has been reported to have a long breeding season, extending from September to January, ie. covering the north-east monsoon months (Jayachandran, 1984). Similar observation was made in cases of Macrobrachium amazonicum (Romero, 1982), M. tenellum (Arroya et al., 1982) and M. felicinum (Inyang, 1984), in which the breeding season coincides with the rainy season. The coincidence of breeding season with rainy season can be explained on the basis of the observation by Pinheiro (1983), who reported that intense reproductive activity in M. acanthurus occurs during the monsoon period when lower temperature and higher dissolved oxygen are recorded.

2.4.3 Breeding Migration.

Migratory movements for breeding, hatching or for both have been recorded among many palaemonids such as Leander squilla and L. longirostris (Gurney, 1923), L. serratus (Forster, 1951), Palaemon carcinus and P. mirabilis (Rajyalakshmi, 1961) and M. rosenbergii (John, 1957; Raman, 1967; Johnson, 1967; George, 1969). According to Hughes and Richard (1973), both upstream and downstream migrations would be of adaptive advantage to species with specific salinity requirements for reproduction and development.

The females of M. rosenbergii are found to migrate downstream to estuaries or brackishwaters at the time the eggs are ready to hatch

(John, 1957). Larvae develop in brackishwater areas and shortly after metamorphosis to the post-larval stage, the prawns migrate to freshwater (John, 1957; Ling and Merican, 1961; Ling, 1969a; Natividad, 1982). Field observations on the distribution and movement have implicated temperature and salinity as influencial factors in their migratory behaviour (John, 1957; Johnson, 1967; Raman, 1967; George, 1969).

Ibrahim (1962) and Rajyalakshmi (1974) reported that M. malcolmsonii, in river Godavari, performs no migration for breeding towards the lower reaches of the river. Larvae reach the estuarine zone of the river along with the flood current. The first stage larvae of M. malcolmsonii has been found to thrive in freshwater for 15 days, unlike as in the case of M. rosenbergii, which can thrive in freshwater, for only 5-6 days (Sankolli and Shenoy, 1980). Monsoon flood transport of larvae was also noted in M. carcinus by Lewis et al. (1966).

2.4.4 Mating and Spawning.

The processes of mating and spawning of different species of palaemonids have been described by many workers. Mating usually occurs between a soft shelled female (moulting stage) and a hard shelled male (intermoult stage). Before mating the male begins to approach and follow the female. The role of chemical and mechanical stimulii emanating from mature females in attracting males, in decapod crustaceans was discussed by Burkenroad (1947). Ling (1962) reported the role of sex pheromone in M. rosenbergii. According to Sarojini et al. (1984), the parturial moulted females of M. kistnensis release sex pheromone into the water which attracts only the intermoult mature males and this pheromone is effective

only for a short duration. The possibility of the existence of such a sex attractant in M. idella was suggested by Shyama (1987).

A few hours before the mating the male holds the female between the widely extended second pair of pereopods and actively touches the female with his antennae and first pair of pereopods. According to Mashiko (1981), the long chelipeds of the male are effective apparatus for its reproductive success in two aspects viz. to guard the soft shelled female immediately after prespawning moult from cannibalistic predators and to guard the female during mating from other male rivals.

The habit of nest building has been reported in M. rosenbergii (Smith and Sandifer, 1979). The number and size of the nest (breeding depression) appeared to be closely related to the size and density of adult male prawns in the ponds. Mature females are found to orient in and around the nests. M. australiense is also known to construct nest for breeding (Lee and Fielder, 1982a).

Rao (1965) observed that the females just before mating are sluggish and males are active. However, Nagamine and Knight (1980) reported that mature females of M. rosenbergii, soon after the preparturial moult, are extremely active and this activity aids the female in seeking out a suitable mate. According to Ogawa et al. (1981), the mating depends largely upon the premating moult of the female and takes place night and day, immediately after the premating moult.

In M. rosenbergii the hard shelled male turns the soft shelled female and presses down from above. During this process, the spermatophore is

transferred from male to female between the second and fourth or fifth pereopods (Chow et al., 1982). Spermatozoa are released from the spermatophore at oviposition and run into the brood chamber of female together with the eggs after 8-12 hours of mating (Bhimachar, 1965). External fertilization seems to take place in this chamber. The eggs are held together by tuft like ovigerous setae developed for this purpose (Rao, 1986). Unmated females of M. rosenbergii also would release the eggs within 24 hours of premating moult, but these eggs would drop-off within 2-3 days (Ling, 1969a). In the case of M. idella also, the unfertilised eggs drop off within two days (Shyama, 1987).

2.4.5 Fecundity.

Dobkin (1969) stated that low fecundity is characteristic of palaemonids. Fecundity of M. rosenbergii has been worked out by many investigators and it has been reported to be of the order of 1,00,000 - 1,60,000 (Chacko, 1955), 7,000 - 1,11,400 (Rajyalakshmi, 1961), 60,000 - 1,00,000 (Ling, 1964), 1,39,000 - 5,03,000 (Raman, 1967), 22,552 - 1,09,491 (Goorah and Parameswaran, 1983) and 82,000 - 1,70,000 (Jinadasa, 1985). Ibrahim (1962) recorded a fecundity of 3,465 - 63,080 for M. malcolmsonii of the size 54-164 mm in Godavari river. Sankolli and Shenoy (1980) has reported the fecundity of M. malcolmsonii to be of the order of 3,400 - 68,000.

Compared to the above two species, M. idella is a low fecund freshwater prawn. According to Natraj (1947), this species, depending on size, has got a fecundity of 2,000 - 20,000. Jayachandran (1984) reported it to be of 6,089 and 29,773 for prawns having a length of 68 mm and 92 mm

respectively; the number of ova per gram body weight ranging from 1450 to 5920.

The number of eggs on the brood pouch of different species of Macrobrachium is known to be directly dependent on the size of the mother shrimp. This is the case in species such as M. idae (Pandian and Katre, 1972), M. lamarrei (Koshy and Tiwari, 1975; Shakuntala, 1977a), M. dayanum (Koshy and Tiwari, 1975), M. novaehollandiae (Greenwood et al., 1976), M. rude (Shakuntala, 1976), M. amazonicum (Rojas and Silva, 1979; Guest, 1979), M. ohione (Truesdale and Mermilloid, 1979), M. acanthurus (Berber, 1984), M. birmanicus birmanicus (Latifa, 1985) and M. heterochirus (Ching and Velez, 1985). Shakuntala (1977a) found that in M. lamarrei, the total biomass of eggs per brood is inversely proportional to the unit body weight of the female.

Based on the number of eggs produced per year, Cabrera-Jimenez et al. (1979) divided various species coming under the genus Macrobrachium into low fecundity species, such as M. acanthurus (52,000 eggs/year), M. tenellum (70,000 eggs/year) and M. rosenbergii (1,12,000 eggs/year) and high fecundity species, such as M. americanum (9,00,000 eggs/year) and M. carcinus (10,50,000 eggs/year).

In M. rosenbergii, Rao (1986) reported that the fecundity is more in the wild than in the pond reared brood stock. A large variation in egg number reported in many species may be due to the large scale loss of eggs during the incubation period. Balasundaram and Pandian (1982a), for example, have reported upto a 53% loss in egg number during the incubation period in

M. nobilii showing that there exists a gap between fecundity (number of eggs produced) and effective fecundity (number of eggs at hatching).

Mashiko (1982) observed that in Palaemon paucidens of Sagami river in Africa, those prawns in the upper lake produce many small eggs and those in the lower areas produce a few but larger eggs. According to him, the two groups may be distinct populations. However, the total amount of egg matter per spawning was found to be almost same.

The intermittent spawning habit of Macrobrachium has been observed by many workers (Ling, 1962 in M. rosenbergii; Pillai and Mohamed, 1973 in M. idella; Balasundaram and Pandian, 1982b in M. nobilii; Inyang, 1984 in M. felicinum). There is no study in Macrobrachium regarding the variation in fecundity, in each successive breeding.

2.4.6 Incubation.

The incubation of eggs by most female decapod crustaceans, according to Ching and Velez (1985), may be one reason for the success of the group. According to Pearse and Gunter (1957), most larval stages in Palaemonidae, are suppressed in the egg, protecting the early developmental stages from osmotic stress.

Extruded eggs of Macrobrachium are of two colours: either green, as in M. idella (Natraj, 1947; Aiyer, 1949) M. acanthurus (Choudhury, 1971 c), M. amazonicum (Guest, 1979) M. heterochirus (Ching and Velez, 1985) and M. malayanum (Samuel et al., 1987) or orange, as in M. carcinus (Lewis et al., 1966), M. ohione (Truesdale and Mermilloid, 1979) and M. rosenbergii (Ling, 1969a). However, for all these species of Macrobrachium, eggs with embryo turn either grey or dark brown prior to eclosion.

Biochemical changes and energy utilization in developing stages of M. idella have been studied by Vijayaraghavan and Easterson (1974), and they reported that protein contributes to about 67.4% of the total energy available for development. But Shakuntala (1977b) reported that in M. lamarrei, 92.5% of energy required is provided by fat and contribution of protein is very less (7.5%).

It is known from the work of Cole (1958) that the period of egg carriage in nature is directly related to temperature. The incubation period of different palaemonid prawns is presented in table 1. During incubation, gravid females clean the eggs with their first pair of pereopods and aerate them by beating their pleopods (Shyama, 1987). The mother prawn is very sensitive to disturbances during the period of incubation (Schone, 1961). When disturbed and in unfavourable environment, the pleopod beat frequency decreases which might delay hatching (Balasundaram, 1980). It has been shown that in M. nobilli batch hatching is controlled by the mother, and not by the stage of development of eggs (Balasundaram and Pandian, 1981). But Adiyodi and Adiyodi (1970) working on M. nobilii, observed that hatching cannot be postponed indefinitely as moulting is obligatory, and normally there is a moulting soon after hatching of eggs in the brood pouch. The presence of developing eggs on the pleopods has been reported to delay moulting (Schone, 1961; Antheunisse and Hoven, 1968; Rao et al., 1985). In Palaemonetes varians, the normal intermoult period is found to be 16.5 $\pm$ 1.9 days for unimpregnated females and 32 $\pm$ 2.2 days for berried females (Antheunisse and Hoven, 1968) and in such cases, the presence of moult inhibiting hormone secreted by the X-organ and stored in the sinus gland might be responsible

Table 1. Incubation period of eggs of various palaemonid prawns.

Species	Incubation period (days)	Temperature (^{o}C)	Source
Palaemon elegans			
(Leander squilla)	18-21	21-22	Schulte, 1976
P. pacificus	30	20	Emmerson, 1985
P. serratus	21	21	Phillips, 1971
Macrobrachium acanthurus	14	27-29	Martinez-Silva, 1981
	16-18	23-27	Choudhury, 1971c
	16-18	-	Carr, 1979
M. amazonicum	12-15	30±1	Guest, 1979
	19-24	24±2	Guest, 1979
	15-17	-	Magalhaes, 1985
	15-20	26	Romero, 1982
M. idae	15	25	Pandian and Katre, 1972

Table 1 (Contd..)

Table 1. (Contd.)

Species	Incubation period (days)	Temperature (^{o}C)	Source
M. idella			
(P. idae)	14-16	-	Natraj, 1947
M. kistnensis	29-30	-	Nagabhushanam and Kulkarni, 1979
M. lanceifrons	22	25-28	Rasalan et al., 1969
M. malcolmsonii	15	-	Kewalramani, 1973
M. rosenbergii	20-21	-	John, 1957
	19-20	-	George, 1969
	19	26-28	Ling, 1969a
	15-24	-	Rao, 1986
	16	31	Diaz, 1987b
	17	29	Diaz, 1987b
	26	25	Diaz, 1987b

Table 1 (Concl.)

for the delay in moulting (Scudamore, 1948). Owing to the delay in hatching, moulting of berried females casting off exuvia with developed eggs was reported in M. nobilii by Balasundaram and Poyyamoli (1984). Balasundaram and Pandian (1982b) reported that delay in hatching affects the survival rate of larvae.

Davis (1965) and Shakuntala (1976) suggested that there is possibility of osmotic pressure playing some role in the hatching of eggs of palaemonids.

2.5 Larval Development

Wide variations in the duration of larval development among the different species of Macrobrachium have been noted (vide table 2).

Based on the number, size of eggs and early life history, 3 types of larval development have been described in the Palaemonidae by Sollaud (1923). These types are (i) typical type - characterised by numerous small eggs with a larval life of many stages, (ii) semi-abbreviated type-characterised by fewer, larger eggs with few larval stages, and (iii) highly-abbreviated type where the newly hatched forms are post-larvae. On the basis of this classification, the different palaemonid prawns could be grouped into 3 categories, as shown in table 2.

Pillai and Mohamed (1973) conducted detailed studies regarding the larval history of M. idella and found that in normal healthy condition, the larvae moulted within 2 to 4 days more or less regularly. Larvae of M. idae, which hatched during the second night, were found to be 2% longer than those which hatched on the first night (Pandian and Katre, 1972).

Table 2. Details of larval development of various species of family Palaemonidae.

Type of larval development	Species	No. of larval stages	Duration of larval development (days)	Salinity (ppt)	Temperature (^{o}C)	Source
Typical-type	Palaemonetes intermedius	6-8	18-24	30	23.7-27.0	Hubschman and Broad, 1974
	P. kadiakensis	6	16-24	0	18.5-24.0	Hubschman and Rose, 1969
	P. pacificus	6	27	35	20	Emmerson, 1985
	Macrobrachium acanthurus	9	37	-	-	Martinez - Silva, 1981
		10	32-42	15-20	23-27	Choudhury, 1970 and 1971C
	M. amazonicum	8-9	23-26	10	24-28	Guest, 1979
		10-11	28-33	-	-	Magalhaes, 1985
	M. americanum	11	53	15	29±0.5	Monaco, 1975
	M. carcinus	12	47-65	14-17.5	24-28	Choudhury, 1971a&b
		12	90	21	-	Lewis and Ward, 1965
	M. equidens	10	25-45	20-25	-	Pillai, 1982
	M. idella	10	39-52	12-18	23-28	Pillai and Mohamed, 1973
	M. malcolmsonii	16	45	-	-	Kewalramani, et al., 1971

Table 2 (Contd.)

Table 2 (Contd.)

Type of larval development	Species	No. of larval stages	Duration of larval development (days)	Salinity (ppt)	Temperature (^{o}C)	Source
Typical-type	M. nipponense	9	18-20	-	-	Kwon and Uno, 1969
	M. novaehollandiae	10-15	41-58	23	15-28	Greenwood et al., 1976
	M. rosenbergii	12	-	-	-	Ling, 1962
		8	30-45	7-14	-	Ling, 1969a
		11	-	-	-	Uno and Kwon, 1969
Semi-abbreviated type	Palaemonetes cummingi	3	9	0	22-24	Dobkin, 1971
	P. paludosus	3	5-10	0	15-31	Dobkin, 1963
	P. sinensis	3	-	-	-	Shen, 1939
	M. asperlum	2	2	0	-	Shokita, 1977
	M. australiense	3	6	0	21-28	Fielder, 1970
	M. canarae	3	7	0	25-27	Jalihal et al., 1982
	M. hendersodayanum	1	1-2	-	-	Jalihal and Sankolli, 1975

Table 2 (Contd.)

Table 2 (Contd.)

Type of larval development	Species	No. of larval stages	Duration of larval development (days)	Salinity (ppt)	Temperature (^{o}C)	Source
Semi-abbreviated type	M. kistnensis	4	7-11		-	Nagabhushanam and Kulkarni, 1979
	M. sankolli	3	<7		-	Sankolli et al., 1980
	M. shokitai	2	1.5		-	Shokita, 1973
	M. tiwarii	3	<7		-	Sankolli, et al., 1980
Highly-abbreviated type	M. borelli	No larval stages and the eggs hatch into post-larvae				Boschii, 1961

Table 2 (Concl.)

Dugger and Dobkin (1975) stated that the larvae of M. olfersii in nature are generally larger than those of the same stage reared in the laboratory.

Marked variations in the number of larval instars and the duration of larval development, have been observed among several groups of decapod crustaceans, being more among caridean shrimps (Knowlton, 1974). Seasonal variations in the number and duration of larval instars have been noted in Palaemon macrodactylus reared in laboratory (Little, 1969). The settlement of the larvae to bottom has been suggested as a mechanism on the part of some palaemonid larvae to reduce downward displacement to sea and thus remain in the estuary. Thus Thorne et al. (1979) observed that in nature, most larvae of Macrobrachium novaehollandiae rest on the bottom for long periods losing their planktonic behaviour. Such an observation has been made in the case of M. petersi by Read (1983 & 1985).

