STUDIES ON REPRODUCTIVE BIOLOGY AND MASS LARVAL REARING OF SELECTED SPECIES OF *MACROBRACHIUM*

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By

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CERTIFICATE

This is to certify that this thesis entitled "Studies on Reproductive Biology and Mass Larval Rearing of Selected Species of Macrobrachium" is an authentic record of work carried out by Sri. C. Mohanakumaran Nair under my supervision, in the Department of Industrial Fisheries, Cochin University of Science and Technology, in partial fulfilment of the requirements for the award of the Ph.D. degree of the Cochin University of Science and Technology and that no part thereof has been presented before for any other degree in any University.

Kochi - 16,

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DECLARATION

I, C. Mohanakumaran Nair, do hereby declare that the thesis entitled "STUDIES ON REPRODUCTIVE BIOLOGY AND MASS LARVAL REARING IN SELECTED SPECIES OF MACROBRACHIUM" is a genuine record of research work done by me under the supervision and guidance of Dr. M. Shahul Hameed, Professor and Head, Department of Industrial Fisheries, Cochin University of Science and Technology, and has not been previously formed the basis for the award of any degree, associateship, fellowship or other similar title of any University or Institution.

Kochi - 16,

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(C. MOHANAKUMARAN NAIR)

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(C. MOHANAKUMARAN NAIR)

CONTENTS

CHAPTER I

INTRODUCTION

I. INTRODUCTION

Freshwater prawns of the genus Macrobrachium are distributed throughout the tropical and subtropical regions of the world and more than 25 species are reported under this genus from Indian waters. Among them Macrobrachium rosenbergii (de Man), M.malcolmsonii (H.M. Edwards), M.choprai (Tiwari), M.idella (Helgendorf), M.rude (Heller), M.equidens (Dana), M.idae (Heller),M. scabriculum (Heller), M.mirabile (Kemp) and M.lamarrei (H.M. Edwards) are considered commercially important, since they support a sizeable fishery in one or other part of the country. However, the most important species among them from aquaculture point of view is the giant freshwater prawn M.rosenbergii, popularly known as Scampi. This species is indigenous to the whole of South and South-East Asia, together with Northern Australia and the Western Pacific islands.

The freshwater prawn Macrobrachium rosenbergii is considered unique for its size. This species is suitable for culture in most inland water bodies such as lakes, swamps, irrigation ditches, canals, ponds and dams. Besides the above habitats, the low saline estuarine areas can also be utilized for cultivating Macrobrachium rosenbergii. It is omnivorous in its feeding habit and hence fishes such as major carps, milk fish, pearl spot, mullets etc can also be cultured along with this species. M.rosenbergii attains a maximum length of 32cm with 450g weight establishing the fact that it is the

largest prawn available. It has got consumer preference as well as demand not only in the local market but also in other countries. The commercial scientific farming of Macrobrachium has become popular in several parts of the world and the global production of this species through culture was 27000 metric tonnes in 1990. Thailand (44%), Vietnam (32%) and Taiwan (17%) are the main producers. Apart from these three countries only Brazil, the Dominican Republic, Mexico, the French Overseas Territories and Puerto Rico currently produce more than 100 tonnes annually. India in 1991-92 has exported a total quantity of 1281 tonnes of headless and one tonne head on scampi, which is mostly contributed by the capture fisheries.

Macrobrachium in general require brackishwater conditions during the larval stages of their life cycle. They exhibit a migratory behaviour and juveniles migrate back to freshwater conditions. But some of them do not undergo migration and complete their life cycle in freshwater or in inland saline lakes. The larvae can survive in freshwater for 1 or 2 days, but saline water is essential for further survival and development. The prawn can surmount weirs and water falls and can even migrate short distances over land where the vegetation is moist.

Macrobrachium exhibit sexual dimorphism. Mature males are considerably longer than females and the second pair of walking legs are much larger and thicker. In the male, the genital pores are between the base of the fifth walking legs, while those of the female are at the base of the third pair of walking legs. The males have an appendix masculina in the second abdominal appendages. But the sex determination of the juveniles is very difficult. Mating takes place between hard-shelled males and soft-shelled (after pre-mating moulting) females. A gelatinous sperm mass is deposited near the gonopores between the walking legs of the female, and external fertilization occurs as the eggs are extruded within 24 hours after mating. Eggs are transferred to a ventral brood chamber formed by the long pleurae of the abdominal segments and the first four pairs of pleopods,and are held in position by a thin membrane between the pleurae. Vigorous aeration is provided to the eggs by the female prawn with the movement of its pleopods throughout the incubation period. Incubation usually requires a period of 18 days for M.rosenbergii and 14 days for M.equidens at 28 to 30°C. The ovaries can further ripen while the brood chamber still contains eggs.

In India scientific commercial farming of Macrobrachium is yet to get a start, the major constraint being the lack of seed. Seed collection from the natural sources alone may not be sufficient, and in such cases seed production in hatcheries is inevitable. This

necessitates knowledge of the reproductive biology of the species, technology development for brood stock and larval rearing, and the optimum conditions required for larval development to undertake large scale seed production.

The present study was taken up with the major objectives of developing a proper, simple and feasible technology for the larval rearing of Macrobrachium, with special emphasis on mass larval rearing suitable for commercial level operations.

CHAPTER II

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

2.1 DISTRIBUTION

The prawns of the genus Macrobrachium are the most important among the freshwater cultivable prawns. They are found in a variety of brackish water habitats, many having a wide geographical distribution (Holthuis, 1952 a). Among the cultivable species of Macrobrachium like M.rosenbergii, M.acanthurus, M.carcinus, M.ohione, M.lar, M.americanum, M.malcolmsonii, M.asperius, M.lanceifrons, M.vollenhoveni, M.rude, and M.tenellum, the largest and commercially the most important species is M.rosenbergii. This species, commonly known as gaint freshwater prawn is widely distributed in the Indo-Pacific region (Cowles, 1914; Chopra, 1939; Ling, 1962, 1969 a; Holthuis and Rosa, 1965; Johnson, 1967; George, 1969; Jayachandran and Joseph, 1989). Ling (1969a) has indicated this species to occur year-round in the lower stretches of most of the rivers where tidal influence is felt and extending even upto about 200 km from the coast, while Djajadiredja and Sachlan (1956) reported this species as economically important in the Indonesian Islands of Sumatra, java, Borneo, Celebes and the Lesser Sunda Islands. Ahmad (1957) mentioned this species to occur in estuaries of Bangladesh. The existence of a commercial fishery of M.rosenbergii has been reported by Johnson (1986) and Longhurst (1970). It has been introduced to

different parts of the world like Hawaii, Africa, the Caribean, Central and South America, Israel, Japan, Mauritius, Tahiti, Taiwan and the United Kingdom for research and culture purposes (New and Singholka, 1985; Sandifer and Smith, 1985).

It is reported to occur along both the coasts of India with the distribution on the West Coast extending from Indus Delta to Malabar coast and in the East Coast in deltaic Bengal (George, 1969). It supports a more or less good fishery in the back waters and Pampa river system of Kerala (Raman, 1967; Kurup, et al., 1989) and the Hooghly estuarine system in West Bengal (Rao, 1967). Jones (1967) reported a regular fishery for the species in the Konkan area, Kerala and the northern half of East Coast, while in other areas of the Indian coast, the fishery is reported to be occasional. Raman in 1967 quantified M. rosenbergii annual landing from Vembanad lake as 300 tonnes and Kurup et al., in 1992 as 39 tonnes.

Among the other commercially important species of Macrobrachium, M. americanum has got a restricted distribution, occurring in the Pacific coast of America between Mexico and Peru (Holthuis, 1952 b). M. carcinus and M. acanthurus are known to occur in the Atlantic coast of America (Holthuis, 1952 b). M. malcolmsonii (Patwardhan, 1958) and M. birmanicum choprai (Tiwari, 1947), the two freshwater prawns growing to the same size as that of M. americanum and M. carcinus are distributed in the Indian

sub-continent.

While medium sized species like M. rude are distributed in East Africa, Madagascar, India and Srilanka (Henderson and Matthai, 1910), M. equidens has its distribution from Madagascar to southern China (Holthuis, 1950). According to Kurien and Sebastian (1986), in India this species is present only in Kerala. But occurrence of the species has been reported from various parts of the country (Nobili, 1903); Tiwari and Pillai, 1973; Dutt and Ravindranath, 1974; Natarajan et al., 1979; Ravindranath, 1982), and in Netravathi - Gurpur estuaries M. equidens tops among the freshwater prawns of the genus Macrobrachium (Natarajan et al., 1979). Jayachandran and Joseph (1989) has reported M. equidens as a common species in most rivers and estuaries of Kerala. According to them it is abundant in the northern coastal regions from Korapuzha river northwards where it supports a good fishery. Kurup et al., (1992) has estimated the landing of M. equidens as 3.34 tonnes from Vembanad lake.

M. idella, another medium sized prawn often referred to as 'slender river prawn' (Holthuis, 1980) is widely distributed in East African countries, Madagascar, India, Indonesia and Malayan Archipelago (Henderson and Matthai, 1910). Jayachandran (1984) indicated this species as a commercially important palaemonid prawn of Kerala.

The two most important ones among the small sized freshwater prawns are M. lamarrei (Ahmad, 1957) and M. dayanum (Chopra and Tiwari, 1947), both of them having a very restricted distribution being present only in the Indian sub-continent.

2.2 SIZE AND GROWTH

Among the freshwater prawns, M. rosenbergii grows to the largest size (Patwardhan, 1958; Raman, 1967; Holthuis, 1980) and the maximum length according to Patwardhan (1937) is 3 feet (90cm) from tip of telson to tip of extended second leg. Holthuis (1980) has recorded a maximum size of 320 mm for male and 250 mm for female. Raman (1967) has also recorded a maximum size of 320 mm in the case of males. Jayachandran and Joseph (1982) has reported capture of male and female measuring 326 mm and 283 mm respectively from the Valapattanam river.

Other species of the genus Macrobrachium which attain comparatively larger 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, 1952 b). M. carcinus attains a maximum size of 233mm in the case of males and 170mm in the case of females (Holthuis, 1952 b). The males of M. malcolmsonii are known to reach a maximum size of

231mm and females 200mm (Holthuis, 1980). The maximum size recorded for M. rude, M. idella, M. equidens and M. lamarrei are 117mm, 111mm, (Henderson and Matthai, 1910), 98mm (Holthuis, 1950) and 69mm (Holthuis, 1980) respectively.

While in many species of Macrobrachium like 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, in the case of M. lamarrei females are larger than males (Koshy and Tiwari, 1975).

Rajyalakshmi (1962) has investigated the age and growth of M. rosenbergii occurring in the Hooghly estuary, and found that sexual dimorphism in growth is exhibited by this species, with the males attaining lengths of 107mm and 149mm at the end of first and second years of life while females 82.5mm, 103.5mm and 168.5mm at the end of first, second and third years respectively. Rao (1967) has reported that in the case of immature individuals having a total length above 30mm, males moult six times a year while females moult only five times, indicating better growth for males. Ling (1969a) on the other hand found the growth rates of young males and females to be the same, and after reaching a length of about 18cm and a weight of about 60 g, the growth rate of females decreases and there is little growth beyond 22cm in length and 120g in weight, but the males keep on growing to about 200g each. Sandifer and Smith (1985) found that females and males grow at the

same rate upto a mean size of about 17g, and slowing of the growth rate in the females occur at sizes above 25g. Growth 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, has been noted in M. rosenbergii (Wickins, 1972 b; Forster and Beard, 1974). Sandifer and Smith (1975) found that M. rosenbergii populations exhibit a positively skewed size distribution with 15-20% of the individuals showing a much higher growth rate compared with the rest of the population. Although the biological basis for the appearance of two size classes is not definitely known (Ra'anan and Cohen, 1984a), experimental evidence suggests that the size variation in M. rosenbergii is more attributable to interaction within the prawn group than to genetic differences between individual prawns (Ra'anan and Cohen, 1984b).