There are many studies to show that environmental factors such as salinity (Sandifer, 1973; Ling, 1969a), temperature (Sandifer, 1973; Knowlton, 1974; Uno et al., 1975; Rochanaburanon and Williamson, 1976; Farmanfarmaian and Moore, 1978; Crowell and Nakamura, 1980; Crowell, 1981), photoperiod (Knowlton, 1974), time of year (Knowlton, 1974), pollutants (Shealy and Sandifer, 1975; Piyan et al., 1985) and anti-pollution agents (Maddox and Manzi, 1976; Manzi et al., 1977) and also the diet (Broad, 1957a&b; Reeve, 1969b; Knowlton, 1974; Sick and Beaty, 1974) influence larval development. Differences among parental population are also known to influence larval development (Reeve, 1969b; Diaz, 1987a; Provenzano et al., 1978). According to Bagenal (1967), the chance of survival of developing eggs of most decapod crustaceans in the natural ecosystem is less than 0.1%.

Variations in the duration of larval development have no significant influence on juvenile growth rate. Thus, in M. rosenbergii early metamorphosis of larvae to post-larvae confers no advantage nor late metamorphosis any disadvantage on growth (Malecha, 1977; Sandifer and Smith, 1979).

2.6 Freshwater Prawn Culture

Many studies regarding the culture of palaemonids, especially of the giant freshwater prawn M. rosenbergii, have been conducted in different parts of the world, particularly, in south-east Asian countries and USA. M. rosenbergii, the most sought after candidate species for aquaculture, is mainly cultured in freshwater areas. However, good growth and production have been obtained from low saline brackishwater areas also (Ling and Costello, 1976; Popper and Davidson, 1982; Smith et al., 1982).

Generally, nursery rearing is not practised in freshwater prawn culture. But increased survival rate and production were obtained when nursery reared juveniles were stocked (Eble et al., 1977). Moreover, nursery rearing of post-larvae reduces cannibalism and also helps in reducing the grow-out period, which is of much value in temperate areas where the culture period is very much restricted.

The major problems in successful Macrobrachium culture are the high cost of feed (Shang and Fujimura, 1977), cannibalism and territorial aggression (Fujimura, 1974) and decreased yield due to wide size variation among individuals (Ra'anan and Cohen, 1983 & 1984a; Peebles, 1979b; Sandifer and Smith, 1985; Rao et al., 1986). The cost on account of supplementary feeding

can be reduced by fertilizing and manuring the ponds which help the development of food organisms. However, over fertilization may lead to algal blooms which may be deleterious very often. The role of algal blooms in freshwater prawn culture has been discussed by Baissac (1976); Willis and Berrigan (1977); Smith et al. (1979) and Rao et al. (1986). According to them, the algae choke the body of the prawn and cause depletion of oxygen and consequent ecological imbalance, which may cause mortality of prawns. Mukhopadhyay and Sarangi (1985) obtained best production of M. malcolmsonii from unmanured ponds with supplementary feed.

Incorporation of low cost components for the manufacture of feed will also reduce the cost incurred. Studies towards this direction by Harpaz and Schmalbach (1986) had shown that supplementation of dry artificial feed with fresh leaves of Ailanthus altissima and Malva parviflora resulted in elimination of moult death syndrome and reduction of black spot disease. Miltner et al. (1983) used air dried rice straw as a feed supplement to feed pellets and recorded an increase in production and average size when both hay and pellets are given, than from the ponds where only hay or pellets are given. Stern et al. (1976) prepared feed with aquatic plants like Azolla, Cladophora, Elodea and Lemna; but these feeds caused increased mortality and decreased weight. Hence it was emphasized by Stahl (1979) that diets which include both natural feeds and commercial pellets exhibit significantly higher growth rate than the prawns raised solely on natural feeds or commercial feeds.

Peebles (1978) reported a mortality of 20-50% from post-larvae to adult and the important reasons for mortality assigned by him, are cannibalism and

territorial aggression. Increase in surface area decreases the density of prawn per unit area and this reduces cannibalism and territorial aggression. Accordingly, greater yield and survival rate were obtained in ponds provided with artificial substrates (Wickins, 1972b; Smith and Sandifer, 1975; Evans, 1976; Ra'anan et al., 1984). At higher stocking densities greater prawn yield was reported, but mean size of harvested prawns decreased (Sandifer and Smith, 1975; Smith et al., 1976, 1978 and 1981; Willis and Berrigan, 1977; Brody et al., 1980; Perry and Tarver, 1981; Cohen et al., 1983b; Subrahmanyam, 1984; Karplus et al., 1986).

Periodic selective harvesting of large sized prawns, to some extent, was found to solve the problem due to size variation among individuals. This perhaps provides better growing facilities to the remaining prawns. Greater survival rate and production were reported from culture ponds which are selectively harvested (Fujimura, 1974; Willis and Berrigan, 1978; Roberts and Bauer, 1978; Malecha, 1983).

Polyculture of freshwater prawns with fishes, to some extent, is effective in reducing the problems involved due to occurrence of algal blooms and wide size variation (Cohen et al., 1983a). Polyculture with fishes also increase production from freshwater prawn culture ponds. Ahmed et al. (1987) obtained best production of M. rosenbergii from polyculture with carps. Wohlfarth et al., (1985) reported that growth and survival of fishes (Common carp, Tilapia and Chinese carps) 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. Stocking of freshwater prawns in

polyculture with fishes also increases the yield of fishes. Guerrero and Guerrero (1977), for example, reported high yield in Tilapia nilotica culture ponds when adult shrimps of Macrobrachium spp were stocked.

In most of the fresh water prawns, the growth rate of males compared to females is more. Sagi et al. (1986) reported higher prawn yield from a culture experiment where an all male population of M. rosenbergii was stocked.

Though, many studies have been conducted to evaluate the potential of culture of the species M. rosenbergii, only very few studies have been made to investigate the farming potential of various other species of freshwater prawns. However, Johnson (1968) and Dobkin (1969) indicated that some of these shrimps, which are highly esteemed as food in many developing countries, might be cultured to some advantage.

Some experiments have been conducted in India regarding the culture of M. malcolmsonii. Production values in various experiments were 80-400 kg/ha/8 months (Rajyalakshmi et al., 1979), 313.7-327.1 kg/ha/year (Rao et al.,1979), 404.2-421.7 kg/ha/year (Mukhopadhyay and Sarangi, 1985), 534.2-690.4 kg/ha/13 months (Rao et al., 1986) and 588-962 kg/ha/13 months (Reddy et al., 1987).

In Mexico, experiments have been conducted to evaluate the culture potential of M. acanthurus and M. tenellum. In one of the trials conducted by Cano (1980) production of M. acanthurus was 275 kg/ha/2 months (1.1 ton/ha/year). Dobkin et al. (1974) reported that males of M. acanthurus grow to 10 cm in 4 months and 12.1 cm in 6 months and

females grow more slowly, atleast, after achieving sexual maturity, in culture ponds. Dobkin et al. (1975) also reported that F_3 generation of selectively bred M. acanthurus showed increased yield per hectare. In a polyculture experiment conducted by Martinez-Silva et al. (1981), wherein, 7826 fingerlings of Mugil incilis were stocked with an undetermined number of M. acanthurus of size of 13.6 mm and 0.03 g, with supplementary feeding (rice meal, cotton seed paste and rice bran), a production of 210 kg/ha of prawn of mean size 12 g and 296 kg/ha of fish was obtained after 133 days of culture. Palacois et al. (1980) stocked M. tenellum of mean length 24-29 mm and mean weight 0.44 g in four 32 M^2 concrete tanks at a stocking density of 26/M^2. They were fed with concentrated chicken feed at a rate of 5% of body weight. Mean survival rate was found to be 61.05% and the production 2 tons/ha/year of which males of commercial size contributed 1434 kg/ha/year.

Guerrero and Villanuera (1978) conducted preliminary studies on the culture of M. idella in philippines. In monoculture system, this prawn was stocked at a mean size of 2.7 g and density of 2000/ha. After a period of 136 days, the prawn was found to have grown to an average size of 8 g (maximum size 18.8 g). In another experiment of monoculture of juveniles and adults of M. idella, conducted seperately by Guerrero and Cagauan (1979), juveniles being stocked at a level of 5 kg/ha and adults also at the same level of 5 kg/ha, the production obtained were 55 kg/ha and 33.3 kg/ha respectively in 150 days of culture.

In Philippines Guerrero and Cagauan (1979) conducted monoculture experiments with juveniles and adults of M. lanchesteri in seperate ponds.

Stocking level of both juveniles and adults was 10 kg/ha. Yield obtained from juvenile stocked pond was 133.5 kg/ha/150 days and from adult stocked pond it was 58 kg/ha/150 days.

Preliminary experiments, with M. lanchesteri and M. lanceifrons montalbanense stocked with and without fish in fertilized ponds, were conducted in Philippines by Guerrero and Guerrero (1979). Stocking ovigerous females and juvenile shrimps in four 0.1 ha earthern ponds with Tilapia mossambica and T.nilotica resulted in net shrimp production of 37 to 179 kg/ha/90 days. Shrimp standing crop of 384 and 391 kg/ha were obtained after 120 days in ponds stocked with adult male and female shrimps at rates of 10 and 9 kg/ha respectively.

Thus, it could be seen that the production rates of most other species of Macrobrachium are low when compared to the production rates of 465-820 kg/ha/119 days and 3800-4700 kg/ha/year achieved through semi-intensive and intensive cultures respectively with M. rosenbergii (Sandifer and Smith, 1975; Smith and Sandifer, 1982; Boonyaratpalin and New, 1982; Sandifer, 1982). However, culture of other larger species of Macrobrachium such as M. americanum, M. malcolmsonii, M. carcinus, M. tenellum, M. acanthurus and M. birmanicum choprai and smaller species such as M. idella, M. equidens, M. villosimanus, M. rude, M. javanicum, M. scabriculum, M. lamarrei and M. dayanum can be successfully done either in monoculture or polyculture with fresh and brackish water fishes.

2.7 Effect of Salinity on Survival, Growth, Reproduction and Larval Development

Salinity is considered to be the most important environmental parameter influencing the life process of species inhabiting brackish water. Although the palaemonid prawns are known as freshwater prawns, many of them especially those of the genus Macrobrachium spend a part of their life cycle in brackishwater environment.

2.7.1 Survival.

Palaemonid prawns are reported to have wide salinity tolerances and preferences. Castille and Lawrence (1981) stated that many species of palaemonids are able to tolerate salinities ranging from fresh to hypersaline water and some species can tolerate only narrow salinity ranges. According to Emery and Stevenson (1957), the family Palaemonidae, as a whole, has been considered by some to be in the process of migration from marine to freshwater environment.

Osmoregulation in palaemonid prawns has been investigated by Panikkar (1941) and according to him, palaemonid prawns achieve osmotic stability by active absorption of ions when in hypotonic media and active transport of water against the osmotic gradient, probably very effective, when in hypertonic media. The osmotic work required for adjustment is brought to a minimum by the low permeability of the integument (gills), which gives the prawns considerable powers of salt retention.

Salinity tolerance of various palaemonid prawns was studied by many workers. Accordingly, the salinity tolerance is 16-39 ppt for

Palaemon affinis (Kirkpatrick and Jones, 1985), 5-45 ppt for P. elegans (Panikkar, 1941; Hernandez and Taylor, 1985) 15-39 ppt for P. intermedius (Dobkin and Manning, 1964), 1-61.2 ppt for P. macrodactylus (Born, 1968), 10-47.5 ppt for P. ritteri (Reynolds, 1975), 16-39 ppt for P. serratus (Panikkar, 1941; Parry, 1954; Spaargaren, 1972), 1-55 ppt for Palaemonetes pugio (Knowlton and Kirby, 1984), 0.2-50 ppt for P. varians (Panikkar, 1941; Parry, 1957; Potts and Parry, 1964; Hagerman and Uglow, 1983), 3-35 ppt for P. vulgaris (Nagabhushanam, 1961), 2-40 ppt for M. equidens (Denne, 1968) and 0-31.6 ppt for M. rosenbergii (Venugopalan, 1988). Among various species of the genus Macrobrachium, only one species viz. M. intermedium has been reported to be fully marine, spending its entire life cycle in the sea (Holthuis, 1952c).

Salinity tolerance of an organism, generally depends on the salinity to which it is preacclimated. Kinne (1964) reported that acclimation to low salinities tends to shift the lower lethal limit downwards and acclimation to higher salinities tends to shift the upper limit upwards. According to Kinne (1966), mortality in extreme low or high salinities appears to be primarily related to (i) critical disturbances in the overall water and mineral balance (ii) direct osmotic damage of protein structure, cells and tissues (iii) direct damage through significant deviation in relative proportion of solute and (iv) indirect damage caused by critical lowering of metabolic rate or activity or by disharmonizing effects on the integrated methodology.

2.7.2 Growth.

According to Kinne (1971), growth is restricted to significantly narrower salinity ranges than is survival in most euryhaline invertebrates. Migration of juveniles of Macrobrachium to areas of lower salinity has been reported by many. In many species upstream migration of juveniles is of much significance. In their migration, juveniles of M. australiense (Lee and Fielder, 1979), M. malcolmsonii (Ibrahim, 1962) and M. rosenbergii (Ling, 1969a), even cross over dams and anicuts. Increase in salinity in the lower reaches is the major factor triggering this juvenile migration.

The concept of maximum growth should occur in iso-osmotic media (since the animals need not expend energy in doing osmotic work) has been suggested by some workers (Canagaratnam, 1959; Ryther and Bardach, 1968; Panikkar, 1968). But Singh (1980) showed that this concept is not true in M. rosenbergii, as in this prawn maximum growth was attained at a very low salinity level of 2 ppt and growth was impaired at high salinity level of 15 ppt (Perdue and Nakamura, 1976). Sandifer and Smith (1974), Goodwin and Hanson (1975) and Venugopalan (1988) also obtained best growth in fresh or slightly brackishwater. Ling and Costello (1976) recommended a salinity range of 0-10 ppt, as the optimum salinity for M. rosenbergii. On the contrary, Popper and Davidson (1982) obtained best growth in salinities from 10 to 15 ppt. But Smith et al. (1982) reported best growth from freshwater to 15 ppt.

2.7.3 Reproduction.

Studies on the influence of salinity on reproduction of palaemonids are very limited. The number and size of eggs of M. nipponense in

freshwater are found to be different from those living in brackishwater regions (Mashiko, 1983 & 1984). The estuarine population is known to produce many small eggs (0.055 mm^3 in mean egg volume) and fresh water population a few but larger eggs (0.104 mm^3 in mean egg volume). The same pattern of reproduction was observed in Palaemonetes varians by Boas (1898).

The effect of salinity on incubation time of M. heterochirus has been studied by Ching and Velez (1985). The different salinity levels tested were 0, 10 and 17.5 ppt. The period of incubation of eggs at these salinities were 15.7 ± 1.37, 16.1 ± 1.60 and 17.0 ± 1.49 days respectively. It was found that the difference in incubation period at these salinities is not statistically significant. However, it was noted that gravid females of M. rosenbergii, that were gradually exposed to salinities of 8 ppt during the last part of incubation had a higher number of larvae released during eclosion (Ling, 1969b).

2.7.4 Larval Development.

Most species of Macrobrachium are dependent on brackishwater for complete larval development (Read, 1986). The salinity requirement for larval development is much varied among the different species of Macrobrachium. The optimum salinity required for larval development of M. rosenbergii has been worked out by Ling (1962, 1969a&b). For larval rearing of this species many workers used a salinity of 11-18 ppt (Fujimura, 1966; Ling, 1969b; Fujimura and Okamoto, 1972; Dugan et al., 1975; Meeran and Sebastian, 1976; Nair et al., 1977; Aquacop, 1977 ; Adisukresno et al., 1982, Aniello and Singh, 1982; Chineah, 1982;

Lee, 1982, Suharto et al., 1982). Wickins (1972b) observed that a salinity of 26 ppt is more suitable in the initial one-half of the rearing period, whereas, Sick and Beaty (1974) reared them in salinities as low as 6 ppt. However, according to Sandifer et al. (1977), the optimal salinity range for the larval development is 12-16 ppt. The necessity of salinity for the larval development of M. malcolmsonii was stressed by Kewalramani (1973). According to Sankolli and Shenoy (1980) the first stage larva of this species can thrive in freshwater for 15 days whereas the larvae of M. rosenbergii only for 5-6 days.