The growth of M. rosenbergii in various culture systems has been investigated by many workers, and these studies revealed that the rate of growth varies in relation to stocking density, intensity of culture operation and also to survival rates obtained. Willis and Berrigan (1977) reported a growth rate of 0.26g/day (43.25g/168 days at a stocking density of $5/m^2$). Ong and Pang (1982) obtained a growth rate of 0.32g/day (11.7cm and

57g/180 days at a stocking density of 5-10/m^2). Ling (1969b) recorded a growth rate of 0.62mm/day for this species. Eble (1979) obtained a growth rate of 0.14g/day in intensive culture system with net substrates at a high stocking density of 36-54/m^2. In laboratory tank culture experiments, Venugopalan (1988) obtained a growth rate of 5.53mg/day.

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

2.3 FOOD AND FEEDING

M. rosenbergii is reported to be omnivorous in its feeding habit (John, 1957; Johnson, 1967; Raman, 1967; Rao, 1967; George, 1969; Ling, 1969a). Examination of stomach contents shows that this species ingests a wide variety of food items, of both animal and plant origin and also detritus, thus functioning as a primary consumer, a secondary consumer and detritivorous in its natural habitat (John, 1957; Rao, 1967). Ling (1969a) reported that the common items of food include aquatic worms, aquatic insects and insect larvae, small molluscs and crustaceans, flesh and offal of

fish and other animals, grains, seeds, nuts, fruits, algae and tender leaves and stem of aquatic plants. Panikkar and Menon (1955) noted that the food of prawn consists of mud, sand and plenty of diatom along with detritus when they are abundant in the environment. John (1957) also observed that the prawn's diet depends on its environment. Qualitative and quantitative analysis of digestive enzymes have confirmed the carnivorous nature of M. rosenbergii (Lee et al., 1980).

Omnivorous feeding habit has also been observed in other palaemonid prawns like Crangon vulgaris (Lloyd and Yonge, 1947), Leander serratus (Forster, 1951), M. malcolmsonii (Ibrahim, 1962) M. americanum (Smitherman et al., 1974) and M. idella (Jayachandran, 1984). M. lanchesteri is known to be an algal feeder (Johnson, 1968).

Eating its own cast-off exoskeleton was reported in Palaemon idea (M. idella) by Natraj (1947) and in M. heterochirus by Ching and Velez (1985). Rao (1965) noted M. rosenbergii to eat their own moult and dead eggs. Cannibalistic nature is reported by many workers in M. rosenbergii (Rao, 1965; Ling, 1969a; Wickins, 1972b; Forster and Beard, 1974; Segal and Roe, 1975; Peebles, 1977) and P. idea (Natraj, 1947).

2.4 BREEDING BIOLOGY

2.4.1 Maturity size: The size at first maturity of M. rosenbergii has been investigated by many workers. To attain sexual maturity it takes two years in the rivers of West Bengal (Rajyalakshmi, 1961 & 1962) and in Kerala one year (Raman, 1967). According to Goorah and Parameswaran (1983) the smallest size of berried females in ponds of Mauritius is 118mm and 20g (5-7 months old). Rao (1967) recorded a mean size of 155mm as the maturity size in Hooghly estuary. According to Ibrahim (1962) the size at first maturity of M. malcolmsonii is 41mm, and according to Sankolli and Shenoy (1980), it is 40-50mm. Rajyalakshmi (1980) has reported that the size at first maturity of this species in river Godavari is smaller compared to that in Hooghly estuary. In M. acanthurus, sexual maturity is attained at a size of 40mm (Berber, 1984). Inyang (1984) recorded a size of 30mm as the maturity size of M. felicinum in Africa. The size at first maturity of M. tenellum in Mexico is 74mm (Arroya et al., 1982). According to Jayachandran (1984), the size at first maturity of M. idella is 41mm.

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. The breeding season of M. rosenbergii is different in various river systems. Rajyalakshmi (1961 & 1962) and Rao (1967) found this species to

have a restricted spawning season in the Hooghly estuary, extending from December to July with peak spawning during March to May. In Kerala, its breeding season is from August to December with a peak in October to November (Raman, 1967). Rao (1967) considers the rise in temperature and salinity during the October to November season as the physiological drive for attaining maturity and undertaking spawning migration. This species is reported to have almost year round breeding in Balgoda lake in Srilanka, with two peak spawning seasons, a major one from May to July and a minor one from November to January (Jinadasa, 1985). M. malcolmsonii in river Godavari has a prolonged breeding season extending from April to November with two spawning peaks, one in June and another during August to October (Ibrahim, 1962). According to Rajyalakshmi (1974), the breeding season of the species extends from May to October, with peak in July to September. Natraj (1947), has reported the breeding season of P. idae in Travancore area to be a prolonged one, beginning from September and extending to the end of February. This species, in Vellayani lake is reported to have a breeding season extending from September to January covering the north-east monsoon months (Jayachandran, 1984). Observations of breeding season coinciding with the rainy season have been made in cases of other species of Macrobrachium like M. amazonicum (Romero, 1982), M. tenellum (Arroya et al., 1982), and M. felicinum (Inyang, 1984). 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 in their natural habitats.

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). Hughes and Richard (1973) suggest that both upstream and downstream migrations would be of adaptive advantage to species with specific salinity requirements for reproduction and development. M. rosenbergii performs spawning migration from their original freshwater habitat to estuarine regions at the time when the eggs are ready to hatch, and spawn in areas where salinity fluctuates between 5 and 20 ppt (John, 1957; George, 1969). After metamorphosis, the young juveniles remain in brackish water areas for 1 or 2 weeks and then migrate to freshwater (John, 1957; Ling and Merican, 1961; Ling, 1969a, Natividad, 1982).

Due to the differences in the spawning periods in the Hooghly estuary and Kerala backwaters, the migration in and out of the estuarine areas occurs at different times of the year in these two places. While the migration of adults into backwaters in Kerala takes place at a time when the salinity is on the decrease, in the

Hooghly, the migration occurs when the salinity is on the increase in the winter and summer seasons. Jinadasa (1985) recorded that in Balgoda lake in Srilanka, the migration of adults to the lake occurs when the monsoon rains lower both the salinity and temperature of the lake. The return migration of adults into the rivers in Kerala occurs when the salinity in the backwaters increases while in Hooghly this occurs during the monsoon months when the salinity is on the decrease (Raman, 1967; Rao, 1967). According to Raman (1967), salinity alone is not the inducing factor for their return migration to the riverine habitats. Possibly, temperature may also be influential in effecting these movements.

Ibrahim (1962) and Rajyalakshmi (1974) reported that M. malcolmsonii in river Godavari, performs no migration for breeding towards the lower reaches of the river, but the larvae reach the estuarine zone of the river along with the flood environment. The first stage larvae of M. malcolmsonii has been found to thrive in freshwater for 15 days, unlike in the case of M. rosenbergii which can thrive in freshwater for only 5-6 days (Sankolli and Shenoy, 1980).

2.4.4 Sex Ratio: When the fishery starts in Pampa river system in May or June, males are more in number than the females. From August onwards females predominate and this state continues upto September to October (Raman, 1967). Males are dominant in

February and again in September, while females are dominating in the month of April to June and again from September to January. According to Rao (1967) during the peak spawning season, March to May, only berried females are found in the spawning grounds and the absence of mature males during this period at this region may be due to non-migration of mature males from their freshwater habitats during the spawning season.

2.4.5 Mating and Spawning: Mating and spawning in many species of Macrobrachium have been described by many workers. The mating behaviour of M. rosenbergii has been studied by Ling (1962, 1969a), Rao (1965) and Chow et al (1982). Mating usually occurs between a soft shelled female and hard shelled male. According to Ling (1962), after premating moult, the female prawn secretes a substance which strongly attracts the males. Release of sex pheromones by the females after the premating moult is also reported in the freshwater prawn, M. kistnensis (Sarojini et al., 1984) and such a possibility is suspected in the case of M. idella (Shyama, 1987). Rao (1965) observed the spawning behaviour of the species in the laboratory and observed that the courting behaviour is initiated only when a female which had just completed prespawning moult is available in the vicinity. A few hours before mating, the male holds the female between the widely extended second pair of pereopods and actively touches the female with its antennae and first pair of pereopods. Mashiko (1981) indicated that the long chelepeds of the male are effective apparatus for its reproductive success in two aspects, namely to

guard the soft shelled female immediately after prespawning moult from cannibalistic predators and to guard the female during mating from other male rivals. 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 premating moult are extremely active and this aids the female in seeking out a suitable male. 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). Extrusion of eggs takes place several hours after mating and generally within 24 hours after the premating moult (Rao, 1965; Ling, 1969a; Sandifer and Smith, 1979; 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). The eggs are held together by tuft like ovigerous setae developed for this purpose (Rao, 1986). Vigorous aeration is made by the female by the movement of the pleopods throughout the incubation period (Ling, 1969a). Unmated females would also 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 laso, the unfertilized eggs drop off within two days (Shyama, 1987).

Macrobrachium is found to be directly related to the size of the brood prawn. This is true in the case of species such as M. idae (Pandian and Katre, 1972), M. lamarrei (Koshy and Tiwari, 1975; Shakuntala, 1977), 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). In M. lamarrei, the total biomass of eggs per brood is found to be inversely proportional to the unit body weight of the female. Based on the number of eggs produced in an year Cabrera - Jimenez et al. (1979) divided various species of 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).

Intermittant spawning habit has been observed in many species of Macrobrachium such as M. rosenbergii (Ling, 1962), M. idella (Pillai and Mohammed, 1973), M. nobilii (Balasundaram and Pandian, 1982) and in M. felicinum (Inyang, 1984). However, studies regarding the variation in fecundity in each successive breeding in Macrobrachium are lacking.

2.4.7 Incubation: According to Ching and Velez (1985), the reason for the success of most decapod crustaceans may be the incubation of eggs by the females. Pearse and Gunter (1957) has pointed out that 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, 1971b), M. amazonicum (Guest, 1979), M. heterochirus (Ching and Velez, 1985) and M. malayanum (Samual et al., 1987) or orange as in M. carcinus (Lewis et al., 1966), M. ohione (Truesdale and Mermilloid, 1979) and M. rosenbergii (Ling, 1969a). For all these species of Macrobrachium, the eggs with embryo turn either to grey or dark brown to hatching.

The work of Cole (1958) revealed that the period of egg carriage is directly related to temperature. The incubation periods of eggs of various palaemonids are different and were reviewed by Ignatius (1989). The mother prawn is reported to be very sensitive to disturbances during the incubation period (Schone, 1961). The gravid females clean the eggs with their first pair of pereopods and aerate them by beating their pleopods (Shyama, 1987). When disturbed during the incubation process, the pleopod beating frequency decreases which might delay hatching (Balasundaram, 1980). Balasundaram and Pandian (1982) reported that delay in hatching affects the survival rate of larvae.

2.4.8 Larval Development: The duration of larval development among the different species of Macrobrachium shows very wide variation. Sollaud (1923), based on the number, size of eggs and early life history has described 3 types of larval development in the members of family Palaemonidae. 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.

The larval development of M. rosenbergii from Malaysian waters was described by Ling and Merican (1961) and Ling (1969a). Ling (1962) has described 12 larval stages for the species, but in a late study he grouped them into 8 stages (Ling, 1969a) and Uno and Kwon (1969) described 11 stages. Occurrence of many stages in the larval life has been noted in several other species of Macrobrachium also. It is reported that there are 10 larval stages for M. acanthurus (Choudhury, 1970, 1971b) and M. idella (Pillai and Mohammed, 1973), 12 for M. carcinus (Choudhury, 1971a) and 16 for M. malcolmsonii (Kevalramani et al., 1971). Seasonal variations in the number and duration of larval instars have been noted in Palaemon macrodactylus reared in laboratory (Little, 1969). All larval stages of M. rosenbergii are active swimmers and are planktonic in habit (Ling, 1969a). They usually swim at a slightly oblique angle with tail first and ventral side upwards. According to Ling (1969a) larval stages require brackish water of

salinity 12-18 ppt for optimum growth and survival, and larvae hatched in freshwater will die-off within 4-5 days, unless removed to brackish water. In nature, hatching may occur in both fresh end brackish water, but those that hatch in freshwater must be carried down to the estuaries within 4-5 days for survival (Ling, 1969a). In certain other species of palaemonids the settlement of the larvae to bottom has been noted, which is suggested as a mechanism to reduce downward displacement of the larvae to the sea. Thorne et al. (1979) observed that in nature, most larvae of M. novaehollandiae rest on the bottom for long periods losing their planktonic behaviour. Read (1983 & 1985) has also made such an observation in the case of M. petersi.