The salinity required for best development and survival of larvae of M. carcinus, according to Lewis and Ward (1965) is 21 ppt and according to Choudhury (1971a&b), it is 14-17 ppt. Salinity levels of 15-20 ppt (Choudhury, 1971c) and 14-20 ppt (Dugan et al., 1975) are found favourable for the development of M. acanthurus larvae. Holtschmit and Pfeiller (1984) reported that early larval stages of M. americanum require 20-30 ppt salinity and only about 15-20 ppt in the later stage. Larvae of M. amazonicum require 7-28 ppt salinity for growth (McNamara et al., 1983). However, the best salinity was found to be between 12 and 18 ppt (Moreira et al., 1986). Guest and Durocher (1979) could rear these larvae in salinities of 1-15 ppt, but not in freshwater. A salinity of 14-17 ppt was found essential for the development of larvae of M. holthuisi (Moreira, et al., 1979); larvae not surviving in salinities above or below 14-17 ppt. Uno (1971) recorded a salinity level of 8-13 ppt for best survival and metamorphosis of larvae of M. nipponense. But Wong (1987) succeeded in rearing these larvae in salinities of zero and 15 ppt.

Pillai (1982) found 20-25 ppt, as the optimum salinity for the development of larvae of M. equidens. Lakshmi et al. (1982) studied the development of larvae of M. idella at salinities of 5, 10, 15, 20, 30 and 35 ppt and found that a salinity range of 10-25 ppt can be considered as normal and the best survival was at 15 ppt. Lee and Fielder (1981) reported that in M. australiense, there is inhibition of metamorphosis at salinities higher than 15 ppt and this species is able to complete its life cycle in freshwater. Likewise, M. iheringi (Bueno, 1980) and M. vollenhoveni (Prah, 1982), also can complete their life cycle in freshwater.

Based on the degree of salinity tolerance, shown by the larvae of various species, Read, (1986) divided them into 3 categories, on the basis of increasing adaptation to fresh water. (i) euryhaline larvae that survive in both low and high saline water such as M. petersi, M. acanthurus, M. olfersii, etc., (ii) stenohaline larvae that survive in low saline water, but cannot tolerate high salinity (>28 ppt), such as M. rosenbergii, M. carcinus etc., and (iii) stenohaline larvae that survive in freshwater, but cannot tolerate salinities greater than 20 ppt, such as M. australiense. The first category, according to him, include the recent invaders to freshwater and the last category, the most advanced in the colonization of freshwater.

III. MATERIALS AND METHODS

III MATERIALS AND METHODS

3.1 Experimental Prawns

Juveniles, sub-adults and adults of Macrobrachium idella used for various studies were collected from the Vembanad lake at Poothotta about 25 km from the Cochin barmouth. Juveniles used for survival and growth studies were within the size range of 45-55 mm and 834-1342 mg. For fecundity and incubation studies, adult females of 48-60 mm and 965-2338 mg and adult males of 64-89 mm and 2364-7031 mg were used.

3.2 Feed

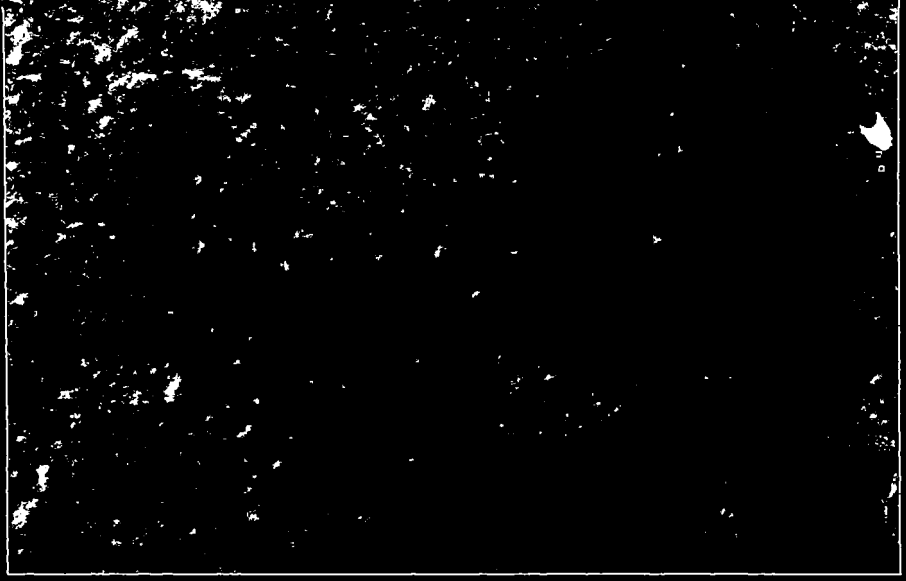
For all the studies clam (Villorita cyprinoides) meat was given as feed. The proximate composition of the clam meat is protein - 14.4%, Fat - 7.8%, ash - 2.9%, calcium - 1.01%, phosphorus - 0.48%, iron - 0.06% and moisture 73% (Sebastian, 1970). Clam meat was kept preserved in frozen state, thawed, cut into small pieces and fed to the prawns daily ad libitum.

3.3 Experimental Cisterns and Containers

For growth and fecundity studies circular cement cisterns of 90 cm diameter and 60 cm height were used. Survival and incubation studies were conducted in rectangular perspex tanks of 28x16x30 cm dimensions.

3.4 Preparation of Saline Media

Hypo-saline water of desired salinity was prepared by diluting the seawater, brought from Cochin barmouth, with tap water. The quantity of seawater to be mixed with freshwater to get the desired salinity was computed by the formula,



1 Macrobrachium idella juveniles used for survival and growth study

$$V = \frac{\text{Required salinity}}{\text{Salinity of seawater}} \times 1000,$$

Where V is the volume of seawater to be diluted to make one litre of water of the required salinity.

Hyper-saline water was prepared by freezing seawater as described by Shapiro (1961).

3.5 Experimental Procedure

3.5.1 Study to Evaluate the Effect of Salinity on Survival.

This experiment was conducted with six prawns in each perspex tank keeping 10 L of water. A gravel bed of about 2 cm thickness was provided in each tank and gentle aeration given throughout.

The effects of both sudden transfer and gradual acclimation to the test salinities were studied. In gradual acclimation experiment, the prawns (6 No./ tank) were first acclimated to the tank environment in freshwater for 5 days. and fed with clam meat. Then the salinity of water was gradually increased from freshwater up to 45 ppt, at an increment of 5 ppt/12 Hr up to 30 ppt and 2.5 ppt/12 Hr from 30 ppt onwards (table 3); by adding calculated quantity of seawater after removing calculated excess volume of water from the experimental tank. Prawns kept in freshwater served as the control group. The experiment was replicated twice. During the course of the experiment, the prawns were not fed. The salinity level at which the mortality of each prawn took place was noted. Dead ones were removed immediately.

Abrupt transfer experiment was conducted with the prawns acclimated at 0 ppt in one set and in another with the prawns acclimated at 20 ppt salinity.

Table 3. Schedule of acclimation of M. idella juveniles to the test salinity levels.

Elapsed time (Hrs)	Test salinity levels (ppt)
0	0.0
12	5.0
24	10.0
36	15.0
48	20.0
60	25.0
72	30.0
84	32.5
96	35.0
108	37.5
120	40.0
132	42.5
144	45.0

In the first set of experiment, the prawns (6 No./tank) were acclimated in freshwater for 5 days in the experimental tanks. Thereafter, the salinity of water was increased abruptly to test salinities of 5, 10, 15, 20, 25, 30, 32.5, 35 and 40 ppt by adding freshly prepared water of required salinity after draining the water in the experimental tanks. Prawns kept in freshwater served as the control group. The experiment was replicated twice. In the second set of experiment, the prawns were gradually acclimated to 20 ppt water from freshwater, at an increment of 5 ppt per day and were acclimated at 20 ppt for 5 days. The salinity was then suddenly changed to test salinities of 0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 32.5, 35, 37.5, 40 and 42.5 ppt, by adding freshly prepared water of required salinity after draining the water in the experimental tanks. Prawns kept in 20 ppt water served as the control group. Two replicates were carried out simultaneously. In both cases, the prawns were fed during the acclimation period and no feed was given afterwards. The exact time of mortality of each prawn was noted in each set of experiment.

Probit analysis technique (Finney, 1971) was used for finding out the 48, 72, 96 and 120 Hr LC_{50} values. Slope of the probit line, standard error and 95% confidence limits were also calculated.

3.5.2 Study to Evaluate the Effect of Salinity on Growth.

Studies to investigate the effect of salinity on growth were conducted in cement cisterns for two and half months during April-June, 1988. Salinity levels tested were 0, 5, 10, 15, 20 and 25 ppt. Since prawns showed distress signs at 30 ppt, this level was not included for growth study. 10 prawns were reared in each cistern containing 100 L of water.

All the 10 prawns in each rearing tank were collectively weighed, using a chemical balance, and from this, average weight of a single prawn was calculated. No aeration was given and 25% of water was exchanged weekly. Prawns were fed ad libitum on all days, except the day before sampling. Un-eaten food and excreta were siphoned out the next day. The chemical and physical parameters of water were determined on a weekly basis. Sampling of prawns, to evaluate the growth increment in cisterns, was carried out at an interval of 7 days. From this data percentage specific growth rate (GW%) at different salinities was calculated for 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days growing period by taking the average weight of prawns on the day of start of experiment as the initial weight (W_o) in all cases. Percentage specific growth rate was calculated by using the formula,

$$GW\% = \frac{\ln W_1 - \ln W_o}{t} \times 100,$$

Where GW% is the percentage specific growth rate; ln W_1, the natural logarithm of final weight; ln W_o, the natural logarithm of initial weight and t, the growing period in days. A one way ANOVA technique was used for statistical analysis (Gomez and Gomez, 1984). For pairwise comparison Duncan's multiple range test was used (Gomez and Gomez, 1984).

3.5.3 Study to Evaluate the Effect of Salinity on Fecundity.

This study was conducted for a period of 70 days in cement cisterns having sand bed, during November 1987 to January 1988. Different salinity levels tested were 0, 6, 12 and 18 ppt. The experiment was carried out with two replicates at each salinity level. In each cistern, containing 200 L of water, 10 mature females and 3 males were stocked. They were allowed to

acclimate at the different test salinities for 40 days. This duration given is twice the period of a normal ovarian cycle (18 ± 2.65 days, Shyama, 1987) of this prawn. This is to give sufficient period for the impact of salinity felt on gonadal development and other associated physiological phenomena. After this period of acclimation, the berried females, whose eggs are green coloured, were collected immediately after spawning and preserved in 7% formalin. 25% of the water in the experimental cement cisterns was exchanged once a week. Water quality parameters were analysed once a week. No aeration was given and feeding ad libitum was resorted with clam meat.

The formalin preserved berried females were repeatedly washed in freshwater and drained for one hour for weight constancy and the fecundity was estimated by gravimetric method. Weighing was done in an electrical balance. The lengths of shortest and longest axis of egg were found out by using a stage micrometer. The area of egg (mm^2) was determined by applying the formula, ΠLl, where L, is the length of longest axis and l, the length of shortest axis.

Since the relationship between fecundity and weight/length of prawns is exponential, log transformations were made for calculation. From the fecundity data, in each salinity, four regression lines were fitted. The four regression lines fitted were, (i) log of fecundity against log wet weight of prawns (ii) log fecundity against log total length (from tip of rostrum to tip of telson) of prawns (iii) log brood weight against log wet weight of prawns and (iv) log brood weight against log total length of prawns. The regression lines of log fecundity against log wet weight of prawns for different

salinities were then compared on a multiple basis (Zar, 1974). Similarly in each salinity, regression lines of log fecundity against log total length, log brood weight against log wet weight and log brood weight against log total length of prawns were also compared. The mean egg area (average area of 25 eggs of each prawn) at different salinities was compared by using one way ANOVA.

3.5.4 Study to Evaluate the Effect of Salinity on Incubation Period, Batch Hatching and Ovarian Development.

For this study only mature females, collected from nature, whose ovary is well developed and green in colour were used. These mature females were stocked in cement cisterns having freshwater, at a density of 5 females and 2 males. Mating and spawning were constantly watched at an interval of 4 hours. After noting the time of spawning, each berried female was then immediately transferred to the perspex tank containing 5 L of water of the desired salinity. Different salinity levels tested were 0, 6, 12, 18 and 24 ppt and the experiment was conducted during August-September, 1988. Gentle aeration was given and water exchanged at a rate of 50% in every 3 days. Temperature was recorded daily. Dissolved oxygen and pH measurements were made in every 5 days. The exact time of hatching of eggs was noted. From this, the period of incubation of eggs was computed. A one way ANOVA was carried out to elucidate the statistical significance of salinity on incubation period. Rate of batch hatching and ovarian development were also noted at the time of hatching of eggs. Chi-square test was conducted to know whether salinity has any effect on batch hatching and ovarian development (Snedecor and Cochran, 1967).

IV. RESULTS

3.6 Determination of Water Quality Parameters

The following water quality parameters were analysed using the method mentioned against each.

Salinity	:	Mohr - Knudson titrimetric method (Strickland and Parsons, 1972)
Dissolved Oxygen	:	Standard Winklers method (Strickland and Parsons, 1972)
Total Alkalinity	:	Acidimetric titration method (APHA et al., 1981)
pH	:	Electrometric method using Elico digital pH meter, Model LI-122
Temperature	:	Using a mercury bulb thermometer with an accuracy of $0.1^{o}C$.

IV RESULTS

4.1 Study to Evaluate the Effect of Salinity on Survival

The study to evaluate the effect of salinity on survival of Macrobrachium idella was conducted in perspex tanks with 12 prawns (total of two replicates) in each treatment. The cumulative percentage mortality of prawns at different salinity levels is given in tables 4, 5 and 6. Water quality parameters of the experimental tanks were; dissolved oxygen, 7.23 - 9.40 ppm; pH, 7.32 - 8.14 and temperature, 26.5 - 29.3^{o}C.

The results of gradual acclimation experiment (table 4) show that mortality of prawns starts at a salinity level of 37.5 ppt. At 40 and 42.5 ppt salinities, the rates of mortality recorded were 33.33% and 75% respectively, registering cent per cent mortality at 45 ppt. Stress sign (opaqueness in body) was noted in case of prawns acclimated at 32.5 ppt onwards.

The results of abrupt transfer experiment, with the prawns acclimated at 0 ppt (table 5) show that mortality starts at a salinity level of 27.5 ppt. But for the prawns acclimated at 20 ppt (table 6), mortality was found to occur only at a higher salinity level of 32.5 ppt. The mortality rate for 120 Hr period for the prawns acclimated at 0 ppt is 16.66% at 27.5 ppt, 33.33% at 30 ppt, 75% at 32.5 ppt, 83.33% at 35 ppt, and 100% at 40 ppt. The mortality rate for 120 Hr period is 16.66% at 32.5 ppt, 41.66% at 35 ppt, 75% at 37.5 and 40 ppt and 100% at 42.5 ppt for the prawns acclimated in 20 ppt water. Stress sign was noted at 27.5 ppt onwards for the prawns acclimated at 0 ppt and 32.5 ppt onwards for the prawns

Table 4. Cumulative percentage mortality of M. idella juveniles acclimated gradually to the test salinity levels of 5,10,15,20,25,30,32.5,35,37.5, 40,42.5 and 45 ppt.

Test salinity levels (ppt)	Cumulative percentage mortality
0-35	0.00
37.5	16.66
40.0	33.33
42.5	75.00
45.0	100

Table 5. Cumulative percentage mortality of Oppt acclimated M. idella juveniles transferred abruptly to various test salinities for a period of 120 Hrs. (No. of prawns/treatment=12).

Exposure time (Hrs)	Test salinity levels (ppt)					
	0-25	27.5	30	32.5	35	40
1	0	0.00	0.00	0.00	0.00	16.66
2	0	0.00	0.00	0.00	8.33	16.66
4	0	0.00	0.00	0.00	16.66	41.66
6	0	0.00	0.00	0.00	33.33	75.00
12	0	0.00	0.00	8.33	33.33	91.66
24	0	0.00	0.00	25.00	58.33	100
48	0	0.00	16.66	41.66	83.33	100
72	0	16.66	25.00	50.00	83.33	100
96	0	16.66	33.33	66.66	83.33	100
120	0	16.66	33.33	75.00	83.33	100

Table 6. Cumulative percentage mortality of 20 ppt acclimated M. idella juveniles transferred abruptly to various test salinities for a period of 120 Hrs (No. of prawns/ treatment=12).

Exposure time (Hrs)	Test salinity levels (ppt)					
	0-30	32.5	35	37.5	40	42.5
1	0	0.00	0.00	0.00	0.00	0.00
2	0	0.00	0.00	0.00	0.00	0.00
4	0	0.00	0.00	0.00	0.00	16.66
6	0	0.00	0.00	0.00	0.00	33.33
12	0	0.00	0.00	8.33	8.33	50.00
24	0	0.00	0.00	33.33	41.66	75.00
48	0	0.00	25.00	58.33	66.66	100
72	0	0.00	41.66	66.66	75.00	100
96	0	16.66	41.66	66.66	75.00	100
120	0	16.66	41.66	75.00	75.00	100

a :- LC_{50} = 32.73 ± 0.5947 ppt

b :- LC_{50} = 37.15 ± 0.9741 ppt

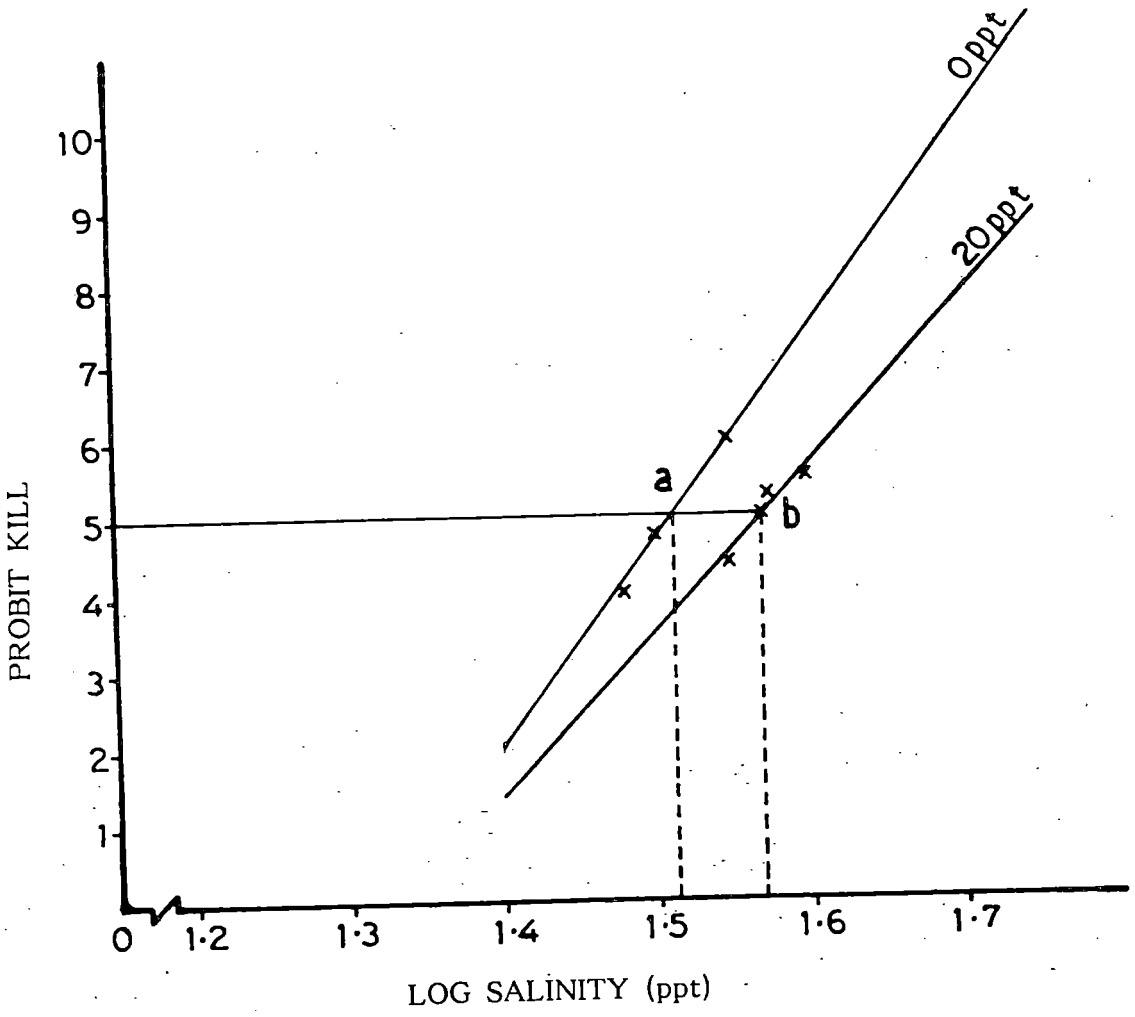


Fig. 3. Probit line for M. idella juveniles abruptly transferred from 0 and 20 ppt to different salinity levels for an exposure period of 48 Hr.

a ;- LC_{50} = 31.98 ± 0.7725 ppt

b :- LC_{50} = 35.89 ± 1.1392 ppt

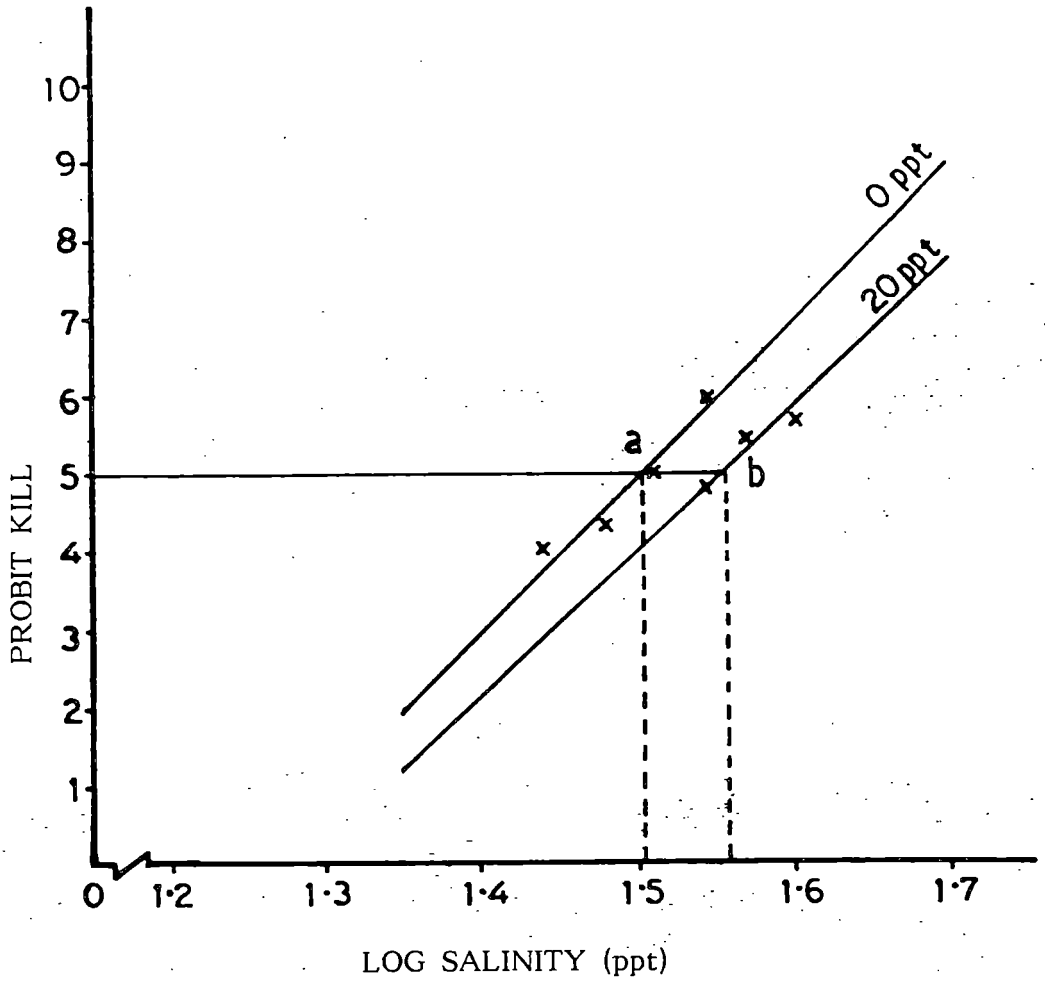


Fig. 4. Probit line for M. idella juveniles abruptly transferred from 0 and 20 ppt to different salinity levels for an exposure period of 72 Hr.

a :- LC_{50} = 30.90 ± 0.8102 ppt

b :- LC_{50} = 35.89 ± 0.8667 ppt

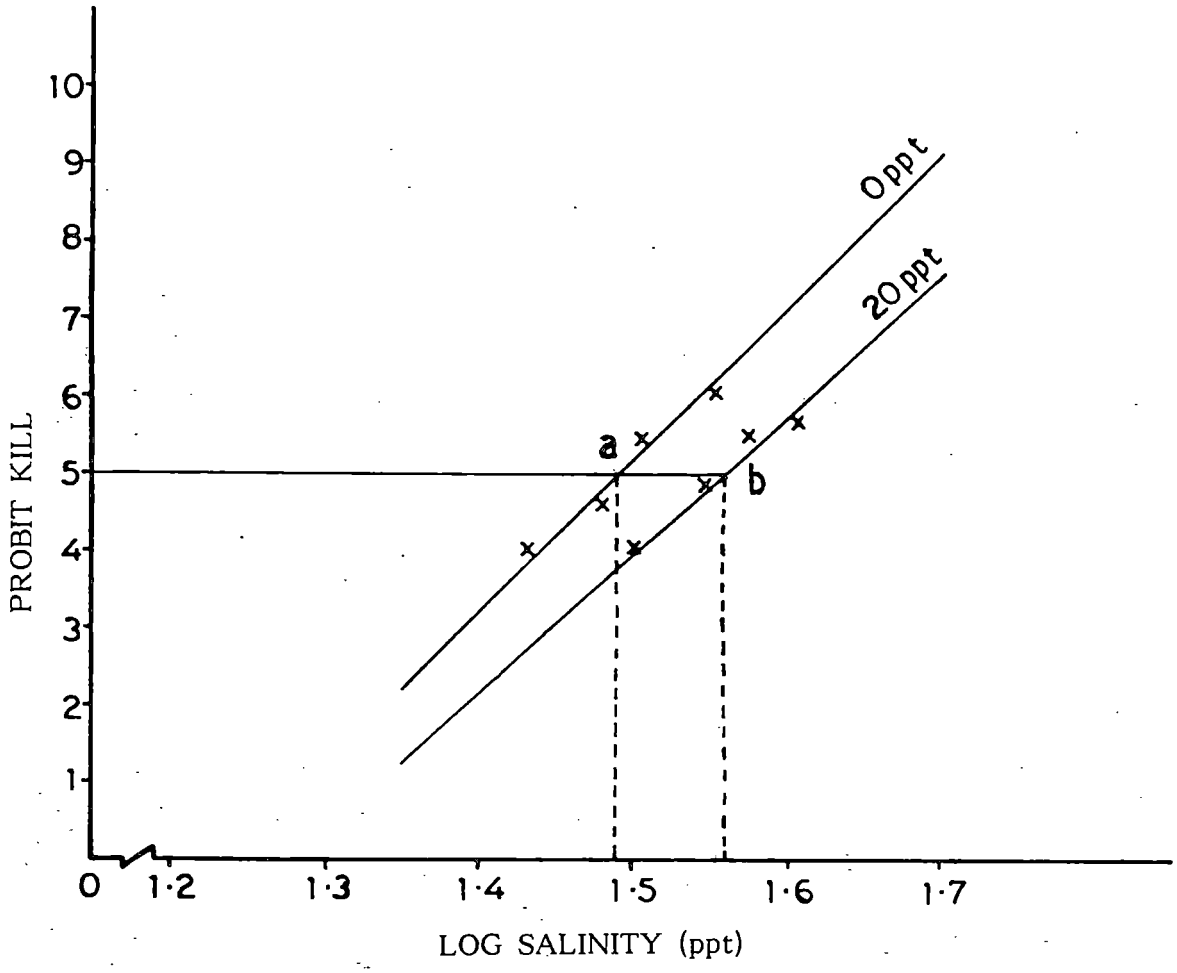


Fig. 5. Probit line for M. idella juveniles abruptly transferred from 0 and 20 ppt to different salinity levels for an exposure period of 96 Hr.

a:- LC_{50} = 30.90 ± 0.7107 ppt

b:- LC_{50} = 35.89 ± 0.7842 ppt

c;- LC_{50} = 40.74 ± 0.7402 ppt

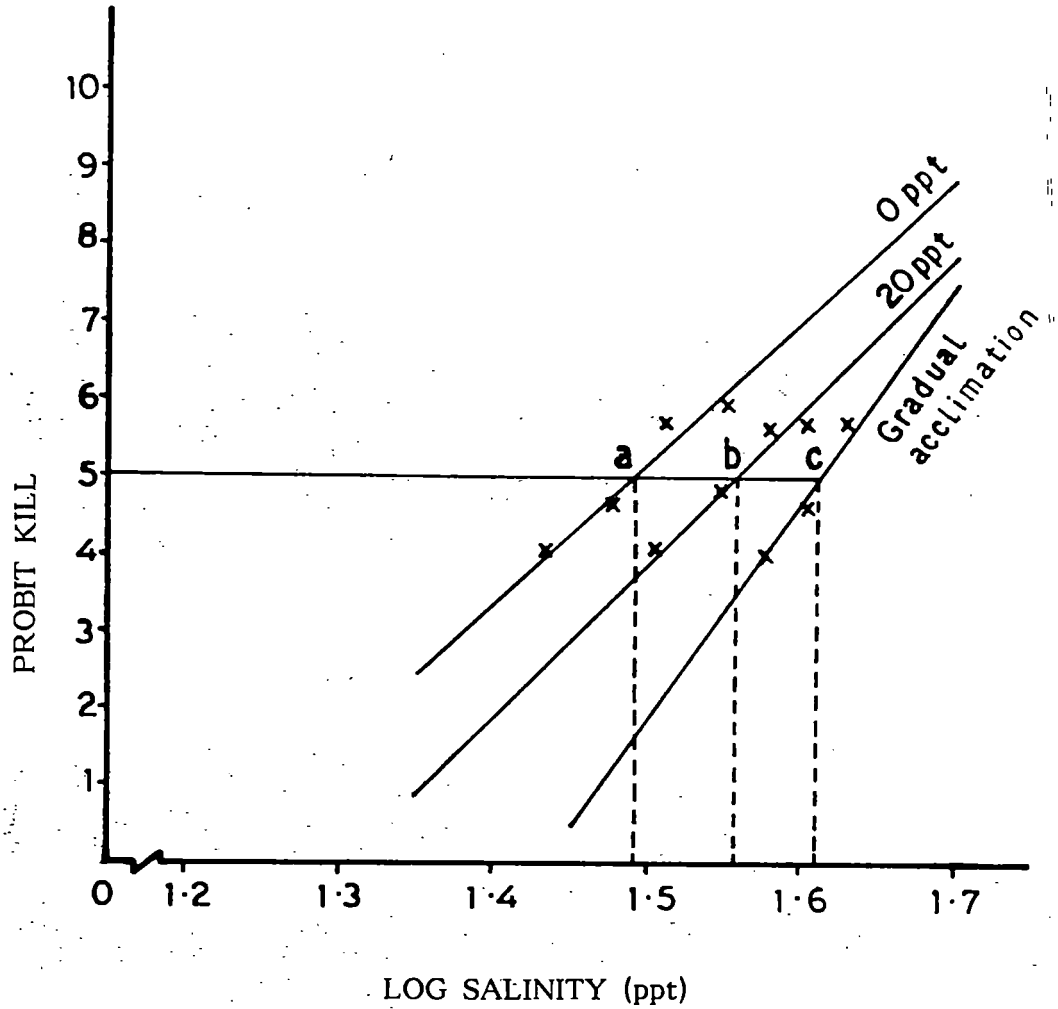


Fig. 6. Probit line for M. idella juveniles abruptly transferred from 0 and 20 ppt to different salinity levels and gradually acclimated from 0 to 45 ppt, for an exposure period of 120 Hr.

Table 7. The 48, 72, 96 and 120 Hr LC_{50} values of M. idella juveniles, acclimated in 0 and 20 ppt water and transferred abruptly to different test salinity levels and 120 Hr LC_{50} value of juveniles gradually acclimated to different higher test salinity levels.