Among the environmental factors that influence larval development 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, 1969; Knowlton, 1974; Sick and Beaty, 1974) are the most important. Differences among parental population are also known to influence larval development (Reeve, 1969; Diaz, 1987; Provenzano et al., 1978). Variations in the duration of larval development have no significant influence on juvenile growth rate.

Thus according to Malecha (1977) and Sandifer and Smith (1979), in M. rosenbergii early metamorphosis of larvae to post larvae confers no advantage nor late metamorphosis any disadvantage, on growth.

2.5. LARVAL REARING

The larvae go through 8 (Ling, 1969a) to 11 (Uno and Soo, 1969) distinct stages in M. rosenbergii and 10 stages in M. equidens (Pillai, 1990), before metamorphosis to post-larvae. The time taken in the larval life varies according to the feeding and environmental conditions, particularly the temperature. Healthy well fed larvae maintained in optimum temperature (26-31°C), become post-larvae in about 16-18 days (New and Singholka, 1985). But larval periods as high as 35 days (Suharto et al., 1982) and 46 days (Adisukresno et al., 1982) for M. rosenbergii; 52 days for M. malcolmsonii (Rao, 1991) and 35 days for M. equidens (Pillai, 1990) are also reported.

Mainly two systems of larval culture are followed in the commercial hatcheries - Green water system and Clear water system (New, 1990). In the former, fertilization (4 parts urea: 1 part 15:15:15 NPK inorganic fertilizers) produces a bloom of phytoplankton (mostly Chlorella spp.) in water used for the larviculture (Adisukersno, 1982; Lee, 1982; Malecha, 1983). In the clearwater system larval rearing is carried out in clear water

free of phytoplankton (Fujimura, 1966; Chineah, 1982; Suharto, 1982; AQUACOP, 1983).

2.5.1 Stocking density and feeding

By stoking at 100/l and 200/l and feeding with brine shrimp nauplii and an artificial feed containing 44.2% protein, a production of 80 and 131 per litre was obtained by Suharto (1982). Lee (1982) by comparing the efficiency of green water and clear water systems has reported a production of 18.9 juveniles/litre in green water system and 9.5 juveniles/litre in clear water system. He also reported that the clear water system is more susceptible to filamentous bacterial infestation, and in the green water system the concentration of nitrite and ammonia is more. But Chineah (1982) reported a high yield of 50/l from the clear water system.

Food density is critically important and must be maintained regardless of the density of prawn larvae. However, the influence of food density decreases and larval population density increases as the larvae grow (Lin and Uno, 1987). An artificial (ie. off-the-shelf) diet has not been developed and marketed for Macrobrachium hatcheries as in the case of P. monodon or P. indicus (New, 1988). A combination of live feed and artificial diet prepared on-site has been reported by many workers. The feed and feeding regimes used in some Hawaiian and Thai hatcheries has

been reviewed by Malecha (1983), New and Singholka (1985), and New (1990). The principal live feed is Brine Shrimp nauplii (New, 1990). But the cladoceran Moina and the rotifer Brachionus are also reported as substitutes to Brine shrimp nauplii. After conducting experiments to study the comparative efficiency of Brine shrimp and cladocerans (Moina), Aniello and Singh (1982) reported a survival of 1.53% in artemia nauplii and 0.88% in Moina. But Alikunhi et al. (1980) reported a high survival of 90% by feeding with Moina. Brachionus was reported as a larval feed by Alikunhi et al. (1980), Reddy (1983) and Subrahmanyam (1984). But Lovett and Felder (1988) reported Brachionus neither as a substitute or supplement for artemia nauplii in feeding larvae of Macrobrachium rosenbergii. 'Dead' or prepared feed is also essential for successful larval rearing (New, 1988). Many feeds prepared from tuna flesh, frozen pollack (Malecha, 1983), egg custard (New and Singholka, 1985), Prawn meat (Alikunhi, 1980) Tubifex worm (Subrahmanyam, 1984) and Channa spp. (Suharto, 1982) are used.

But the success of Macrobrachium larval rearing without the use of live feed has been reported by Adisukresno (1982). A survival of 35% (10,300 PL in 1300 litre of water) was obtained by feeding with a compounded wet feed containing 22.8% protein, 4.5% fat, 49% carbohydrate, 3.3% fibre, 3.3% ash and 14.7% water. But when this feed was combined with one time feeding of artemia nauplii the survival went upto 70.8%.

Most of the coastal hatcheries use sea water mixed with well water or dechlorinated tap water. But many small or back-yard prawn hatcheries in Thailand are far from the sea and rely on transported sea-water (Singholka and Sukapunt, 1980). Yambol and Cruz (1986) evaluated the possibility of utilizing brine solution and sea salt in the larviculture of M. rosenbergii and reported that brine solution can economically be used for Macrobrachium larval rearing.

In M. malcolmsonii, an yield of 25 PL/1 was reported by Kanaujia et al. (1992) by feeding the larvae with Brine Shrimp and egg custard in a closed re-circulatory system. Similar success in the larval rearing of M. malcolmsonii was also reported by Rao (1991).

2.5.2 Water Quality

M. rosenbergii larvae are generally reared in 10-15 ppt. saline water (New, 1990). Nair (1977) reported 15 ppt as the optimum salinity for M. rosenbergii. Salinity tolerance of various palaemonid prawns was studied by many workers. A tolerance of 0-31.6 ppt was reported for M. rosenbergii by Venugopalan (1988) and 2-40 ppt for M. equidens by Danne (1968). A water temperature range of 26-31°C is reported as optimum for M. rosenbergii larvae. Temperature below 24°C or above 35°C causes retarded mortality (New, 1990). However Hsieh et al. (1989)

reported high survival in Taiwanese hatcheries at 30-33°C. A pH of 7.0 - 8.5 is considered optimum. Mass mortality occur at pH 9.5 (Hsieh et al., 1989). The maximum limit of nitrite (NO_2-N) and nitrate (NO_3-N) are 0.1 ppm and 20 ppm respectively (New, 1990). Armstrong et al. (1976) reported nitrite nitrogen (NO_2-N) at levels as low as 1.8 ppm to retard growth in M. rosenbergii larvae and an incipient LC_{50} value of 3 ppm NO_2-N. The same workers observed that at 10 ppm NH_3 (8.23 ppm TAN) level no inhibition of growth of larval M. rosenbergii occurred (Armstrong et al., 1978). The pH and to a lesser extent temperature, determine the proportion of highly toxic un-ionized ammonia present in the aquatic medium. For M. rosenbergii larvae, values for 24 hr and 144 hr LC_{50}s show that as pH increases, the tolerance of larvae to total ammonia levels decreases because of the increasing concentration of unionized ammonia. Armstrong et al. (1978) found reduced growth of the larvae at a total ammonia level of 32 ppm and pH 6.83.

2.5.3 Oxygen consumption and ammonia excretion

Oxygen consumption was reported as proportional to the body weight and a relationship of log O_2 = 1.54 + 0.904 log wt. was established by Stephenson and Knight (1980).

Ammonia excretion in Macrobrachium spp. has been studied by Wickins (1976), Nelson et al. (1977a&b), Anantharaman et al.

(1981, 1982) and Stern and Cohen (1982). Wickins (1976) found the nitrogen excretion rate of M. rosenbergii of size range 2-27g to be between 0.84-0.25 mg N/g/day. The influence of diets on the ammonia production of juvenile M. rosenbergii was studied by Nelson et al. (1977a), who reported no difference between the diets tested but related it to the body weight of the organism. In a subsequent study, Nelson et al. (1977b) found that irrespective of the diet, the majority of the nitrogen assimilated was excreted. Nelson et al. (1979) investigated the ammonia excretion of a benthic estuarine shrimp Crangon franciscorum and found the rates to be 7 times higher in fed shrimps than in starved ones. Anantharaman et al. (1982) found that the rate of ammonia excretion in M. lanchesteri is not related to the feeding rate.

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

Environmental salinity is reported to affect the nitrogen excretion in euryhaline crustaceans and Armstrong et al. (1981) linked this increase or decrease in response to salinity to the activity of the Na^+/NH_4^+ exchange pump in the gills. Sharma

(1966) reported an increase in nitrogen excretion in the freshwater cray fish, Orconectes rusticus after subjecting it to an increase in salinity. But in M. rosenbergii, Armstrong et al. (1981) found a decline in ammonia excretion rate in response to an increase in salinity. Stern et al. (1984) observed an increase in ammonia excretion in this species in higher salinities. Armstrong et al. (1981) observed that when M. rosenbergii was subjected to a hyperosmotic transfer from .0 ppt to 24 ppt salinity, the ammonia concentration in exposure water declined for the first 24 hours and then increased. Exogenous ammonia was absorbed by the animal. This absorption of exogenous ammonia during hyperosmotic shock has been confirmed by the use of $(NH_4)_2SO_4$ containing the stable isotope ^{15}N (Taylor et al., 1987).

2.6 NURSERY REARING:

Many farmers stock 1-4 week old post-larvae directly into rearing ponds while others use only intermediate, 1-3 month old seed after a nursery phase. Smith and Sandifer (1979) reported that the use of artificial habitats will increase the survival rate in nurseries. But contrary to this, Mulla and Rouse (1985) observed that feed is an important factor in improving the survival rate, the artificial habitat not having any role in the improvement of the survival rate.

2.7 BROOD STOCK:

In most hatcheries, berried females from the ponds are used than those from the wild (New, 1990). Separate prawn brood stocks are rarely maintained in hatcheries (Hsieh et al., 1989). But at the Brackishwater Aquaculture Development Centre (BADC), Jepara in Indonesia, brood tanks are maintained in which a sex ratio of 1 male to 3-5 females and a stocking density of 4 nos./m^2 are maintained (Adisukresno, 1982). Small females (40g) caught from ponds are being used in most of the hatcheries as brooders (New, 1990). But Malecha (1983) suggested that separate brood stock ponds should be kept, the sex ratio be maintained to one male to four or five females and that females should be reared to 100g to increase production of larvae per animal.

Methods of detection of mature females as early as seven days before the premating moult, based on the external examination of gonadal development and on the reproductive behaviour of dominant males have been developed by Sagi and Ra'anan (1985). These techniques help in broodstock management for studies on breeding, artificial insemination and genetics. Methods for increasing hatchability and spawning frequency through artificial incubation (Balasundaram and Pandian, 1981; Pandian and Balasundaram, 1982), artificial insemination (Sandifer and Smith, 1979; Chow, 1982) and spermatophore cryopreservation (Chow et al., 1985) have been developed. Unilateral eyestalk ablation has been shown to

significantly advance gonadal maturation and increase the number of moults, clutches and eggs of Macrobrachium nobilii (Kumari and Pandian, 1987).

2.8 CULTURE:

Studies on the culture of palaemonids, especially giant freshwater prawn M. rosenbergii, have been conducted in different parts of the world, particularly in South-East Asian Countries, U.S.A. and Israel. M. rosenbergii is the best species for aquaculture since it can be grown in both fresh and low saline waters, is compatible for polyculture, omnivorous and hardy, has the maximum growth potential among the cultured prawns, has no serious problem of diseases and has good consumer preference and demand in the local as well as export markets (Sebastian et al., 1993).

The major problems in successful Macrobrachium culture was 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, 1984a; Peebles, 1979; Sandifer and Smith, 1985; Rao et al., 1986). Generally Macrobrachium is cultured in earthern ponds, but in Thailand commercial pen-culture in lakes is also practiced (Kulkao et al., 1985). In 1985 there were over 38 ha of pens in cultivation in Thailand and the envisaged production was 1,075

kg/ha (New, 1988) whereas the average pond production was only 780 kg/ha in 1986 (Janssen, 1987). Several workers have reported varying degrees of growth, survival and production by culturing the prawn under mono and polyculture systems in earthern ponds.