Mode of transfer	Exposure period (Hrs)	Acclimation salinity (ppt)	Slope	LC_{50} (ppt) with standard error	95% confidence limits (ppt)	
					Lower limit	Upper limit
Abrupt transfer	48	0	30.50	32.73 ± 0.5947	31.5640	33.8960
		20	18.75	37.15 ± 0.9741	35.2410	39.0590
	72	0	19.75	31.98 ± 0.7725	30.4749	33.5031
		20	19.75	35.89 ± 1.1392	33.6594	38.1250
	96	0	17.25	30.90 ± 0.8102	29.3120	32.4880
		20	18.50	35.89 ± 0.8667	34.1913	37.5887
	120	0	20.00	30.90 ± 0.7107	29.5070	32.2930
		20	21.50	35.89 ± 0.7842	34.3530	37.4270
Gradual acclimation	120	0-45	30.00	40.74 ± 0.7402	39.2892	42.1908

acclimated at 20 ppt. Since the prawns acclimated at 20 ppt exhibited no mortality at 0, 2.5, 5, 7.5, 10 and 15 ppt within a period of 120 Hr, it can be concluded that this prawn can well withstand direct transfer from water of 20 ppt to 0 ppt.

The probit lines and LC_{50} values calculated for 48, 72, 96 and 120 Hr periods at different test salinity levels in the case of prawns directly transferred from 0 and 20 ppt and LC_{50} value for 120 Hr period for the gradually acclimated prawns together with the slope, standard error and 95% confidence limits are given in figures 3, 4, 5, 6 and table 7. The 120 Hr LC_{50} values are 30.90 $\pm$ 0.7107 for the prawns acclimated at 0 ppt, 35.89 $\pm$ 0.7842 ppt for the prawns acclimated at 20 ppt and 40.74 $\pm$ 0.7402 ppt for the prawns gradually acclimated from 0 ppt to 45 ppt water. This data indicate that by acclimating at a higher salinity of 20 ppt, M. idella can tolerate up to 35.89 $\pm$ 0.7842 ppt compared to 0 ppt acclimated prawns, which can tolerate only 30.90 $\pm$ 0.7107 ppt, by abrupt transfer. It was also found that by gradual acclimation this prawn can tolerate up to 40.74 $\pm$ 0.7402 ppt.

4.2 Study to Evaluate the Effect of Salinity on Growth

The experiment to evaluate the effect of salinity on the growth of the prawn M. idella has been conducted by growing it in cement cisterns at salinities ranging from 0 to 25 ppt. Details regarding the stocking density, average initial weight, average final weight and percentage of survival are given in table 9. Percentage specific growth rate for 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 days growing period at different

Table 8. Water quality parameters of experimental cement cisterns during the growth study.

Salinity (ppt)	pH		Dissolved Oxygen (ppm)		Temperature (°C)		Total alkalinity (ppm)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
0	7.56-8.32	7.98	4.80-6.45	5.71	26.0-29.5	27.66	41.5-59.0	53.7
5	7.87-8.45	8.12	5.25-6.40	5.80	26.0-29.0	26.91	51.2-61.8	58.3
10	7.93-8.39	8.21	4.85-6.80	6.35	26.5-29.0	27.13	42.0-55.6	48.7
15	7.60-8.32	7.97	5.30-6.25	5.65	26.5-29.0	27.86	47.8-58.2	52.6
20	8.09-8.48	8.29	5.15-6.25	5.45	26.5-29.0	27.30	57.0-59.3	58.8
25	8.05-8.32	8.18	4.80-5.85	5.40	26.5-29.0	28.01	52.3-58.9	56.9

Table 9. Details of stocking density, average initial and final weight and percentage survival of M. idella reared in cement cisterns at different salinities.

Salinity (ppt)	Number stocked per tank	*Total initial weight (g)	*Average initial weight of one prawn (g)	*Total final weight (g)	*Average final weight of one prawn (g)	Average increment in growth (g)	*Percentage survival
0	10	11.645	1.165	16.155	1.702	0.537	95
5	10	12.425	1.243	16.590	1.739	0.498	95
10	10	12.765	1.277	16.890	1.689	0.412	100
15	10	12.215	1.222	13.270	1.475	0.253	90
20	10	12.985	1.299	12.115	1.422	0.123	85
25	10	12.820	1.282	11.358	1.358	0.076	85

*Each value is an average of two replicates.

salinity levels is given in table 10. The water quality parameters of experimental cement cisterns are given in table 8.

A one way ANOVA (table 11) of percentage specific growth rate of prawns for different periods, in various test salinities, shows that there exists significant difference ($P < 0.05$) among growth rates at different salinities. Duncan's multiple range test is used for pairwise comparison (table 12) and it is inferred that among the different salinity levels tested, there exists no significant difference ($P > 0.05$) between growth rates from 0 to 10 ppt. A uniform growth rate is achieved in 0, 5 and 10 ppt. The growth rates in 0, 5 and 10 ppt salinity levels are found to be statistically significant from 15, 20 and 25 ppt. The growth rates recorded in 20 and 25 ppt are very low.

After the period of experiment, prawns reared at 25 ppt salinity were found to be infected by a sporozoan parasite causing bulging of branchial region of the carapace (Fig.7). The parasites were abundant in the gills and mortality of prawns was observed frequently. In some cases, even the gill lamellae were found to be eroded. Later this parasite also infected the prawns at 20 and 15 ppt; probably through the handnet used for collecting the prawns.

4.3 Study to Evaluate the Effect of Salinity on Fecundity

The experiment to evaluate the effect of salinity on the fecundity of M. idella has been conducted by keeping the females and males in cement cisterns containing water of varying salinities (0, 6, 12 and 18 ppt) for a

Table 10. Percentage specific growth rate of M. idella at different salinity levels for different periods (average value of two replicates).

Growing period (days)	Salinity (ppt)					
	0	5	10	15	20	25
7	0.5407	1.2593	1.5457	0.9536	0.0136	0.3750
14	0.9700	0.9168	0.8872	0.4850	0.2179	0.3172
21	1.1131	0.6238	0.7819	0.3393	0.3038	0.2660
28	0.7600	0.5807	0.5607	0.3156	0.0497	0.0775
35	0.8385	0.4949	0.6272	0.3791	0.2501	0.2144
42	0.6594	0.5082	0.4957	0.2632	0.1396	0.1199
49	0.6104	0.4663	0.4716	0.1743	0.0362	0.0693
56	0.5481	0.4876	0.3484	0.2577	0.0604	0.0104
63	0.5860	0.4704	0.4106	0.2690	0.0745	0.0553
70	0.5425	0.4765	0.3954	0.2661	0.1294	0.0582

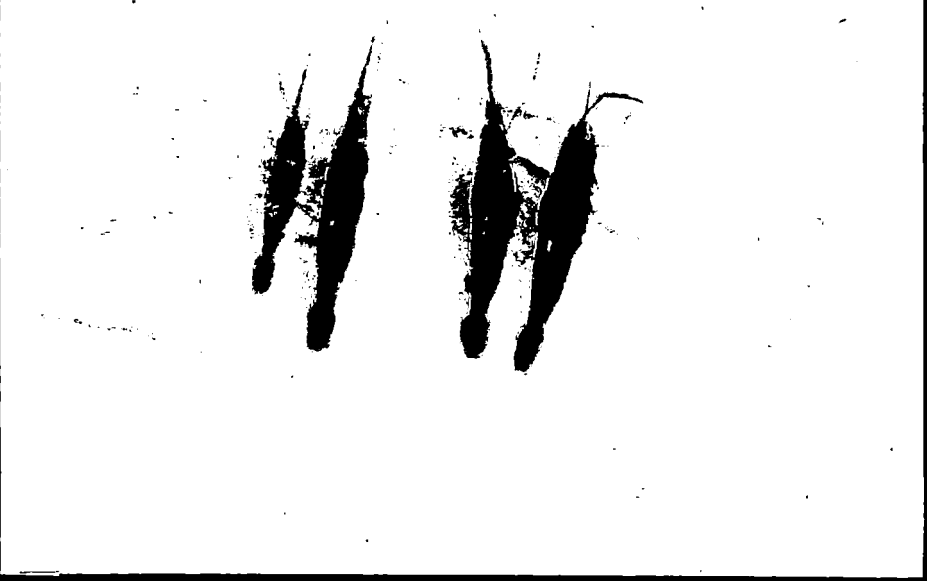
Table 11. Analysis of variance (CRD with equal replication) of percentage specific growth rate at different salinities given in table 10.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F Value	
				Computed	Tabular (5% level)
Treatment	3.4025	5	0.6805	13.1371	2.368
Error	2.7980	54	0.0518		
Total	6.2005	59			

Table 12. Pairwise comparison of percentage specific growth rate at different salinity treatments by Duncan's multiple range test.

Salinity (ppt)	Mean percentage specific growth rate	DMRT*
0	0.7169	a
5	0.6285	a
10	0.6524	a
15	0.3703	b
20	0.1275	c
25	0.1563	bc

*Any two means having a common letter are not significantly different at the 5% level of significance.



Macrobrachium idella sub-adults (two on the right side) showing characteristic bulging of the branchial region caused by sporozoan infection, in comparison with the two on the left side without infection.

period of 30 days, after 40 days of initial acclimation. The results of the study are presented in figures 8, 9, 10 and 11. A summary of the water quality parameters is given in table 13. At 0, 6 and 12 ppt, ovarian development, breeding, egg development and hatching were found to be normal. At 18 ppt, no berried females were obtained, whereas from 0, 6 and 12 ppt, out of 20 females each stocked; 9, 12 and 10 berried females respectively were obtained over a 30 days period of observation.

Later, to find out the exact salinity at which breeding is inhibited, 10 females and 3 males were reared at 15, 18 and 21 ppt. After 40 days of acclimation, observations were made for 30 days as done in the earlier experiment. During this period 2 berried females were obtained from 15 ppt and only one from 18 ppt. No berried female was obtained from 21 ppt. The results of this experiment, along with that of the earlier experiment indicate that salinities above 18 ppt may inhibit reproduction.

The fecundity values recorded for the prawns of the size 48-60 mm and 965-2338 mg at 0, 6 and 12 ppt were 672-2180, 799-3738 and 463-1924 respectively. The log fecundity values corresponding to the log wet weight of brooders in each salinity were transformed into regression line and are given in Fig. 8. The correlation coefficient (r) obtained for fecundity values at 0, 6 and 12 ppt were 0.7584, 0.7605 and 0.9158 respectively, the linearity of all of which are found to be significant at 5% level. Hence in each salinity there exists an exponential relationship between the fecundity and wet weight of prawns. The regression lines thus obtained were then compared on a multiple basis (table 14) and it is found that there exists no significant difference ($P > 0.05$) between the fecundity at 0, 6 and 12 ppt salinities.

Table 13. Water quality parameters of experimental cement cisterns recorded during the fecundity study.

Salinity (ppt)	pH		Dissolved oxygen (ppm)		Water temperature (^{o}C)		Total alkalinity (ppm)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
0	7.97-8.49	8.13	4.80-6.45	5.65	25.5-28.5	27.3	49.6-79.3	69.7
6	7.63-8.37	8.23	4.65-6.85	5.80	25.5-28.0	27.6	52.9-78.1	72.1
12	7.79-8.53	8.47	5.20-7.00	6.20	25.5-27.5	26.8	59.3-76.4	69.1
18	7.61-8.12	7.90	4.40-6.90	5.99	25.5-28.5	27.1	55.3-78.6	68.5

Similarly, regression lines (Fig.9) fitted for log fecundity against log length of brooders were also found to be linear (r values are 0.7483, 0.6388 and 0.8338 for 0, 6 and 12 ppt respectively). Multiple comparison of these regression lines (table 15) shows that there exists no significant difference ($P > 0.05$). So it is inferred that salinity has no significant effect on fecundity at 0, 6 and 12 ppt. However, a slight reduction in the number of eggs can be observed at 0 and 12 ppt salinities compared to that of 6 ppt.

Apart from fecundity, the influence of salinity on brood weight was also investigated. The brood weight values recorded for the prawns of the size 48-60 mm and 965-2338 mg at 0, 6 and 12 ppt were 74-215 mg, 88-416 mg and 90-201 mg respectively. Here also there exists an exponential relationship between brood weight and wet weight of prawns at 0, 6 and 12 ppt (Fig.10), the r values computed were 0.7833, 0.8207 and 0.9225 for 0, 6 and 12 ppt respectively. Multiple comparison of these regression lines (table 16) shows that at different salinities tested, there exists no significant difference ($P > 0.05$) in brood weight.

Similarly regression lines (Fig.11) fitted for log brood weight of prawns against log length of prawns were also found linear. (r values obtained were 0.8631, 0.7759 and 0.8539 for 0, 6 and 12 ppt salinity levels respectively). Multiple comparison of these regression lines (table 17) shows that there exists no significant difference ($P > 0.05$) in brood weight at different salinities. A slight increase in brood weight is however evident at 6 ppt when compared to those at 0 and 12 ppt.

The mean egg area of M. idella at 0, 6 and 12 ppt is given in table 18. A one way ANOVA (table 19) was carried out for testing the

0 ppt : Y = -1.2478 + 1.3586 x ; n = 9 ; r = 0.7584

6 ppt : Y = -1.9604 + 1.6176 x ; n = 12 ; r = 0.7605

12 ppt : Y = -1.8629 + 1.5327 x ; n = 10 ; r = 0.9158

o :- Fecundity values at 0 ppt

× :- Fecundity values at 6 ppt

▽ :- Fecundity values at 12 ppt

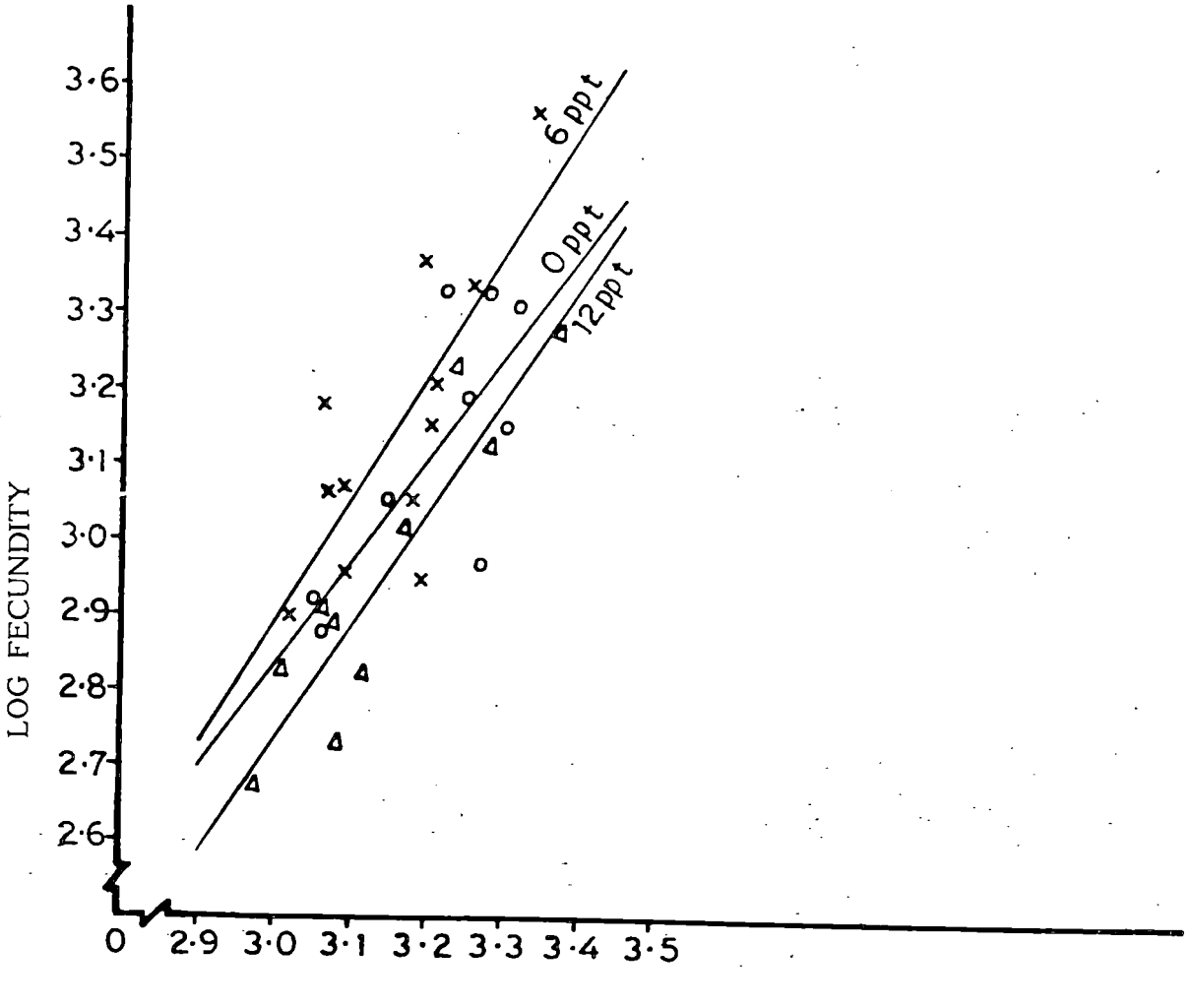


Fig. 8. Regression lines fitted for log fecundity against log wet weight of M. idella at 0, 6 and 12 ppt salinity levels.

Table 14. Comparison of slopes of the regression lines (Fig.8) fitted for log fecundity against log wet weight of M. idella at 0, 6 and 12 ppt salinity levels.

	Salinity (ppt)	X^2	XY	Y^2	Residual sum of squares	Degrees of freedom
Regression	0	0.0863	0.1174	0.2753	0.1156	7
	6	0.0982	0.1572	0.4353	0.1837	10
	12	0.1389	0.2093	0.3804	0.0650	8
Pooled regression					0.3643	25
Common regression		0.3234	0.4839	1.091	0.3670	

Comparison of slopes : $$F = \frac{(0.3670-0.3643)/2}{0.3643/25}$$

$$= 0.0959^*$$

* Not significant at 5% level

0 ppt : Y = -8.5464 + 6.6906 x ; n = 9 ; r = 0.7483

6 ppt : Y = -4.7452 + 4.5513 x ; n = 12; r = 0.6388

12 ppt : Y = -6.6629 + 5.6040 x ; n = 10 ; r = 0.8338

o:- Fecundity values at 0 ppt

x:- Fecundity values at 6 ppt

▽:- Fecundity values at 12 ppt

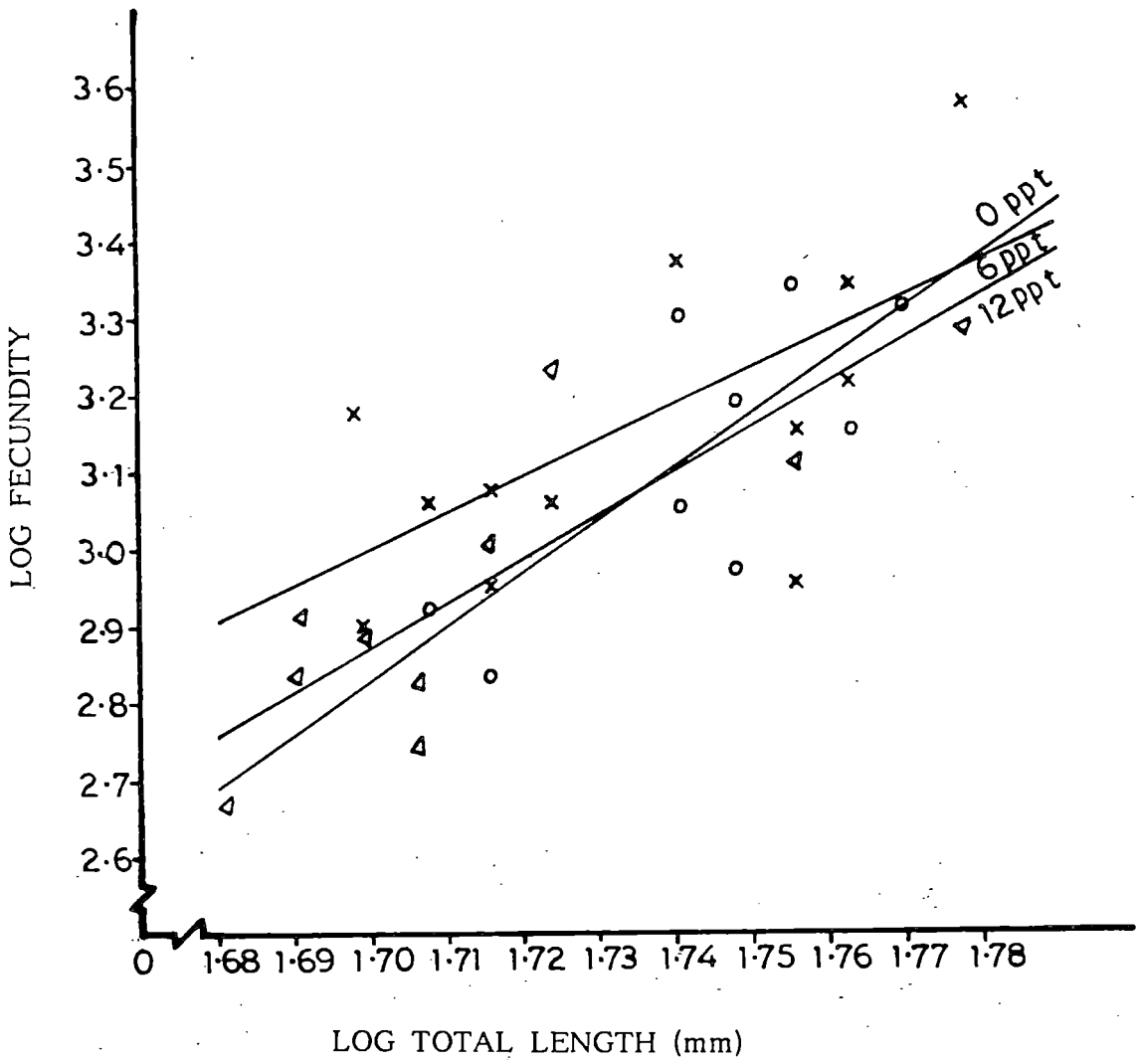


Fig. 9. Regression lines fitted for log fecundity against log total length of M. idella at 0, 6 and 12 ppt salinity levels.

Table 15. Comparison of slopes of the regression lines (Fig.9) fitted for log fecundity against log length of M. idella at 0, 6 and 12 ppt salinity levels.

	Salinity (ppt)	X^2	XY	Y^2	Residual sum of squares	Degrees of freedom
Regression	0	0.0048	0.0248	0.2753	0.1472	7
	6	0.0096	0.0404	0.4353	0.2653	10
	12	0.0092	0.0475	0.3804	0.1352	8
Pooled regression					0.5477	25
Common regression		0.0236	0.1127	1.091	0.5528	

Comparison of slopes :

$$F = \frac{(0.5528-0.5477)/2}{0.5477/25}$$

$$= 0.1187^*$$

*Not significant at 5% level.

0 ppt : Y = -1.6764 + 1.1723 x ; n = 9; r = 0.7833

6 ppt : Y = -2.6079 + 1.5413 x ; n = 12 ; r = 0.8207

12 ppt : Y = -0.7794 + 0.9127 x ; n = 10; r = 0.9225

o :- Fecundity values at 0 ppt

x :- Fecundity values at 6 ppt

▽ :- Fecundity values at 12 ppt

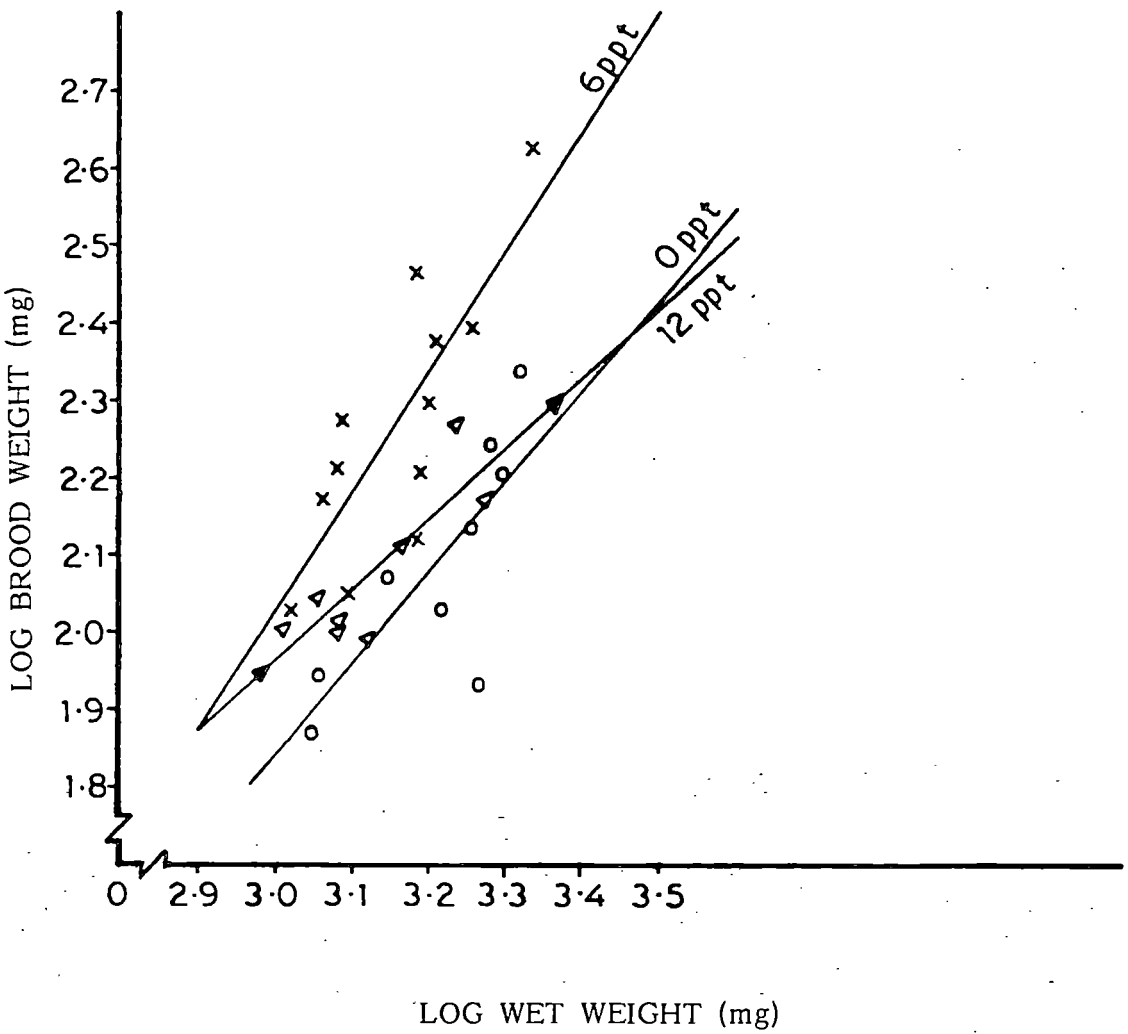


Fig. 10. Regression lines fitted for log brood weight against log wet weight of M. idella at 0, 6 and 12 ppt salinity levels.

Table 16. Comparison of slopes of the regression lines (Fig.10) fitted for log brood weight against log wet weight of M. idella at 0, 6 and 12 ppt salinity levels.

	Salinity (ppt)	X^2	XY	Y^2	Residual sum of squares	Degrees of freedom
Regression	0	0.0863	0.1001	0.1903	0.0742	7
	6	0.0982	0.1480	0.3369	0.1138	10
	12	0.1389	0.1257	0.1340	0.0202	8
Pooled regression					0.2082	25
Common regression		0.3234	0.3738	0.6612	0.2291	

Comparison of slopes : $F = \frac{(0.2291-0.2082)/2}{0.2082/2}$

$= 1.2651^*$

*Not significant at 5% level.

0 ppt : Y = -9.1479 + 6.4463 x ; n = 9 ; r = 0.8631

6 ppt : Y = -6.2055 + 4.8807 x ; n = 12 ; r = 0.7759

12 ppt : Y = -3.7326 + 3.3924 x ; n = 10 ; r = 0.8539

o:- Fecundity values at 0 ppt

×:- Fecundity values at 6 ppt

▽:- Fecundity values at 12 ppt

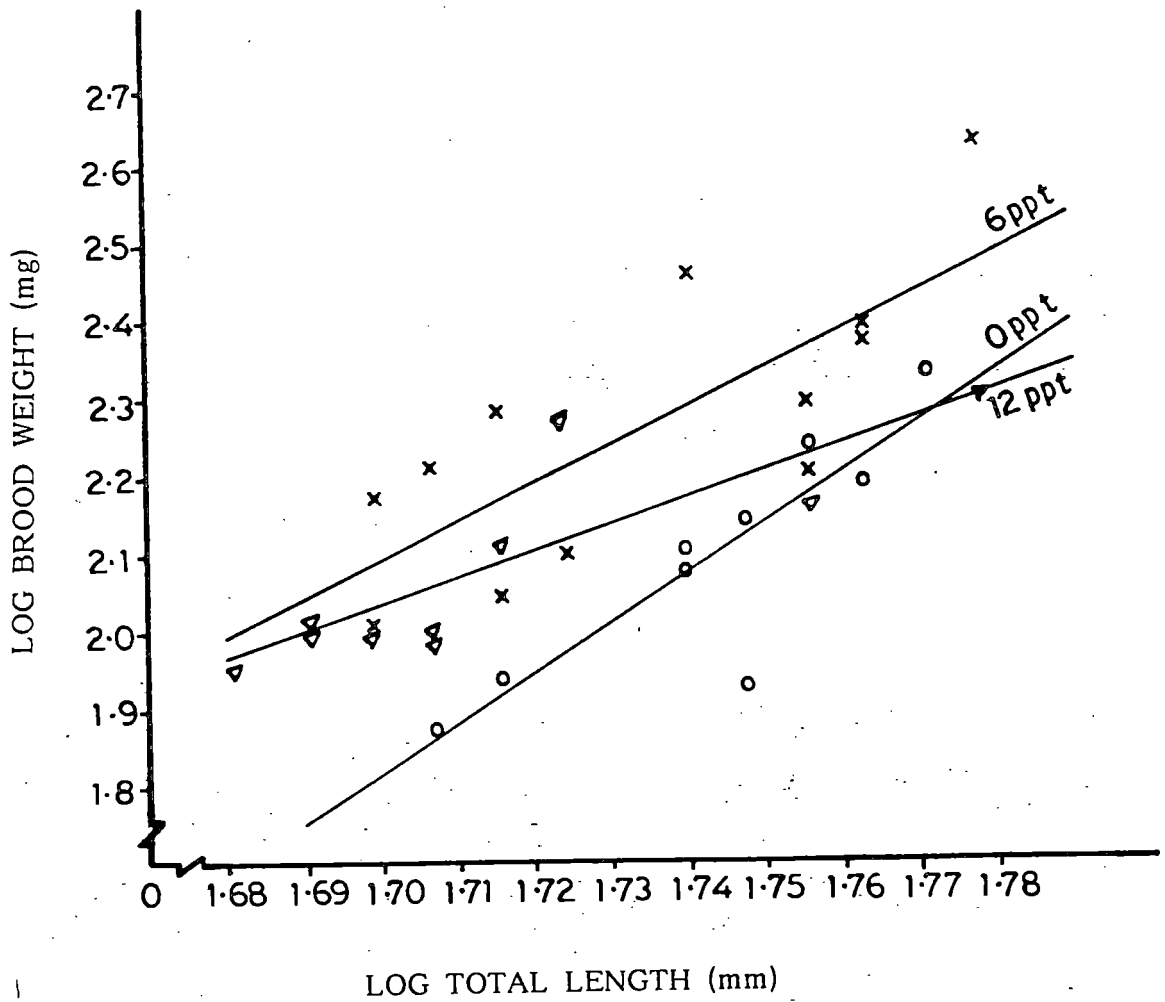


Fig. 11. Regression lines fitted for log brood weight against log total length of M. idella at 0, 6 and 12 ppt salinity levels.

Table 17. Comparison of slopes of the regression lines (Fig.11) fitted for log brood weight against log length of M. idella at 0, 6 and 12 ppt salinity levels.

	Salinity (ppt)	X^2	XY	Y^2	Residual sum of squares	Degrees of freedom
Regression	0	0.0048	0.0228	0.1903	0.0820	7
	6	0.0096	0.0421	0.3369	0.1523	10
	12	0.0091	0.0293	0.1340	0.0397	8
Pooled regression					0.2740	25
Common regression		0.0235	0.0942	0.6612	0.2836	

Comparison of slopes : $F = \frac{(0.2836 - 0.2740)/2}{0.2740/25}$

$= 0.4364^*$

*Not significant at 5% level.

Table 18. Mean egg area (mm^2) of M. idella at 0,6 and 12 ppt salinity levels.