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

Sagi et al. (1986) observed the production potential of prawns in monosex populations in cages under tropical conditions, and found that marketable sized prawns obtained from the all-male population were 80% higher than those produced by the mixed population. It was also observed that females when cultured alone yielded more than double the crop which was obtained from females cultured in presence of males. These observations clearly indicate the possibility of culturing prawns in monosex populations. But contraditory to this Cohen et al. (1988) reported a production 1640 kg/ha/140 days from all female population in pond culture and 2,041 kg/ha/140 days from the mixed population. But they also reported a high production of 2,200 kg/ha/140 days from all male population.

The low average weight, the wide size distribution and occurrence of undesired, unicellular and multicellular algal blooms associated with prawn stocked at high densities can be solved by resorting to polyculture (Cohen et al., 1983). They also reported that stocking of prawns at a low density of about 5000/ha in polyculture with fin fishes resulted in 90% of the prawns growing to market size (45g) within 130 days with a survival rate of 43-96% and yields of 96-312 kg/ha. Joseph (1978) demonstrated the commercial feasibility of polyculture of M. rosenbergii with carps like Labeo rohita, L. fimbriatus, Cirrhinus mrigala, Catla catla and Cyprinus carpio in Kerala. Guerrero and Guerrero (1977) by culturing M. rosenbergii with tilapia in

polyculture systems reported an increase in the total yield in polyculture systems compared to monoculture ones. In a study to evaluate the effect of culturing grass carp, silver carp, big head carp and Gambusia sp. on the production of M. rosenbergii, Tunsutapanich et al. (1982) obtained total yields of prawn ranging from 1000-1587.5 kg/ha with a survival rate of 56-64% in rearing periods of 6-8 months and 20 days. Results of polyculture of Macrobrachium with various species of fishes showed that the growth and survival of the fish and prawns are independent, with the prawns influenced only by their own stocking density and were not influenced by the species of fish co-stocked with them (Wohlfarth et al., 1985).

The growth rate of individual prawns is extremely variable and exhibits a bull-runt phenomenon. Removal of faster growing individuals permits the slower growing prawns to develop faster (New, 1990). This is the basis for continuous cull-harvesting technique employed in many farms with year round water availability. In this technique, large prawns are regularly harvested by seining and the ponds are rarely drained. Batch culture is practiced in places where continuous culture cannot be employed and water availability is seasonal (New, 1990). In batch culture all prawns are harvested when the ponds are drained.

Though many studies have been conducted to evaluate the potential of culture of the species M. rosenbergii, only a very few studies have been made to investigate the farming potentials of various other species of fresh water prawns. However, Johnson (1968) and Dobkin (1969) indicated that some of these prawns, 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 80-400kg/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 cultures conducted by Cano (1980) production of M. acanthurus was 275 kg/ha/2 months (1.1 tonne/ha/year). Guerrero and Villanuera (1978) conducted preliminary studies on the culture of M. idella in Philippines. Guerrero and Cagauan (1979) also reported the culture potential of M. idella.

Thus it could be seen that the production rates of most other species of Macrobrachium are low when compared to the production

rates of M. rosenbergii. However, high density culture of these species could be done as it is being practiced for small varieties of penaeid shrimps.

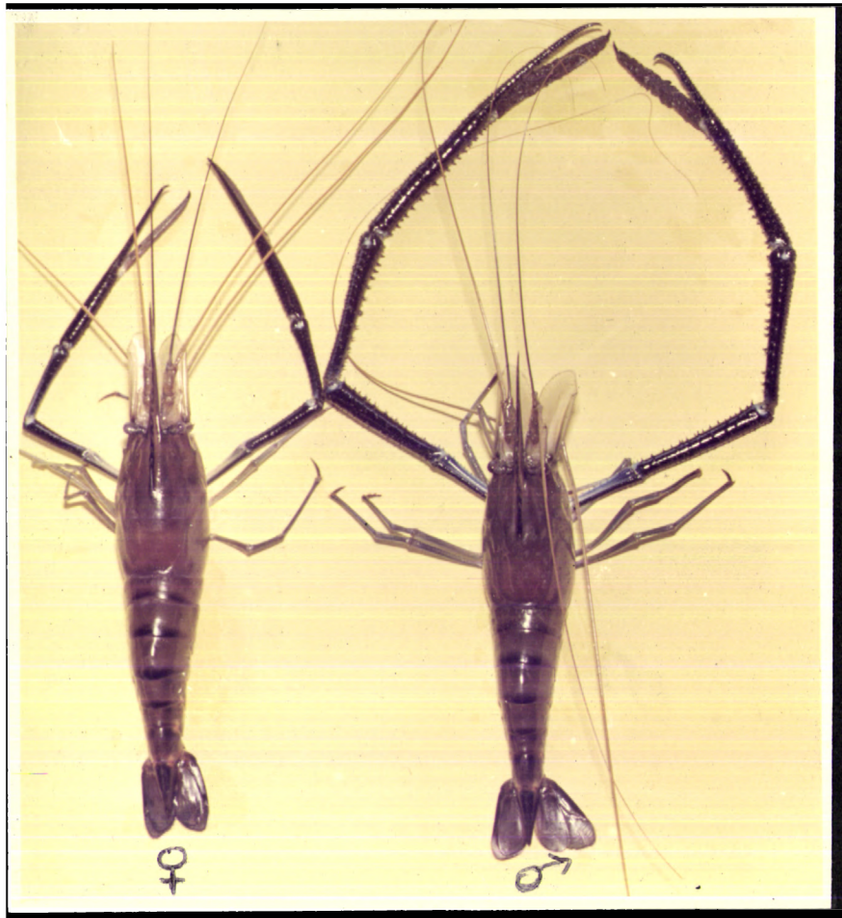

PLATE I M. ROSENBERGII - MALE & FEMALE

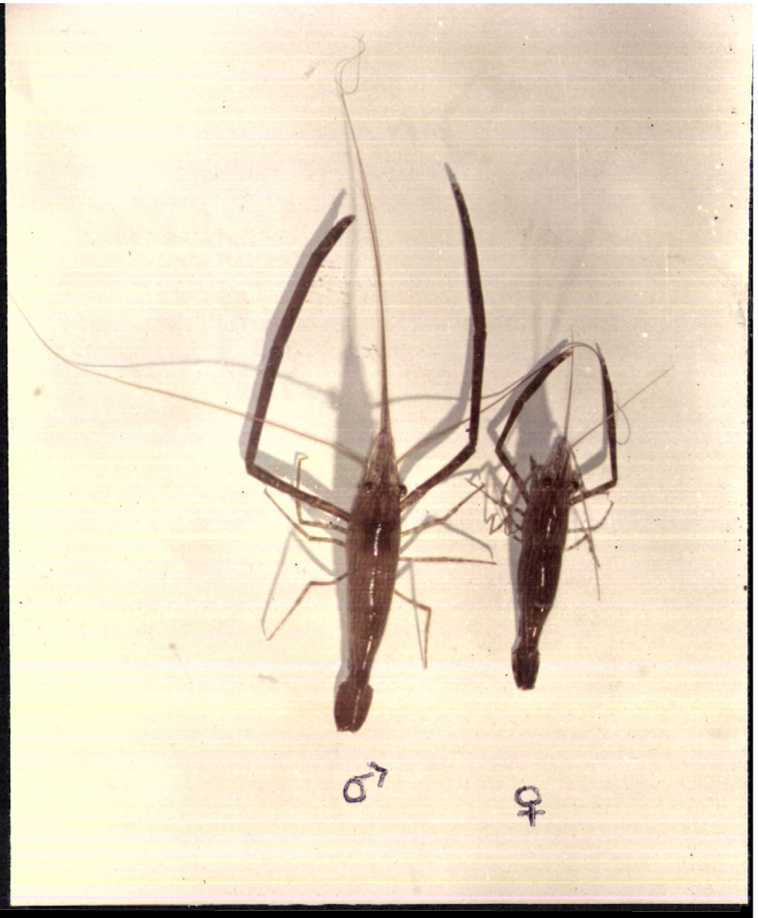

PLATE II M. EQUIDENS - MALE & FEMALE

CHAPTER III

REPRODUCTIVE BIOLOGY

III. REPRODUCTIVE BIOLOGY

3.1 INTRODUCTION

There are over a hundred species of Macrobrachium and most of them require brackish water in the initial stages of their life cycle. While all these species exhibit a breeding migration, some complete their cycle in inland saline and fresh water bodies. Like all other crustaceans, Macrobrachium also undergoes regular moulting. There are two types of moultings. One is somatic moulting which is accompanied by a sudden increase in size and weight. The other type, pre-mating moulting (females only) is accompanied by mating and deposition of semen in a gelatinous mass on the underside of the thoracic region of the females between the walking legs. Egg laying occurs within a few hours of copulation, the egg being fertilized on extrusion by the semen attached to the exterior of the female's body. The eggs are transferred to a brood chamber on the underside of the abdominal region formed by the expanded pleopods of the females. In M. equidens the extruded eggs are dark green in colour which turns to slate blackprior to eclosion. In M. rosenbergii the eggs which are initially golden yellow turns to orange and then to slate black before hatching. The length of time that the eggs are carried by the female (incubation period) varies with species and temperature. It is 18 days for M. rosenbergii and 16 days for M. equidens at 28-30°C. The number of eggs in the brood pouch of different species of

Macrobrachium is known to be directly dependent on the size of the mother prawn.

In this chapter the seasonal fluctuation in the availability of natural berry and its relation to the salinity regimes in the fishing centres are studied. Also, the fecundity in relation to total body length, rostral length, claw length and body weight, and the egg diameter frequency are studied.

3.2 MATERIALS AND METHODS

The catch of M. rosenbergii and M. equidens coming at two major landing centres, one in the upper Vembanad lake (Murinjapuzha) which is not subjected to much tidal influence, from the bar mouth and the other in the lower Vembanad (Panangad) near to the bar mouth (Fig. 1) were analysed. The catch of M. rosenbergii was observed daily. Since M. equidens occurred in small quantities along with other minor species of Macrobrachium, it was sorted out from the other species once in a week and the total landing in a week was estimated by multiplying with the number of fishing days in that week. The catches were examined giving due importance to total length (tip of rostrum to tip of telson), rostral length (tip to base of rostrum), claw length (second cheliped leg), body weight, sex ratio, state of maturation and berry colour. In M. rosenbergii the eggs were then classified based on the developmental stages like yellow, orange and slate

black and the diameter of eggs was measured using an ocular micrometer, each micrometer division being 39 microns. But in M. equidens the eggs have not been classified on the basis of the developmental stages since the colour change from dark green to grey was not very conspicuous. The total slate black berry landing of the day was examined once in every 7 days for estimating fecundity. Following Holthuis (1980) for identifying M. equidens both the spotted and striped varieties have been considered as M. equidens. It may however be noted that recently Pillai (1990) has classified the two varieties as two species namely M. equidens and M. striatus. This was not followed in the present study.

From the landing data, the percentage landing of male, female and berried females in each month was calculated and regressed with the salinity regime. The sex ratio was estimated monthwise and tested using the Chisquare (Snedecor & Cochran, 1967). The sexes were determined by the presence of appendix masculina on the second pleopods in males. Since appendix masculina could be seen only in individuals of 30mm and above in total length, those below 30mm were considered indeterminates. Fecundity (F) was estimated by raising the number of eggs (n) in small fraction of weight of the berry (w) to the whole weight of the berry (W), thus fitting the formula $F = \left(\frac{n}{w}\right)W$. The relationship of fecundity to the total length, claw length, rostral length and body weight were established. The size at first maturity was determined by using

the relative condition factor Kn (Lecren, 1951).

3.3 RESULTS AND DISCUSSION

The occurrence of males and females in different months in two major landing centres, namely Murinjapuzha and Panangad are presented in Table 1. The sex ratio (Snedecor and Cochran, 1967) in different months showed significant difference from the expected ratio of 1:1; specifically this was more during June and September to May at Murinjapuzha and June and August to December for M. rosenbergii at Panangad. The reason for the difference in June was due to the predominance of males while predominance of females contributes to the latter period. The chi-square value for the pooled data was 19.367 which showed significant variation from the expected 1:1, due to the predominance of females(1:2.57). M. equidens has a difference registered in the beginning of the season namely during February at Panangad and December at Murinjapuzha due to the dominance of males. But in the month of May the difference was due to the contribution of females. The Chi-sqaure test for the pooled data did not show significant difference (0.50) indicating that the population of M. equidens as a whole has almost equal proportion of males and females (1:1.15).

From the Table 1 it could be seen that the fishery of M. equidens was less than M. rosenbergii. The fishery of both the

species is influenced by the salinity variations brought in by monsoon rainfall. M. rosenbergii appears in the wild catches by the onset of South West monsoon (June), when the salinity in the back water reaches almost 0 ppt. Its presence increases after the South West monsoon maximum being in November in Murinjapuzha and October in Panangad. Thereafter its abundance decreases with the increase in salinity and is completely absent from the catches once the salinity reaches 18 ppt. In regions like Panangad which are in the lower reaches of the lake and often subjected to high and quick fluctuations in salinity, M. rosenbergii is obtained from June to December only, but in the upper reaches of the lake like Murinjapuzha it is available almost throughout the year (June to April) with maximum in November. In the lower reaches of the back waters (Panangad) salinity increases after the South West monsoon to about 16 ppt in September and decreases slightly during the following two months, possibly due to the effect of North East monsoon. Correspondingly, the M. rosenbergii registered a decline in the availability in September. But the decline in catches is not seen in the upper reaches of the lake where increase of salinity is not seen in the month of September. In M. equidens the availability starts only after the salinity reaches 15 ppt. i.e. at Murinjapuzha in February and Panangad in December. Unlike M. rosenbergii its availability increased with increase in salinity with peak in April/May. As in the case of catches, in M. rosenbergii the maximum number of berried females are also obtained in the month of October and November. But the percentage

of berried females in the catches is more during September/October. The spent females appear as the season advances, reaching a maximum of 85% in May in the case of M. rosenbergii at Murinjapuzha and 21% in December at Panangad. The availability of berried M. equidens was maximum in the month of April, but the percentage of berried females was more in March.

The average fecundity of M. rosenbergii was found to be 50799 eggs (598/g wt) and that of M. equidens was 4698 eggs (987/g body wt). The Scatter diagram indicating the relationship of fecundity with total length, rostral length, claw length and body weight of both the species are given in Fig. 2 to 9 and the results of corresponding regression analyses are presented in Table 2. The r^2 was found significant (Fisher, 1954) in M. rosenbergii but not in M. equidens.

The average values of Kn for both sexes of M. rosenbergii and M. equidens were plotted against the respective length in Fig.10 to 13. The Kn values increased rapidly reaching peaks at 54mm and 62mm for males and females respectively of M. rosenbergii and 40mm and 44mm for males and females of M. equidens respectively, indicating probably that M. rosenbergii males attain first sexual maturity at 54mm and females at 62mm and M. equidens males at 40mm and females at 44mm.

The data on frequency distribution of egg diameter are given

in Fig. 14 to 17. The Fig. 14 represents early yellow eggs of M. rosenbergii, Fig. 15 the orange, and Fig. 16 the slate black eggs of M. rosenbergii. The yellow eggs have size distribution from 450 to 720 microns with mode at 516 microns whereas the orange eggs have a modal length of 630 microns (range 540 to 870 microns) and slate black eggs, 603 microns (range 540 to 960 microns). The pooled eggs of M. equidens (Fig. 17) have a size range from 450 to 810 microns with a mode of 600 microns.

Earlier reports on the sex ratio indicate that females always outnumbered the males in the commercial catches of M. rosenbergii (Miller, 1971; Wanninayake and Costa, 1987). During the present study also females dominated the males with an overall male-female ratio of 1:2.57. Murthy et al. (1987) got an overall male female ratio of 1:1.06 for M. equidens which almost agrees with the present finding of 1:1.15.

Field observations on the distribution and movement of the palaemonid prawns have indicated salinity and temperature as influencing factors in the migratory behaviour (John, 1957; Johnson, 1967; Raman, 1967; George, 1969). In the present observations also salinity is seen influencing the migration. When the salinity increases to around 18 ppt in the backwaters M. rosenbergii is seen migrating to the upstream. In response to the increasing salinity after South West monsoon in Panangad area it was available only upto December whereas in Murinjapuzha where

salinity fluctuations were not marked the species was available upto May. M. equidens exhibits just the reverse character of M. rosenbergii and its availability started only when the salinity reached around 15 ppt. In the lower reaches, the fishery started from December and extended upto May but in the upper reaches, from February to May only. Most species of fresh water prawns in India reach their maximum abundance in the lakes and rivers during monsoon and post monsoon seasons (Ibrahim, 1962; George, 1969; Rajyalakshmi, 1980; Prakash, 1989). The present results in the case of M. rosenbergii are in agreement with these findings. However the present study showed that M. equidens is abundant during pre-monsoon also.

The breeding season of palaemonid prawns varies from region to region, coinciding with the prevailing rainfall. It is reported that in Hooghly river, M. rosenbergii breeds during December to July with peak in March-May following the North East monsoon (Rajyalakshmi, 1961; Rao, 1965; 1967). In Balgoda lake of Sri Lanka it is reported to spawn twice a year, during May to June and November to January when monsoonal shower lower both salinity and temperature of the lake (Natrai, 1947). Raman (1967) reported the breeding season of M. rosenbergii as August - December with a peak in October - November. The present observation of the maximum percentage of berried females in October - November is more in agreement with the observations of Raman (1967).

Unmated females of M. rosenbergii also release the eggs into the expanded brood pouch within 24 hours of premating moult, but the eggs would drop-off within 2-3 days (Ling, 1969a). In the case of M. equidens also the unfertilized eggs drop off within two days. The high percentage of spent females of M. rosenbergii during December to May may be due to the very poor number of males in the sex ratio resulting in weak fertilization and large number of berried females shedding the eggs 2 days after extrusion.

The fecundity of M. rosenbergii has been worked out by many investigators and it was found to be varying widely. Chacko (1955) reported it as 1,00,000-1,60,000; Rajyalakshmi (1961) as 70,000-1,11,400; Ling (1964) as 60,000-1,00,000; Raman (1967) as 1,39,000-5,03,000; and Goorah and Parameswaran (1983) as 22,552-1,09,491. The present estimation of 50799 (average fecundity) agrees with Ling (1964) and Goorah and Parameswaran (1983) but it differs very much with Raman (1967) and Chacko (1955). Ang et al. (1991) reported a decrease in fecundity by 32.3% when yellow eggs become orange and 34.3% when they turn to grey. Higher values obtained by Raman (1967) and Chacko (1955) may be because they must have taken those yellow eggs whereas in the present study only the grey eggs which give the final fecundity were selected. The estimation of the average fecundity of 4698 for M. equidens agrees with the 448-8281 of Murthy et al. (1987).

The number of eggs in different species of Macrobrachium has relation with length and weight (Pandian and Katre, 1972; Koshy and Tiwari,1975; Rojas and Silva, 1979; Guest, 1979). Shakuntala (1977) found that in M. lamarrei the total biomass of eggs per brood is inversely proportional to the unit body weight of the female. Murthy et al. (1987) reported a linear relation between fecundity and total length (r = 0.9713) and fecundity and body weight (r = 0.9338) for M. equidens. While these reports are agreeing with the results obtained in the present observation for M. rosenbergii, the scatter graph for M. equidens shows no definite relationship (Fig. 6 to 9). It may however be noted that in the present study both M. equidens (variety striped) and M. equidens (variety mossaic) were classified as M. equidens following Holthuis (1980), although recently Pillai (1990) has suggested that these are two separate species.

The size at first maturity of 120mm (Fig. 10) for males and 122mm (Fig. 11) for females of M. rosenbergii are very neat to the 118mm obtained by Goorah and Parameswaran (1983) but is far below the 155mm reported by Rao (1967) from Hooghly estuary. The initial maturity size of 44mm (Fig. 12) and 40mm (Fig. 13) estimated by plotting relative condition factor agrees with the 42mm reported by Murthy et al. (1987) by plotting Kn against length and the 41-48mm obtained by Pillai (1980) in laboratory reared Macrobrachium equidens.