Salinity (ppt)		
0	6	12
0.5211	0.5977	0.7092
0.7597	0.6151	0.5587
0.7594	0.7142	0.5339
0.6056	0.6522	0.6290
0.4942	0.7570	0.7310
0.5815	0.6714	0.4748
0.5964	0.6035	0.6252
0.7809	0.6169	0.5903
0.5647	0.5689	0.6111
	0.5989	0.5730
	0.5703	
	0.5475	

Table 19. Analysis of variance (CRD with unequal replication) of mean egg area of M. idella at different salinities given in table 18.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F value	
				Computed	Tabular (5% level)
Treatment	0.0039	2	0.0020		
Error	0.1906	28	0.0068	34	19.496
Total	0.1945	30			

statistical significance and it is found that salinity has no significant influence on mean egg area.

4.4 Study to Evaluate the Effect of Salinity on Incubation Period, Batch Hatching and Ovarian Development

The study to evaluate the effect of salinity on incubation period, batch hatching and ovarian development was conducted in perspex tanks by keeping one berried female in each tank holding 5 L of water. The incubation period of eggs of M. idella at different salinities is given in table 20. Water quality parameters of the experimental tanks were; temperature, 26.0-28.5^{o}C; pH, 7.23-8.37 and dissolved oxygen, 6.45-9.00 ppm.

Eggs during the initial days of incubation were green in colour, and then changed to greyish black during the last days of incubation.

Among the different salinities tested, it was found that the hatching of eggs is inhibited at 24 ppt. There was embryonic development at 24 ppt, but hatching was inhibited and the eggs turned black and then dropped off.

Subsequently, this was tested in 20 and 22 ppt salinity levels to confirm the critical salinity level with regard to the development of eggs and hatching. Out of 4 berried females each incubated at 20 and 22 ppt, 3 females at 20 ppt hatched, while only one hatched at 22 ppt, indicating that hatching of eggs in M. idella is inhibited at a salinity of about 22 ppt.

The incubation period of eggs of M. idella recorded in this study is 14.18 $\pm$ 0.83 in 0 ppt, 14.54 $\pm$ 0.51 in 6 and 12 ppt and 14.54 $\pm$ 0.67 days in 18 ppt. A one way ANOVA of incubation period at different salinities (table 21) shows that there exists no significant difference ($P > 0.05$) among them.

Table 20. Period of incubation of eggs of M. idella at different salinities.

Salinity (ppt)	Number of gravid females	Range in incubation period (days)	Incubation period (days) (X ± S.D)
0	11	13-15	14.18 ± 0.83
6	11	14-15	14.54 ± 0.51
12	11	14-15	14.54 ± 0.51
18	11	13-15	14.54 ± 0.67
24	11	No hatching of eggs	-

Table 21. Analysis of variance (CRD with equal replication) of incubation period of eggs of M. idella at different salinities given in table 20.

Source	Sum of squares	Degrees of freedom	Mean sum of squares	F Value	
				Computed	Tabular (5% level)
Treatment	1.0909	3	0.3636	1.2251	8.527
Error	17.8182	40	0.4455		
Total	18.9091	43			

Observations indicate that the incidence of batch hatching is more in 0 ppt. Out of 11 prawns, which were incubated in each salinity level, the number of prawns in which batch hatching occurred was, 7 in 0 ppt, 2 in 6 ppt, 5 in 12 ppt and 6 in 18 ppt (table 22). Chi-square test (table 23) conducted to know the effect of salinity showed that a slight salinity level of 6 ppt reduces the incidence of batch hatching ($P < 0.05$). In this level, compared to 0 ppt, incidence of batch hatching was found to be significantly less.

The ovary of prawns, the eggs of which are incubated at 6, 12 and 18 ppt, were found developed at the time of hatching of eggs. At 0 ppt, among 11 prawns incubated, the ovary of only two prawns was found to have developed (table 22). Chi-square test (table 23) shows that rate of ovarian development is significantly more in 6 and 12 ppt ($P < 0.05$).

Mortality of eggs was observed due to infection during the incubation period. Infection was there only in eggs which were incubated in 0 ppt. Infection usually occured during the last days of incubation. In one case eggs hatched, but the larvae were all dead. In another case, eggs did not hatch and later the mother prawn was found dead. In the third case decaying of eggs was noticed. In all these cases, fungus growth on eggs was observed. Microbiological studies indicate that bacteria, fungi and yeast were associated with the infected eggs.

Table 22. Number of prawns in which batch hatching and ovarian development occurred.

Salinity (ppt)	Total No. of prawns	No. of prawns showing batch hatching	No. of prawns showing ovarian development
0	11	7	2
6	11	2	9
12	11	5	7
18	11	6	6

Table 23. Results of the Chi-square test conducted to evaluate the effect of salinity on batch hatching and ovarian development.

Pairs	Batch hatching		Ovarian development	
	Value of Chi-square		Value of Chi-square	
	Computed	Tabular (5% level)	Computed	Tabular (5% level)
Between 0 and 6 ppt	4.700	3.841	8.910	3.841
0 and 12 ppt	0.733	3.841	4.701	3.841
0 and 18 ppt	0.188	3.841	3.140	3.841
6 and 12 ppt	1.886	3.841	0.917	3.841
6 and 18 ppt	3.140	3.841	1.886	3.841
12 and 18 ppt	0.182	3.841	0.188	3.841

V. DISCUSSION

V DISCUSSION

5.1 Effect of Salinity on Survival

Results of the present study on the effect of abrupt transfer to higher and lower salinity levels, than to which the prawns had been acclimated earlier, indicate that Macrobrachium idella is an euryhaline prawn capable of withstanding very wide variations of salinity in the environment. M. idella being a typical palaemonid prawn showing breeding migration to brackishwater areas, such a capability is a must.

Various other species of palaemonid prawns such as Palaemon affinis (Kirkpatrick and Jones, 1985), P. elegans (Panikkar, 1941; Hernandez and Taylor, 1985), P. intermedius (Dobkin and Manning, 1964), P. macrodactylus (Born, 1968), P. ritteri (Reynolds, 1975), P. serratus (Panikkar, 1941; Parry, 1954; Spaargaren, 1972), Palaemonetes pugio (Knowlton and Kirby, 1984), P. varians (Panikkar, 1941; Parry, 1957; Potts and Parry, 1964; Hagerman and Uglow, 1983), P. vulgaris (Knowlton and Schoen, 1984), M. equidens (Denne, 1968) and M. rosenbergii (Sandifer et al., 1975; Venugopalan, 1988) are also known to have wide salinity tolerance. Of these, Palaemon macrodactylus can be considered as the most tolerant species capable of tolerating very wide range of 1-61.2 ppt (Born, 1968), followed by Palaemonetes pugio (Knowlton and Kirby, 1984), P. varians, (Panikkar, 1941; Parry, 1957; Potts and Parry, 1964; Hagerman and Uglow 1983) and P. vulgaris (Knowlton and Schoen, 1984) which tolerate salinities of the range of 1-55 ppt, 0.2-50 ppt and 0.8-51 ppt respectively. Brackishwater species such as M. equidens (Denne, 1968), Palaemon elegans (Panikkar,

1941; Hernandez and Taylor, 1985) and P. ritteri (Reynolds, 1975) could tolerate salinities of the range of 2-40, 5-45 and 10-47.5 ppt respectively, whereas Palaemon affinis (Kirkpatrick and Jones, 1985) P. intermedius (Dobkin and Manning, 1964) and P. serratus (Panikkar, *1941*; Parry, 1954; Spaargaren, 1972) could tolerate only narrow ranges of the order of 16-39, 15-39 and 16-39 ppt respectively. The freshwater species, M. rosenbergii could tolerate only a narrow range of 0-28.18 ppt (Venugopalan, 1988).

In the case of M. idella, which can also be considered as a freshwater species, the range is much wider (0-37.5 ppt) and in this respect it can be compared to that of M. equidens, which could tolerate a range of 2-40 ppt.

Normally, most species of freshwater palaemonids undertake breeding migration from freshwater to brackishwater. So such salinities normally should not be expected to cause much difficulty to tolerate. In the case of M. idella also there was neither mortality nor stress up to a salinity of 25 ppt, even when transferred abruptly from 0 ppt, although such an abrupt change in salinity is not likely to occur in nature.

The results of the present experiment do amply testify the advantage of acclimation at a higher salinity, in tolerating much higher salinity levels, as when prawns were subjected to abrupt transfer to higher salinities, in those prawns acclimated at 0 ppt, mortality was noticed at a salinity of 27.5 ppt, whereas in those acclimated at 20 ppt, mortality was noticed only at a higher salinity of 32.5 ppt. In M. rosenbergii, Sandifer et al. (1975) observed such a shift in tolerable upper salinity level when acclimated at a higher salinity level. They found that compared to

post-larvae acclimated at 20 ppt for 9 days, those at 25 ppt for the same period, showed reduced mortality when transferred abruptly to 35 ppt.

Results of the experiment of slow acclimation, show that by acclimating gradually, this prawn could tolerate a salinity level of 37.5 ppt. This shift in the tolerable salinity level from 27.5 ppt (abruptly transferred from 0 ppt) to 32.5 ppt (abruptly transferred from 20 ppt) and then to 37.5 ppt by way of slow and steady acclimation at different levels, show that by giving sufficient time for the prawns to adapt itself by acquiring the capabilities (by structural and physiological modification), they can very well tolerate even salinities higher than seawater. In nature, prawns like M. idella which undertake breeding migration over a long period, would normally get adequate time to adapt themselves structurally and physiologically to the different salinity levels of the water en route.

The fact that the 120 Hr LC_{50} value shifted from 30.90 $\pm$ 0.7107 ppt for the prawns acclimated at 0 ppt to 35.89 $\pm$ 0.7842 ppt for the prawns acclimated at 20 ppt and then to 40.74 $\pm$ 0.7402 ppt for the prawns gradually acclimated from 0 ppt to 45 ppt (vide table 7), strongly supports the finding that in this prawn acclimation has got advantage in tolerating higher salinity levels. This is further supported by the observation of the shift in the salinity level at which 100% mortality of prawns occured, from 40 ppt (abruptly transferred from 0 ppt) to 42.5 ppt (abruptly transferred from 20 ppt) and then to 45 ppt in the case of prawns gradually acclimated stepwise at different salinity levels. In M. rosenbergii, Sandifer et al. (1975) found that, when post-larvae were transferred abruptly from larval rearing water of 16 ppt to 35 ppt, there was 100% mortality within 2 days

(mortality started 6 Hr after transfer) and there was *no mortality* within 4 days in the same level of 35 ppt, when they were acclimated gradually by increasing the salinity at a rate of 2.5 ppt/day. Commending on this, they stated that although post-larvae of M. rosenbergii are able to regulate their blood concentration regardless of external salinity upto a range of about 27-30 ppt and then cannot do so as the blood concentration begins to rise rapidly with the medium, they are able to withstand higher salinities (35 ppt) by gradual acclimation.

From the results of the present study, wherein M. idella was subjected to varying salinities from 0 to 45 ppt, it can be seen that they probably resort to hyper-regulation at lower salinities and hypo-regulation at higher salinity levels, as is the case with most of the palaemonids. Denne (1968) observed that M. australiense, a primarily freshwater species regulate hyper-osmotically at all salinities from freshwater to about 25 ppt, its upper salinity limit, by maintaining a nearly constant internal concentration to the external salinities from freshwater to about 17.5 ppt, and by increasing the blood concentration, with that of the external salinity, at higher salinities. In the case of M. equidens, which is a brackishwater species, Denne (1968) reported that the osmoregulation is somewhat different, it maintaining a constant blood osmotic condition from about 7 to 30 ppt salinity, by hyper-osmotic regulation at salinities below 18 ppt and hypo-osmotic regulation at higher levels. But at salinities < 7 and > 30 ppt, the blood osmotic concentration decreased or increased in response to that of the medium. According to Sandifer et al. (1975) the haemolymph of M. rosenbergii is hyper-osmotic below 18 ppt and

hypo-osmotic above that salinity up to 27-30 ppt, after that the blood concentration quickly conforms to that of the medium. The pattern of adaptation in M. idella seems to be similar to that of M. rosenbergii.

Kirkpatrick and Jones (1985) stated that all brackishwater and marine species of palaemonid prawns studied to date are hyper-hypo-regulators. According to Gilles (1975), Kirschner (1979) and Evans (1980), euryhaline crustaceans withstanding changes in environmental salinity behave as hyper-regulators or hyper-hypo-regulators, none of them having the capability of maintaining a blood osmolality constant at its original level. This general pattern of osmoregulation is stated to be typical of many brackishwater animals by Beadle (1943), Schoffeniels and Gilles (1970) and Vernberg and Vernberg (1972) and according to Hagerman (1971), it is a common feature of species living in habitats which experience extreme salinity flunctations. Kinne (1963a&b) considered this type of osmoregulation as the most advanced form of genetic adaptation to osmotic change possessed by invertebrates. Ability to regulate under both hypo-and hyper-osmotic conditions, according to Verwey (1957), has obvious advantages, as it produces relatively constant conditions with in the animal and minimises the possibility of cellular damage.

In the present experiment on M. idella, stress sign was observed from 27.5 ppt onwards for the prawns acclimated at 0 ppt and 32.5 ppt onwards for the prawns acclimated at 20 ppt and those acclimated gradually. This condition was also observed in M. rosenbergii by Sandifer et al. (1975), when abruptly transferred to higher salinity levels. The presence of whitish

opaque areas in the muscle has been attributed to various stress factors including adverse salinity conditions by Rigdon and Baxter (1970), Venkataramaiah (1971) and Lightner (1977). Although M. idella is a freshwater prawn, according to Pillai and Mohamed (1973) and Lakshmi et al. (1982), its larval development requires an optimum salinity of 15 ppt. McNamara et al. (1986) stated that such dependence to salinity during larval development of many species of palaemonids seems to indicate that they are still in the process of invading freshwater. This observation along with the present finding of wide salinity tolerance of M. idella indicate that this prawn along with many others, is in the process of colonization of freshwater. The observation of Ortmann (1902), Hedgpeth (1957) and Dobkin (1969) that the prawns of the family Palaemonidae as relatively recent in the process of emigration to freshwater seems to be correct.

5.2 Effect of Salinity on Growth

The results of the experiment to study the effect of salinity on growth indicate that the rate of growth obtained for prawns reared in 0, 5 and 10 ppt, is almost uniform and in 15 ppt growth is lower. The rate of growth is significantly very low in 20 and 25 ppt. This observation is in agreement with the observations made in M. rosenbergii by Wickins (1972b), Goodwin and Hanson (1975), Perdue and Nakamura (1976) and Venugopalan (1988) who obtained best growth in slight brackishwater. However, Popper and Davidson (1982) obtained best growth for this species in 10-15 ppt in a laboratory experiment with salinities ranging from 0-25 ppt.

From the results of the present study it can be seen that growth is restricted to a narrow salinity range of 0-10 ppt, although, by acclimation, it could survive in salinities up to 37.5 ppt. This is in conformity with the observation of Kinne (1971), who stated that in most of the euryhaline invertebrates growth is restricted to significantly narrower salinity ranges than is survival.

In the present experiment the water temperature in rearing cisterns was 26.0-29.5^{o}C. These levels were found to be within the optimum range for the growth of Macrobrachium rosenbergii (Uno et al., 1975; Armstrong, 1978; Farmanfarmaian and Moore, 1978; New and Singholka, 1982). But according to Natividad (1982), M. rosenbergii in Philippine rivers prefers thriving at a temperature range of 23.5-25.5^{o}C. pH values recorded were within the range of 7.56-8.48. Cripps and Nakamura (1979), Sarver et al. (1979 & 1982), Malecha et al. (1980) and Sandifer and Smith (1985) observed that high pH values are not favourable for the growth of M. rosenbergii. However, Natividad (1982) reported that in Philippine rivers, M. rosenbergii prefers a wide range of pH (4-8.5). Total alkalinity levels in cement cisterns were not above the dangerous levels (>180 ppm) as reported by Sandifer and Smith (1985). The dissolved oxygen values were also within the range required for growth as reported by Smith et al. (1976, 1978 & 1981), and Subrahmanyam (1987).

In the present experiment, the prawns reared in 25 ppt were infected by a sporozoan parasite. Gradually the infection was found to spread to prawns reared in 20 and 15 ppt salinity water. However, there

was no such infection in the prawns grown at 0, 5 and 10 ppt. It is possible that exposure at higher salinities might have made the prawns weak and susceptible to sporozoan attack. From this, it can be inferred that these salinities are not ideal for growth and has caused stress conditions. Infection by sporozoan parasites was also reported by Reeve (1969a) in Palaemon serratus and Schulte (1976) in Leander squilla in laboratory cultures. But they have not related this occurrence to any stress condition on the part of the prawn.