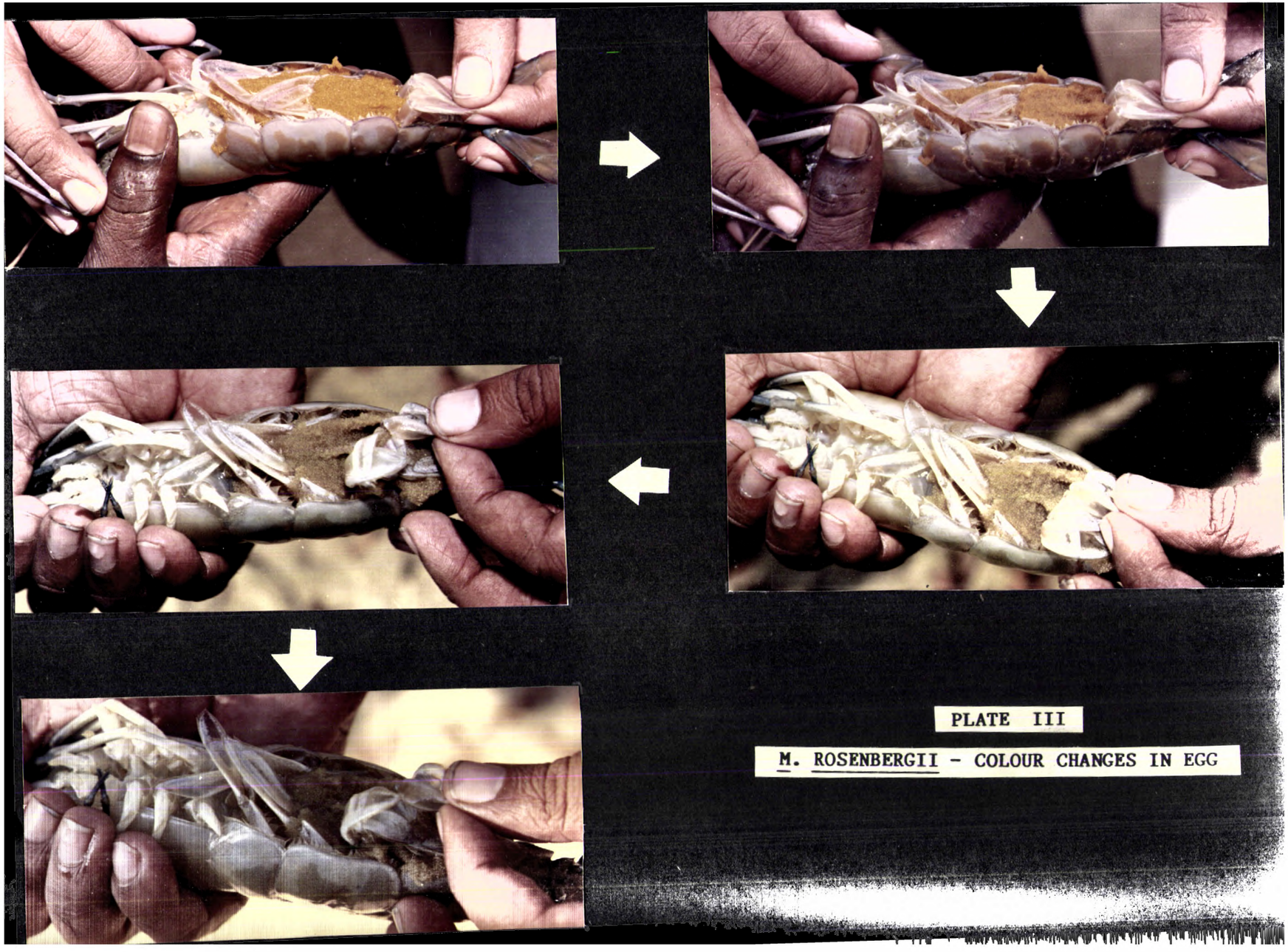

PLATE III

M. ROSENBERGII - COLOUR CHANGES IN EGG

PLATE IV M. EQUIDENS - COLOUR CHANGES IN EGG

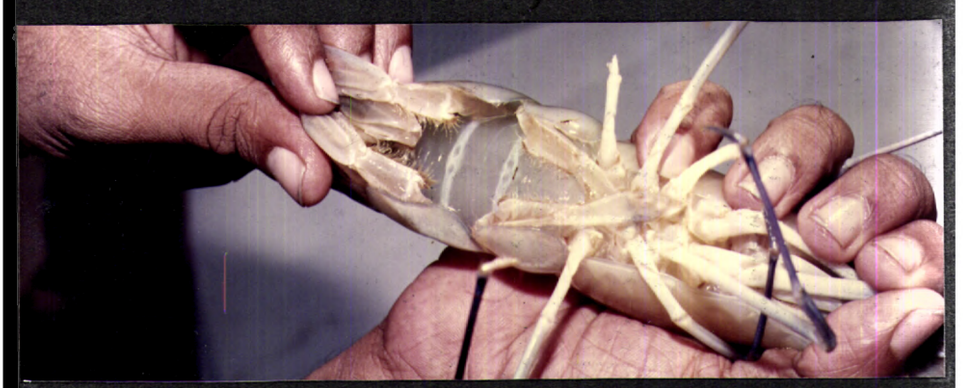

PLATE V M. ROSENBERGII - SPENT FEMALE

PLATE VI M. EQUIDENS - SPENT FEMALE

CHAPTER IV

BROOD STOCK DEVELOPMENT

IV. BROOD STOCK DEVELOPMENT

4.1 INTRODUCTION

Breeding of prawns in captivity is relatively simple, and it is a normal practice of hatcheries to maintain brood-stock of Macrobrachium because of the availability of berried females in the farm. Though broodstock can be obtained from natural waters also, in practice most hatcheries rely on the capture of berried females from prawn farm ponds. This helps in obtaining disease free berries from a known genetic stock. Moreover, while natural broodstock exhibits seasonal variation associated with the onset of the rainy season, the berried females can be obtained throughout the year from the culture ponds. Most of the commercial hatcheries use berried females of 40g, obtained from the farms as a source of mother prawns. In general, Macrobrachium develop ovary in culture ponds and availability of berried female is not a major problem for the hatcheries. In the present study some of the techniques used in other crustaceans for ovarian development like eyestalk ablation, use of exogenous glands, influence of light, age and season to accelerate the process of ovary development were tried in Macrobrachium also.

4.2 MATERIALS AND METHODS

Experiments were conducted to study the effect of eyestalk

ablation (unilateral and bilateral), exogenous hormones (HCG, Progesterone and Ovaprim) and influence of salinity, males, age and season on ovary development. Plastic pools of 300 litre capacity (60 cm height x 80 cm diamter) filled to a height of 50cm with an effective volume of 250 litres were used for all the experiments except those designed to study the influence of age and season on ovary development. This was done in 10,000 litre capacity plastic pools having a height of 1.2m and 3m diameter, filled to 3/4 of its capacity. Bigger pools were used to accommodate more number of specimens used in the study.

The specimens having no external appearance of gonadal development were selected for the studies by adopting the technique of Sagi and Ra'anan (1985). A male to female ratio of 1:5 was maintained in each tank as suggested by Malecha et al. (1980) and Adisukresno (1982).

For understanding the influence of eyestalk ablation on ovary development, prawns weighing approximately 50g were selected. Two sets of treatments and a control were maintained. In one set unilateral ablation and in the other bilateral ablation was performed using an electro cautery unit. In each experimental pool of 300 litre capacity 1 male and 5 females were released and each treatment was replicated 4 times. The tanks were treated with 2 ppm chloramphenicol as a prophylactic measure. A photoperiod of 12L : 12D and temperature of 31-32°C were

maintained following, Chavez Justo et al. (1989). The experiments were conducted for a period of 45 days and the prawns were fed ad libitum with fresh clam meat. Fifty percent of the water in the tanks was exchanged daily. The prawns were kept without any external disturbance and were examined for ovary development only once in 15 days. For maintaining optimum salinity conditions for the species, M. rosenbergii was maintained in fresh water and M. equidens in 18 ppt salt water. The total number of berries obtained for each tank was noted. Those which extruded the eggs were allowed to hatch and remature in the same tank. Observations were continued for a period of 45 days.

The effect of three exogenous glands viz. 17,α hydroxyprogesterone, HCG and ovaprim have been studied. The prawns weighing around 50g were collected from the culture ponds of the College of Fisheries, Kochi and these were randomly divided into five groups each containing 20 females and 4 males. In the first group each female was injected with 0.01 μg of 17,α hydroxyprogesterone/g body weight. The 17,α hydroxyprogesterone was dissolved in pure ethanol immediately prior to injection. The second group was injected with 0.01 μg human chorionic gonadotropin (HCG) after dissolving in pure ethanol. The third group was injected with ovaprim (ovulating agent) @ 0.01 μl/g body weight. In the fourth group (control I), each prawn was injected with 0.1 μl pure ethanol/g body weight. In the case of fifth group (control II) the prawns were not injected. The prawns were

subsequently released in 4 plastic pools containing freshwater, with 5 females and a male. The experiments were set up inside an open shed roofed with intermittent transparent and opaque roofing sheet. For each treatment,3 replicates were maintained. The animals were injected once in 15 days and observed for a period of 30 days. Since it is practically impossible to inject M. equidens because of its small size, the experiments on the effect of exogenous glands were limited to M. rosenbergii.

Experiments to study the influence of light on ovary development were also carried out in plastic pools with 5 females and one male in each pool. Six replicates were maintained for both M. rosenbergii and M. equidens. The experimental tanks were kept inside a dark shed which would not permit day light inside it. Provision for one 5 W blue light bulb was also provided inside the shed and used only at the time of examining the prawns, cleaning, feeding and water exchange. The control tanks were kept inside an open shed, with transparent roofing which permitted sufficient sunlight. Observations were made for a period of 30 days.

For studying the influence of salinity on ovary development, the experimental prawns were introduced in 5 ppt saline water and the control ones in fresh water. Since it is known that M. equidens would not survive in fresh water, the study was limited to M. rosenbergii.

The influence of males on ovary maturation was studied by keeping one set of experiments without males and a control set along with males in plastic pools. M. rosenbergii was introduced in fresh water with 5 females in experimental pool and 5 females and a male in the control pool. The experiments with M. equidens were carried out in its preferred salinity of 18 ppt.

Because of the difficulty in getting M. equidens of known age from the culture farms, the observations on the influence of age and season were limited to M. rosenbergii alone. In each pool 20 females and 4 males were introduced. The experiments were conducted in big plastic pools of 1.2m height and 3m diameter. For observing the influence of age, the prawns were grouped into 4 sets. The first set consisting of virgin females of 5-6 months age, the second set of 10-11 months, the third set of 17-18 months age and the fourth set collected from the wild (probably of one year age group). The experimental pools were randomly arranged and 4 replicates were maintained for each treatment. Observations were made once in every 10 days for a period of 36 days (two berry carrying period) and the total number of berries obtained from each year group was recorded and these results were subjected to statistical analysis using Students 't' test. The experiments were carried out in summer season (15.3.90 to 19.4.90) and again in winter season (15.10.90 to 19.11.90), to understand the seasonal variation. Feeding was done with clam meat ad libitum.

4.3 RESULTS AND DISCUSSION

All the prawns of M. rosenbergii bilaterally ablated were dead within 10 days and hence no berry was obtained. In M. equidens out of the 20 prawns 4 died after the 30th day. A total of 116 berries have been obtained from this experiment. All the berries formed, were shed within 5 to 6 days and new berries formed in its place on the next day. In berried females the ovary development was quick and it took place much earlier than those observed in control. The fully formed ovaries were extruded shedding the berries already present.

In unilateral ablation no mortality was observed and the development and hatching of the eggs were normal. From a total of 20 ablated specimens, 22 berries were obtained in M. rosenbergii and 33 in M. equidens. In both the species the extrusion of eggs (many specimens) was in two instalments during the 45 day period of study. In M. rosenbergii the percentage success was 110% compared to that in the control (80%). In M. equidens it was 165% against the control value of 125%. On statistical analysis using 't' test (Snedecor & Cochran, 1967) the performance of ablated specimens showed significantly a better performance ($P < 0.05$) than the control (Table 3).

Out of the 15 specimens in each group subjected to the effect of 3 exogenous glands, it was seen that ovaprim gave two berries.

Both Control I and Control II also gave 2 berries each, indicating that ovaprim does not have any influence on ovary development. H.C.G. which produced 6 berries showed a significant influence ($P < 0.05$) compared to the other glands used, and the control (Table 4). The HCG has a significant influence on the ovary maturation. In M. rosenbergii two of the berries extruded in prawns injected with H.C.G. were green colour first, which turned into normal yellow colour on the next day while the other berries had the normal yellow colour. These two berries took 19 days to hatch where as the other normal berries took only 18 days.

From the 30 specimens kept in dark shed for 30 days, 33 berries were obtained in M. rosenbergii and 50 in M. equidens. The control (open shed) gave only 10 and 13 respectively for M. rosenbergii and M. equidens. The differences in both the species were significant ($P < 0.05$) suggesting that light is having a significant negative effect on ovary maturation in Macrobrachium. (Table 5).

In M. rosenbergii it is seen that the salinity is not having any influence on inducing the development of ovary. Out of the 30 females kept in 5 ppt saline water 14 extruded and in the control 15.

For both the species the presence of males were found to have no influence in inducing the ovary maturation. From a total of 30 prawns in which males and females were kept in the ratio 1:5, a

total of 33 berries were obtained and in the control without males 36 berries, in the case of M. rosenbergii. In M. equidens, there were 42 berries in the control and 45 berries in the treatment. These differences were not statistically significant as seen in an analysis using 't' test. The apparent higher number of berries obtained in the controls was due to the shedding of unfertilized eggs within 2 days of extrusion because of the absence of males in the pools. This might have resulted in the formation of new berries.

Age and season of the animal were found to have influence on gonadal maturation. During the summer season, only prawns belonging to the 5-6 month and 10-11 month age groups responded, whereas in winter all the age groups showed encouraging response. During summer, out of the 80 specimens observed a total of 80 berries were obtained in 5-6 month group and 19 in 10-11 groups. Statistical analysis using students 't' test also showed significant difference ($P < 0.05$) between the two groups. Performance of 5-6 month group was better in summer (Table 6).

In winter, performance of all the age groups were satisfactory. A total of 65, 82, 92, and 99 berries were obtained from 5-6 month, 10-11 month, 17-18 month and wild collection groups respectively, over a period of 36 days. It was seen that the responses of wild group and 17-18 month groups were better during this period. The difference between these groups were also

found to be statistically significant (Table 6).

In the eyestalk of decapod crustaceans, a gonadal inhibiting hormone (GIH) is produced by the neurosecretory cells of X organ (Adiyodi & Adiyodi, 1970). In Parapenaeopsis hardwickii, the activity of the ovary (gonad) inhibiting hormone was highest in the eyestalk of females with inactive and spawned ovaries, whereas it was negligible in those at full vitellogenesis (Kulkarni and Nagabhushanam, 1980). Otsu (1960, 1964) reported the presence of a gonadal stimulating hormone (GSH) in the thoracic ganglion and Gomez and Nayar (1965) reported the presence of GSH in the brain also besides the thoracic ganglion in crustaceans. Cutting of one eye (Unilateral ablation) leading to the removal of X organ and the reduced production of GIH in body thus inducing the prawn to undergo ovary development under the influence of GSH from the brain and thoracic ganglion is now taken as a standard package of practice in commercial penaeid hatcheries (Emmerson, 1983; Caillovet, 1972; Santiago, 1977; Primavera et al., 1978; AQUACOP, 1979). Unilateral eyestalk ablation has shown to significantly advance sexual maturation and increase the number of moults, clutches and eggs of Macrobrachium nobilli (Kumari and Pandian, 1987). However this technique is not used in commercial practice with M. rosenbergii (New, 1990). In the present study, the number of berries formed in unilaterally ablated Macrobrachium is significantly higher and latency period shorter than that in the unablated controls. These findings on unilateral ablation also

agrees with the reports of unilateral ablation in penaeids and Macrobrachium. The high mortality and non-hatching in the bilaterally ablated specimens observed in the present study are also in agreement with the reports of Alikunhi et al. (1975) in Penaeus merguiensis and Marchiori and Boff (1983) in P. panlensis. The eyestalk produces in addition to the GIH, a number of other hormones which are essential for the normal physiological functions of the body. Removal of both the eyestalks has negatively affected the normal physiological activities leading to death of the animal.