In tank rearing experiments, susceptibility of newly moulted prawns to attack from their companions has been noted by Reeve (1969a), Meixner, (1969), Forster (1970), Smith and Sandifer (1975) and Schulte (1976). But in the present study, cannibalism of prawns occurred very rarely, probably because of low stocking density, supply of sufficient quantity of food and presence of suitable substratum.

5.3 Effect of Salinity on Fecundity

The results of the experiment to evaluate the effect of salinity on fecundity variation show that fecundity is not affected much by salinity. The fecundity values of M. idella recorded at 0, 6 and 12 ppt are 672-2180, 799-3738 and 463-1924 respectively. Brood weight of this prawn recorded at 0, 6 and 12 ppt are 74-215 mg, 88-416 mg and 90-201 mg respectively. Statistical tests conducted show that salinity has no significant effect on fecundity and brood weight. Since in 0, 5 and 10 ppt, this prawn exhibits almost a uniform growth rate, any difference in fecundity and brood weight cannot be normally expected

at the above salinity levels. However, a slight increase in fecundity and brood weight was observed at 6 ppt. This is reasonable in prawns such as M. idella which undertake breeding migration to slight brackishwater areas for facilitating the larval development. In the experiment to study the effect of salinity on incubation period, batch hatching and ovarian development, it was observed that rate of ovarian development is more at 6 ppt. This observation also indicates that a slight salinity is best for reproduction.

No berried females were obtained from 18 ppt salinity onwards. Since growth is inhibited at 15 ppt, salinity of 18 ppt may not be quite congenial for ovarian development, although occassionally ovarian development and mating were observed upto a salinity of 24 ppt. However, spawning has never been observed in salinities above 18 ppt and in the salinity of 18 ppt also, it was observed only in one case. Resorption of ovary has been observed in M. idella in such unfavourable higher salinity levels. Such resorption of ovary under unfavourable conditions is reported in prawns to conserve energy. From incubation studies of M. idella, it is known that hatching of eggs is interrupted at salinities of about 22 ppt. So salinities above 18 ppt may not be congenial for development and hatching of eggs. This may be the reason for resorption of ovary in higher salinities. In various other crustaceans also, resorption of ovary was reported by Adiyodi and Subramoniam (1983).

In the present study of M. idella there exists a logarithmic linear relationship between fecundity and body size of the mother shrimp.

Similar relationship was also reported by Rajyalakshmi (1961) and Wickins and Beard (1974) in M. rosenbergii, Hoglund (1943) in Palaemon elegans (Leander squilla) and Emmerson (1985) in P. pacificus. Direct linear relationship between fecundity and body size was reported in M. rosenbergii by Goorah and Parameswaran (1983) and in M. heterochirus by Ching and Velez (1985).

Fecundity of M. idella reared in tanks in the laboratory, in the present study is 463-3738, which is much less when compared to that recorded from nature. In nature according to Natraj (1947), the fecundity is 2,000-20,000 and according to Jayachandran (1984) it is 6089-29,773. Lack of favourable environmental factors in rearing tanks may be the reason for this reduced fecundity. Schulte (1976) also reported reduction in fecundity in laboratory reared Leander squilla compared to that collected from nature.

In the present experiment, no difference could be seen in prawns reared at 0, 6 and 12 ppt regarding the size of eggs. But in M. nipponense, Mashiko (1983 & 1984) observed that estuarine population produces many smaller eggs and fresh water population produces few but larger eggs. Boas (1898) also observed such a difference in eggs of estuarine and freshwater populations of Palaemonetes varians.

The size of eggs does not show any relationship with the size of the mother shrimp in the case of M. idella. It is almost uniform being 0.4748-0.7809 mm^2 (mean egg area). Contrary to this observation, Goorah and Parameswaran (1983) reported that the eggs of

M. rosenbergii tended to become larger with increase in size of the mother. But Shakunthala (1977a) observed a reduction in size of eggs with increase in length of M. lamarrei.

The water quality parameters of the rearing cisterns in the experiment on the effect of salinity on fecundity, are almost within the range as suggested by Pinheiro (1983), who reported that intense reproductive activity in M. acanthurus occurs at a temperature range of 21-27^{o}C and high dissolved oxygen content. Lee and Fielder (1982b) observed that egg production is optimum at 25^{o}C whereas blocking of egg production occurs at 15^{o}C in case of M. australiense. But Dugan et al. (1975) reported that by dropping water temperature to 24^{o}C, spawning was retarded in Macrobrachium until the temperature was raised to 27.5^{o}C.

5.4 Effect of Salinity on Incubation Period Batch Hatching and Ovarian Development

Results of the experiment on the study to evaluate the effect of salinity on incubation period, batch hatching and ovarian development indicate that salinity has no significant effect on incubation period of eggs of M. idella and hatching of eggs is inhibited at a salinity of about 22 ppt. But it could be seen that the incidence of batch hatching and ovarian development are significantly influenced by salinity. The present observation is in accordance with the observation by Ching and Velez (1985) who reported that incubation period of eggs of Macrobrachium heterochirus is not significantly affected by salinity.

The incubation period of M. idella observed in this study at different salinities is 13-15 days. This confirms the observation of Natraj (1947), who reported 14-16 days as the incubation period of the eggs of this species. In species such as M. acanthurus (Martinez-Silva, 1981), M. idae (Pandian and Katre, 1972) and M. malcolmsonii (Kewalramani, 1973) also the incubation period is of this duration. The slight variation in the incubation period noticed in M. idella in this experiment is the normal one and this can be seen to do nothing with regard to salinity.

Although salinity has no significant influence on incubation period of M. idella, such other physical factors like temperature may have some effect on incubation and hatching of eggs. For instance, higher temperature has been found to shorten the incubation period in Palaemon serratus by Phillips (1971), M. amazonicum by Guest (1979) and M. rosenbergii by Diaz (1987b). Diaz (1987b) noted that there exists difference in the response of zoeae of M. rosenbergii obtained by incubating the eggs at different temperatures such as 25, 29 and 31^{o}C; the larvae from eggs incubated at 25^{o}C during embryonic development showed tolerance to a broad range of temperature and salinity conditions than those incubated at 29 and 31^{o}C. Pandian and Katre (1972) have noted that in M. idae, greater agitation of eggs by ovigerous females enhances hatching of larvae earlier.

The process of casting of exuvia with developed eggs during moulting by berried females as reported by Balasundaram and Poyyamoli (1984) in M. nobilii was not observed in the present study in M. idella.

In the present study, mortality of eggs due to infection by bacteria and fungi was noted in prawns incubated at 0 ppt only and not at other salinities of 6, 12 and 18 ppt, perhaps suggesting that freshwater is not a congenial medium for the incubation of eggs. It may possibly be due to the presence of such pathogenic organisms in the freshwater rather than at higher salinities. Such infection by bacteria (Leucothrix sp.) in Palaemon serratus was reported by Anderson and Conroy (1968). Infection by fungi of the genera such as Aphanomyces and Achlya of the eggs of M. rosenbergii was reported by Bland (as cited in Sindermann, 1977). The fungus infection of eggs was also reported in atyiid shrimp, Alpheus saulcyi by Herrick (as cited in Unestam, 1973).

In the present study, incidence of batch hatching of eggs was found to be lower when incubated at 6 ppt compared to those incubated at 0, 12 and 18 ppt, suggesting that a slight salinity is congenial for incubation and development of eggs. The incidence of batch hatching in the present study was more at 0 ppt followed by 18 and 12 ppt. According to Balasundaram and Pandian (1981) batch hatching is related to unfavourable environment in M. nobilii.

Experiment on ovarian development clearly indicates that at 6 and 12 ppt water, the rate of ovarian development is more compared to 0 and 18 ppt. But among the berried females collected from freshwater regions in nature with developing eggs, in majority of the cases, the ovaries were found to be fully developed, suggesting that the soil salinity might have helped the ovarian development. In the case of M. rosenbergii, Rao

(1967) found that rise in temperature and salinity is helping in attaining the maturity.

The results of the present experiment indicate that fresh water is not an ideal medium for incubation, hatching and ovarian development of the freshwater prawn, M. idella. Various observations made in the case of other species of Macrobrachium from nature also substantiate this. For example, Bhimachar (1965) reported that M. rosenbergii migrates down to estuarine region and spawns in areas where salinity flunctuates between 6 and 17.5 ppt. John (1957), Johnson (1967), Raman (1967), George (1969) and Padilla (1982) have observed that ovigerous females of M. rosenbergii are available more in slight brackishwater areas than in freshwater areas. Ching and Velez (1985) also observed the abundance of ovigerous M. heterochirus in brackishwater areas. Sidthimunka and Bhukaswan (1982) reported that in Thailand, berried females of M. rosenbergii are found only in brackishwater areas and never in completely freshwater areas.

VI. SUMMARY

VI SUMMARY

1. The present study is intended to elucidate the effect of salinity on survival, growth, ovarian development, fecundity, incubation period and batch hatching of the freshwater prawn, Macrobrachium idella.

2. The study to evaluate the effect of salinity on survival was conducted in perspex tanks of 28x16x30 cm with 6 prawns/tank. Effect of both gradual acclimation and abrupt transfer to different test salinity levels was studied.

3. In gradual acclimation, wherein prawns were acclimated to the tank environment at 0 ppt for 5 days, and there after the salinity was gradually increased at a rate of 5 ppt/12 Hr up to 30 ppt and then at a rate of 2.5 ppt/12 Hr upto a salinity of 45 ppt, mortality was found to start at a salinity level of 37.5 ppt registering cent per cent mortality at 45 ppt.

4. Abrupt transfer experiment was split into two. In the first case, wherein the prawns were acclimated at 0 ppt for 5 days and then abruptly transferred to 5, 10, 15, 20, 25, 30, 32.5, 35 and 40 ppt levels, mortality had started at a salinity level of 27.5 ppt registering cent per cent mortality at 40 ppt.

5. In the second case of abrupt transfer wherein prawns acclimated at 20 ppt for 5 days were abruptly transferred to 0, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 32.5, 35, 37.5, 40 and 42.5 ppt levels, the mortality had started at a salinity level of 32.5 ppt registering cent per cent mortality at 42.5 ppt.

6. Probit analysis technique was used for computation of LC_{50} value. The 120 Hr LC_{50} value for the gradually acclimated prawns was 40.74±0.7402 ppt. The 48, 72, 96 and 120 Hr LC_{50} values recorded for the prawns abruptly transferred from 0 ppt were 32.73±0.5947, 31.98±0.7725, 30.90±0.8102 and 30.90±0.7107 ppt respectively, and for the prawns abruptly transferred from 20 ppt, the 48, 72, 96 and 120 Hr LC_{50} values were 37.15±0.9741, 35.89±1.1392, 35.89±0.8667 and 35.89±0.7842 ppt respectively.

7. The results show that gradual acclimation has got advantage in extending the tolerable salinity range (0-37.5 ppt) and that acclimation at a higher salinity level (20 ppt) increases the upper tolerable salinity level (32.5 ppt).

8. Study to evaluate the effect of salinity on growth was conducted in cement cisterns having 90 cm diameter and 60 cm height with 100L of water keeping 10 prawns. The salinity levels tested were 0, 5, 10, 15, 20 and 25 ppt. They were grown for a period of 70 days and fed daily with clam meat ad libitum.

9. A one way ANOVA of percentage specific growth rate of prawns at different salinities had shown that salinity has got significant effect on growth rate. The rate was uniform from 0 to 10 ppt, being lower at 15 ppt and significantly low at 20 and 25 ppt.

10. It was found that prawns grown at 25, 20 and 15 ppt were infected by a sporozoan parasite. Stress at higher salinities might have made the prawns weak and susceptible to infection.

11. Water quality parameters recorded in the experimental tanks were within the range required for growth.

12. Study to evaluate the effect of salinity on fecundity of M. idella was conducted in cement cisterns containing 200L of water. In each tank, 10 mature females and 3 males were stocked and fed daily with clam meat ad libitum.

13. The salinity levels tested were 0, 6, 12, 18 and 21 ppt. From 0, 6 and 12 ppt levels out of the 20 females stocked, 9, 12 and 10 berried females were obtained over a period of 30 days whereas, only one berried female was obtained from 18 ppt and none from 21 ppt, which indicates that breeding is inhibited at about 18 ppt and a salinity level of about 6 ppt seems to be ideal.

14. The fecundity values at 0, 6 and 12 ppt for the prawns of the size 48-60 mm and 965-2338 mg were 672-2180, 799-3738 and 463-1924 respectively. There exists an exponential relationship between fecundity and wet weight/length of berried females. Multiple comparison of regression lines fitted for fecundity against wet weight/length of brooders at different salinities, showed that salinity has no significant effect on fecundity although a slight increase in fecundity was noticed at 6 ppt.

15. The brood weight values at 0, 6 and 12 ppt salinity levels were 74-215 mg, 88-416 mg and 90-201 mg respectively for the prawns of the size 48-60 mm and 965-2338 mg. The relationship between brood weight and wet weight/length of berried females was also found to be exponential. Multiple comparison of regression lines fitted for brood weight against wet weight/length of brooders at different salinity levels showed that salinity has no significant effect on brood weight.

16. Salinity was found to have no significant effect on the mean egg area, the values of which were 0.4942-0.7809, 0.5475-0.7570 and 0.4748-0.7310 mm^2 at 0, 6 and 12 ppt respectively.

17. Study to evaluate the effect of salinity on incubation period, batch hatching and ovarian development was done in perspex tanks at salinities of 0, 6, 12, 18, 22 and 24 ppt. Development of eggs and hatching were normal in all salinities except at 22 and 24 ppt. Out of 4 berried females incubated at 22 ppt only one hatched normally and none at 24 ppt.

18. The incubation period at 0, 6, 12 and 18 ppt salinity were 14.18 ± 0.83, 14.54 ± 0.51, 14.54 ± 0.51 and 14.54 ± 0.67 days respectively, indicating that salinity has no significant effect on incubation period.

19. Out of 11 prawns incubated in each salinity level, the number of prawns in which batch hatching observed was 7, 2, 5 and 6 at 0, 6, 12 and 18 ppt respectively, which indicate that the rate of batch hatching is less at 6 ppt compared at 0, 12 and 18 ppt salinity levels.

20. The number of prawns in which ovarian development occured was 2 in 0 ppt, 9 in 6 ppt, 7 in 12 ppt and 6 in 18 ppt which showed that a slight salinity level of 6 and 12 ppt is congenial for ovarian development.

21. During incubation mortality of eggs due to infection by bacteria, fungi and yeast was observed only in case of eggs incubated at 0 ppt. The occurrence of mortality of eggs, batch hatching and the nature of ovarian development indicate that fresh water is not a congenial medium for incubation of eggs.

VII. REFERENCES

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EFFECT OF SALINITY ON SURVIVAL, GROWTH AND BREEDING OF THE SHRIMP

MACROBRACHIUM IDELLA (HILGENDORF)

By

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ABSTRACT OF A THESIS

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ABSTRACT

The present study has been undertaken with the major objective of elucidating the effect of salinity on survival, growth, ovarian development, fecundity, incubation period and batch hatching of the freshwater prawn Macrobrachium idella.

Mortality of juveniles of M. idella which were acclimated at 0 and 20 ppt and transferred abruptly to varying salinities, was found to start at 27.5 and 32.5 ppt respectively, whereas in those which were gradually acclimated from 0 to 45 ppt, it was found to start only at a higher salinity level of 37.5 ppt.

The 120 Hr LC_{50} values were 30.90±0.7107, 35.89±0.7842 and 40.74± 0.7402 ppt for the prawns acclimated at 0 and 20 ppt and for those gradually acclimated respectively. Thus it could be seen that this prawn is a highly euryhaline one and that acclimation at a higher salinity level and gradual acclimation have got advantage in extending the upper tolerance level.

Growth was found to be significantly influenced by salinity, being highest at 0-10 ppt, lower at 15 ppt and almost nil at 20 ppt and beyond. Sporozoan infection was noticed in the prawns reared at 15, 20 and 25 ppt.

Salinity was found to have no significant influence on fecundity, while ovarian development is being influenced by it. The rate of ovarian development was high at 6 and 12 ppt and low at 0 ppt. Incubation period, which takes about 13-15 days was not found to be influenced by salinity, while there was some effect on batch hatching, the rate of which was more at 0 ppt and less at 6 ppt. During incubation, mortality of eggs due to infection was observed only at 0 ppt. Thus, it could be seen that a salinity level of around 6 ppt is ideal for ovarian development, maximum fecundity and synchronous hatching.