The use of exogenous hormones to induce ovarian maturation and spawning of crustaceans is not well established. However Yano (1985) reported successful spawning of 2 females of Metapenaeus ensis after 30 days by injecting 0.1 µg/g body weight of progesterone. Kulkarni et al. (1979) observed stimulation of oogenesis in Parapenaeopsis stylifera by injecting with progesterone. Rapid ovarian development and induced spawning (even at a low temperature of 20°C) by the administration of 17, ∝hydroxy progesterone in P. stylifera was reported by Nagabhushanam et al. (1982) and Nagabhushanam et al. (1987). The Human Chorionic Gonadotropin (HCG) was reported to stimulate the oogenesis in sand shrimp Crangon crangon (Bomirski and Klekkawniska, 1976) and the rate of fat body vitellogenin synthesis in female isopods, Idotea balthica (Souty and Picaud, 1984). In the present study on M. rosenbergii encouraging results

showing significant increase ($P < 0.05$) with HCG was obtained. However two of the berries produced were of green colour which turned to yellow on the next day and took one more day for hatching than the control. Perhaps a reduction low dosage of HCG may produce a normal extrusion and hatching. The 17,α hydroxy-progesterone also gave a positive result as reported in other crustaceans but it has not shown any significant increase in ovary development.

A photopheriod of 12L : 12D has been reported to give the maximum ovary development in M. rosenbergii (Chavez Justo et al., 1989). However, in the present observation better results were obtained in the dark shed than in 12D : 12L shed (Table 5). Though present observations do not agree with Chavez Justo et al. (1989) it is in agreement with the reports in penaeid prawns that a reduced level of light would lead to fast maturation in P. monodon (Emmerson, 1983, Primavera, 1983).

The percentage of male and salinity variation were found to have no influence in inducing the ovary development in Macrobrachium. In some prawns like Cardina natarajani the ovary development takes place only in the presence of males (Thampy, 1972). The present observations showed that such a male influence is not seen in M. rosenbergii and M. equidens. It was also observed that salinity was not having any influence in the ripening of the ovary. Although better hatching in brackish water

compared to freshwater was reported by New (1990), reports on the influence of brackish water on ovary maturation are not available.

Observations on the influence of age on ovary development were not so far reported. Winter season is reported as the main breeding season of Macrobrachium rosenbergii also. Raman (1967) reported August to December with peak in October to November as the breeding season of M. rosenbergii in Kerala. Jinadasa (1985) also reported the breeding season of M. rosenbergii as December to January in Srilanka. Present observations are also in agreement with this.

CHAPTER V

LARVAL REARING

V. LARVAL REARING

5.1 INTRODUCTION

The success of larval rearing and post larval production of prawns depends on type of feed, stocking density, temperature, light and physico-chemical parameters of water. The larvae are planktonic and do not feed until the second day after hatching, and they rely only on their embryological food reserves during this time. A wide variety of live and dead feeds are used either singly or in different combinations which vary widely in different prawn hatcheries (New, 1990). The feeding regimes used in Hawaii and Thai hatcheries are summerised by Malecha (1983) and New and Singkolka (1985). Both live feed and dead feed are essential for successful Macrobrachium larval rearing. The principal live feeds used are the freshly hatched brine shrimp (Artemia sp.) nauplii, and dead feeds are particulated pieces of tuna flesh (Malecha, 1983), mullet roe (Pillai, 1983), egg custard (Ling, 1962), prepared feed (Adisukresno, 1982) etc. In the present study the suitability of several locally available diets either individually or in various combinations are tried as larval feeds for Macrobrachium.

On a global level both open type hatcheries under direct sunlight and closed hatcheries inside sheds partially covered with transluscent roofing material are common. As on today no report

seems to be available on the relative performance of these systems. Hence to understand this aspect so that one or the other could be recommended under given circumstances, experiments were carried out to study the effect of light on larval rearing of Macrobrachium. Observations were also made to assess optimum stocking density. Several metabolic products of larvae come into the hatchery system. Also therapeutics used may remain in the system, which may have adverse effect on hatchery production. In order to understand their effect, the lethal levels of metabolic wastes like ammonia, nitrite and nitrate and therapeutics like formalin, chloromycetin and oxytetracycline and also salinity have been studied.

5.2 MATERIALS AND METHODS

5.2.1 Larval feeds

The larvae were reared in 100 litre capacity cylindroconical F.R.P. tanks having a middle standpipe and swing arm for central drain. The tanks had a diameter of 30cm and height of 60 cm. These tanks were filled upto 50% of its capacity with filtered seawater of 12 ppt salinity for M. rosenbergii and 18 ppt salinity for M. equidens. The temperature of the water was kept at 28-30°C. The water quality in the tanks were maintained by replacing 30% of the water daily with fresh filtered sea water of the same salinity. Three replicates were run for each treatment. The

tanks were arranged using the Randomised Block Design technique. 16 different feeds/feed combinations were tried. These feeds were selected after determining the acceptability of the feed by the larvae. The food acceptability was determined by finding out the percentage of larval feeding within five minutes of feed introduction in a one litre beaker containing 20 larvae. The feeds with 75% or more acceptability was selected for feeding experiments.

5.2.1.1 Feed Preparation

5.2.1.1.1 Live Feed:

a) Artemia salina cyst (Sanders, U.S.A) was hatched, separated and washed as per Sorgeloos and Pandian (1984). The first day nauplii having a size of 0.25 mm were selected for feeding the larvae. The proximate composition of the artemia nauplii used is given in Table - 7.

b) Monia spp.

Monia spp. was cultured and harvested using the technique of Alikunhi et al. (1980). Young moina having a size of 200-250 microns were selected for feeding the larvae. The proximate composition of the moina used is given in Table - 7.

5.2.1.1.2 Prepared Feed:

a) Thelly meat: The meat of Metapenaeus dobsoni (thelly) was blended with whole chicken egg in the ratio 100g meat: 1 egg in a mixer grinder and coagulated by steaming.

b) Macrobrachium idella meat: The meat of M. idella was blended with whole chicken egg in the ratio 100g meat: 1 egg in a mixer grinder and coagulated by steaming.

c) Squid meat: The squid tube was skinned and washed well and blended with basis egg in the ratio, 100g tube : 1 egg in a mixer grinder and coagulated by steaming.

d) Squilla meat: The head, tail and appendages of Oritosquilla nepa was removed and 120g flesh body with shell was thoroughly blended in a mixer grinder with water. It was then passed through a 0.5mm mesh sized coarse filter cloth discarding the connective tissue and retaining only the materials which pass the strainer. The Squilla material which passed through the strainer was blended again with chicken eggs and then coagulated by steaming. 120g raw Squilla was taken to get a flesh of 100g after removing the shell.

e) Clam meat: 120g of clam meat (Villorita cyprinoides) was blended in a mixer grinder with water. It was then passed through a 0.5mm mesh sized coarse filter cloth and the connective tissue discarded, retaining only the materials which pass through the strainer. To the clam meat that passed through the strainer, one chicken egg was added and again blended. It was then coagulated by steaming.

f) Carp meat: 100g meat of rohu (Labeo rohita) was made into solution without bones, scales and skin and then blended with a chicken egg and coagulated by steaming.

g) Soya Cake feed: 100g protein rich (64% protein) Soyabean cake blended with one chicken egg and coagulated by steaming.

h) Egg Custard: Chicken egg is blended thoroughly in a egg blender and coagulated by steaming.

i) Carp roe: During the breeding season gonads of the freshly killed female rohu (Labeo rohita) were taken out by cutting open the abdomen, and raw eggs ranging from 200 to 800 microns were separated and washed to remove mucilage and connective tissues.

j) Artificial feed: Macrobrachium eggs scrapped out from the berried specimens in the processing plants were collected, washed well and blended with the ingredients shown in Table 8 and coagulated to get a larval feed. The different coagulated feeds mentioned in 5.2.1.1.2 were particulated by passing through superimposed standard test sieves of appropriate mesh sizes to get particles of 3 sizes viz. 200-400 microns, 401-600 micron and 601-1000 microns.

5.2.1.2 Feeds

The following 16 different feeds have been used for M. rosenbergii and M. equidens. Each treatment was replicated thrice.

a) Thelly meat and Brine Shrimp Nauplii (BSN)

b) M. idella meat and BSN

c) Squid meat and BSN

d) Squilla meat and BSN

e) Clam meat and BSN

f) Carp meat and BSN

g) Soyabean cake and BSN

h) Carp roe and BSN

i) Egg custard and BSN

j) Thelly meat and Moina

k) Moina alone

l) BSN alone

m) Egg custard alone

n) Carp roe alone

o) Artificial feed and BSN

p) Artificial feed alone

5.2.1.3 Feeding Schedule:

In the first and the 15th treatments, the dead feeds were fed ad

libitum at 3 hourly intervals during day time. In the evening at 18 hours the excess feed remaining at the bottom of the tank was carefully siphoned out using a 3mm dia P.V.C. tubing, 30% water was exchanged and newly hatched BSN was fed at a rate of 3-5 nauplii per ml of water in the tanks. In treatment 10, thelly meat was fed ad libitum at 3 hourly intervals during day time and moina at the rate of 3-5 specimens per ml of water during night hours at 3 hourly intervals. The excess feed remaining at the bottom of the tanks were siphoned out daily both in the morning at 6 hours and evening 18 hours. Thirty percent of the water was exchanged in the evenings. In treatments 11, 13, 14 and 16 the moina, BSN, egg custard and artificial feed were fed respectively at 3 hourly intervals, both during day and night hours. The bottom of the tanks were cleaned in morning 6 hours and evening 18 hours. In treatment 12 where BSN alone was fed, because of the excellent keeping quality of BSN in water of 12 ppt salinity, the feeding was made only in the morning and evening hours. However, care was taken to see that BSN was always available in the culture system at a rate of not less than one nauplii per 1 ml of water.

A stocking density of 50/1 was used. The number of post larvae obtained at the end of 35th day in M. rosenbergii and 30th day in M. equidens were counted in each tank and the percentage of production calculated. Advanced larvae which were not metamorphosed to the P.L. stage on the day of termination of the experiments were discarded and not taken into account for

calculating the percentage of production. The results obtained were analysed using the method of one-way analysis of variance and wherever the differences showed significance, the treatments were compared pairwise using 't' test. Since the first post larvae was obtained on an average on the 20th day in M. equidens and 25th day in M. rosenbergii, the experiments on M. equidens were terminated 5 days earlier than those on M. rosenbergii.

5.2.2 Effect of light:

The experiments were conducted in 100 litre capacity cylindroconical tanks filled to the half of its capacity with 50 litre of seawater of 12 ppt salinity for M. rosenbergii (New, 1990) and 18 ppt for M. equidens (Pillai, 1990). Continuous aeration was provided to all the rearing tanks from a roots air blower. An initial stocking density of 25/l was maintained. Three sets of experiments each with 4 replicates were maintained simultaneously. The first set of experiments was in direct sunlight without any shade. The second set was inside an open shed, roofed with transluscent fibre glass sheet which allowed approximately 50% of the day light and the third set was kept inside a closed shed, roofed completely with opaque roof. The larvae were fed ad libitum at 3 hourly intervals during day time with a particulated feed prepared from thelly meat and chicken egg, and at night with one time feed of newly hatched BSN fed at the rate of 3-5 nauplii per ml of water. Since BSN has good

keeping quality in 12 and 18 ppt saline water it was fed only once in the night. The bottom of the tanks were cleaned and 30% of the water exchanged daily in the evening. The post larval production in each tank was assessed at the end of 35th day in M. rosenbergii and 30th day in M. equidens and percentage of production calculated. The results were compared using 't' test.

5.2.3 Stocking density:

The experiments were conducted in 100 litre capacity cylindroconical tanks filled with 50 litre of water. M. rosenbergii was reared in the preferred salinity of 12 ppt and M. equidens in 18 ppt. Initial stocking densities of 10/l, 25/l, 50/l, 100/l, 150/l, 175/l and 200/l were maintained. Since M. equidens had exhibited a comparatively sharper decline after reaching maximum production at 150/l, additional treatments of 225/l and 250/l have also been carried out for ascertaining whether such a trend persists further in the species. Three replicates were run for each stocking density. The experiment was carried out using Randomized Block Design technique.

The larvae were fed with a feed prepared by mixing M. dobsoni meat (thelly meat) and chicken egg in the ratio 100g: 1 no. The feed is blended and coagulated by steam cooking and particulated by passing through standard superimposed test sieves. The prepared feed was fed at 3 hourly intervals during day time.

Artemia nauplii was fed to the larvae one time in the evening, at 18 hours at a rate of 3-5 nauplii per ml of water. The bottom of the tanks were cleaned and 30% of the water exchanged daily in the evening. The post larvae obtained in each tank at the end of the 35th day in M. rosenbergii and 30th day in M. equidens were counted and the production per litre for each stocking density was calculated. The results obtained were statistically analysed using the method of one-way analysis of variance and wherever the difference showed significance, the treatments were compared pairwise using 't' test.

5.2.4 Tolerances:

The experiments were carried out to study the toxicity of different levels of ammonia, nitrite, nitrate, formalin, Oxytetracyclin, chloromycetin, salinity and pH to the larvae of M. rosenbergii and M. equidens. They extended over a period of 72 days for Stage I, Stage V, Stage X and PL for M. rosenbergii and Stage V for M. equidens. The incipient LC_{50} ie. the concentration at which 50% of the larvae died after 12, 24, 36, 48, 60 and 72 hrs. were obtained.

The test solutions were prepared by dissolving requisite amounts of analar grade NH_4Cl for ammonia, $NaNO_2$ for Nitrite, $NaNO_3$ for Nitrate, analytical grade formaldehyde for formalin, medical grade capsules for oxytetracycline and chloromycetin,

Hydrochloric acid or $CaCO_3$ solution for pH and concentrated brine for salinity. The experiments were conducted in 1 litre capacity beakers containing 800 ml of the test solution. Each beaker contained 10 larvae and was aerated. The larvae were fed with newly hatched artemia nauplii. The test solution in the experimental tanks were renewed once in 12 hours.

The experiments were repeated twice for each concentration. Mortality was assessed once in 12 hours upto 72 hours. The larvae that did not respond to prodding were considered as dead. Total ammonia was measured by the method of Zolarzano (1969) and the percentage of un-ionized ammonia was calculated following Whitfield (1974), taking into account the pH, temperature, and salinity of the medium. Nitrite was measured by the method of Strickland and Parsons (1972), salinity was measured by the standard methods (Strickland and Parsons, 1972), formalin, oxytetracylin and chloromycetin by dissolving calculated quantities in water, and pH by using a sensitive Elico pH meter. The LC_{50} was calculated by weighed Probit analysis (Finney, 1984) at 90% confidence level (Reish and Oshida, 1986).

5.2.5 Oxygen Consumption and Ammonia Excretion

Experiments were designed to study the oxygen consumption, and the influence of salinity changes on oxygen consumption. The method described by Cheriya (1973) for respiratory measurements

of small aquatic organisms like isopods and prawn larvae was used for the present study (see Fig. 18).

The respiratory chamber was filled with water of the desired salinity and oxygen tension. After introducing the egg/larvae/juveniles inside the chamber and taking an initial water sample of 10 ml each for oxygen and ammonia the piston was set at the 40ml mark. One more sample of 10 ml each was collected after an interval of 1 hour. The concentration of oxygen in the water samples was estimated using Winkler's micromethod as described by Welsh and Smith (1953). A burette with the accuracy of 0.005 ml was used for the titration. Ammonia was estimated by the phenol hypochloric spectrophotometric method (Strickland and Parsons, 1972).

Filtered clear sea water with salinity of 12 ppt was used for M. rosenbergii and 18 ppt for M. equidens. The water was treated with 2 ppm tetramycin. Healthy larvae fed on artemia nauplii and artificial feed (thelly meat and egg) were used for the experiments. The larvae were starved for 24 hours before the experiment, oxygen consumption and ammonia excretion of different stages (viz. egg, I Stage, V Stage, X Stage, PL, juveniles) and the effect of salinity changes on oxygen consumption and ammonia excretion were studied.

5.3 RESULTS AND DISCUSSION

5.3.1 Larval feeds

From Fig. 19 & 20 it could be seen that the maximum survival of 67.47% and 84.27% were obtained with BSN and thelly meat for M. rosenbergii and M. equidens respectively. This was followed by BSN and M. idella meat with a survival of 65% and 75% respectively for M. rosenbergii and M. equidens. The survival rate reduced to 59.41% for M. rosenbergii and 74.67% for M. equidens with BSN and artificial feed. The percentage of survival obtained with BSN and carp roe; BSN and artificial feed; moina and thelly meat; BSN and squid; artemia and soyabean; BSN and carp meat; BSN and egg custard; moina alone; BSN and clam meat; carp roe; BSN alone; BSN and squilla; artificial feed alone and egg custard were 64.16: 59.41; 59.01; 57.63; 57.15; 55.44; 45.95; 25.6; 33.68: 6.03: 48.21; 18.35; 20.08 and 13.6 respectively for M. rosenbergii; and for M. equidens it was 70.32; 74.67; 75.95; 65.55; 64.32: 60.75; 54.69; 46.8; 44.43; 22.08: 54.69: 24.53: 20.88 and 6.35. The highest survival of 67.49% obtained with BSN and thelly meat for M. rosenbergii did not show any significant difference with the survival of BSN and M. idella meat (65.17%), BSN and carp roe (64.60%), moina and thelly meat (59%) and BSN and artificial feed (Table 9). The highest survival of 84.77% obtained for M. equidens with BSN and thelly meat also showed no significant difference with BSN + M. idella meat (75.57%), moina + thelly meat

(75.95%) and BSN + artificial feed (74.65%). However, unlike M. rosenbergii, it showed significant differences with BSN + fish roe at 5% level (Table 9). It could be seen from the Table 7 and Fig. 19 & 20 that the production and survival has no relation to the level of protein in the feed. As observed by Chakraborty et al. (1992) it shows ingredient dependent pattern of performance of the feed. For both the species in general the percentages of survival were better in tanks fed with a combination of live feed and a dead feed. The lowest results were obtained in tanks fed with dead feed alone. This is in agreement with the finding of New (1988) that both 'dead' or prepared and live feed are essential for successful Macrobrachium rosenbergii larval rearing and that dead or live feed alone will not give satisfactory results.

Raje and Joshi (1990) screened 25 feed combinations including live and dead material of both animal and plant origin for M. rosenbergii. They obtained the highest survival of 69% by using crab meat and fish liver. Using BSN and chironomid larvae, the survival obtained by them was only 5%. Rao and Ram (1990) reared M. rosenbergii using 24 types of feeds. Of these 7 were plant diets, 15 animal diets and 2 mixed diets. They got encouraging results only with diets of animal origin. The maximum survival of 29.6% was obtained by feeding BSN in combination with a prepared feed made mainly from fish and egg custard. The present results of 67.49 received with BSN and thelly meat is higher than

the 29.6% got by Rao and Ram (1990) and is comparable with the 69% got by Raje and Joshi (1990) by using crab meat and fish liver. It may be noted that compared to thelly meat, the crab meat and fish liver are costlier and scarce items, which make it difficult to use their combination of larval feed in the hatcheries on a regular basis. Moreover, the keeping quality of crab meat in ordinary refrigerated conditions is also poor.

The results obtained with BSN and thelly meat in the present study are comparable with the results from the commercial hatcheries in Hawaii which get good productions of 70% by using BSN and prepared feed from fish, egg etc. (Malecha, 1983) and the survival of 50-70% obtained in commercial hatcheries of Thailand using BSN and dead feed (New, 1988).

5.3.2 Effect of light:

There was no production in the tanks which were kept in completely closed sheds. All the larvae died by 10 to 14 days. An average survival of 52.2% was obtained in tanks kept in direct sunlight and 54.2% in the tanks which were under partial sunlight, for M. rosenbergii. For M. equidens, it was 61% in direct sunlight and 69% in partial sunlight. But on statistical analysis using 't' test these differences were not found to be significant.

New (1988) observed that no special photoperiodic routine is necessary for larval culture, but the lighting should have the same spectral quality as sunlight and the tanks where the 'clear water' technique is used should be 90% covered or housed indoors to prevent the sun - cancer effect. The larvae of M. rosenbergii are photostatic and they have reduced feeding in direct sunlight (Ling, 1969b). The satisfactory results obtained here in direct sunlight may be due to the fact that in tropical countries a thick growth of diatoms develop in the culture tanks on exposure to sunlight. This must have reduced the intensity of light to the larvae. A thick growth of diatoms (10^6/ml) was observed in the present experiments.

5.3.3 Stocking density:

It could be seen from Fig. 21 that in M. rosenbergii the survival percentage initially increased slightly and reached a maximum of 57.12 at a stocking density of 25/l and decreased sharply to about 20% at 100/l. It then gradually declined and reached 10.11% in 200/l. But the production/litre which gives a better picture of the efficiency of a larval rearing system showed a sudden increase from 10/l to 75/l. It remained more or less the same upto 200/l except a slight increase in 150/l. From critical difference analysis using 't' test it is seen (Table 10) that the 21.82/l obtained by stocking 150/l is not significantly higher than 19.80/l obtained by stocking 75/l. However the increase from

18.56/l at 50/l to 21.82/l at 75/l is significant, suggesting that 75/l is the optimum stocking density in the case of M. rosenbergii for seed production.

In M. equidens (Fig. 22) it could be seen that the production per litre showed a constant increase from 6.8 in 10/l to a maximum of 54/l at a stocking density of 150/l and thereafter it dropped slightly. On critical difference analysis using 't' test it could be seen that (Table 10) the 54/l obtained by stocking 150/l did not show any significant difference with the 49/l obtained with 125/l and 46/l obtained with 100/l suggesting that 100/l could be the optimum stocking density for M. equidens in hatchery seed production. The percentage of survival showed an increase from 10/l to 25/l, and then a sharp decrease.

Many Hawaiian hatcheries use an initial larval stocking density of 60/l, although some of the hatcheries practice a two-stage rearing with an initial stocking density of 160/l which is later on thinned to two tanks (New, 1990). The stocking densities followed in these commercial hatcheries are comparable with the optimum stocking density of 75/l obtained here. Thai hatcheries however use a lower stocking density of 30-50/l and which is mainly due to the ready availability of berried females at a cheap rate. Intensive prawn hatcheries both in French Overseas Territories and in French-inspired hatcheries stock at the rate of 100/l (Palanisamy, 1989). Taiwanese inland hatcheries

often use a multistage hatchery system with initial stocking rates of 300-1000 which will be transferred to larger tanks later as the larvae grow (Hsieh et al., 1989). In Indian conditions where the berried females are scarce and costly, an optimum stocking density of 75/1 obtained in the present study seems ideal.

5.3.4 Tolerances

It would be seen from Table (11) and (Fig. 23 to 52) that the tolerance limits of the larvae in general increased as the size of the larvae advanced. However, in the case of salinity the lower stages showed a much higher tolerance than the advanced ones. Liao and Guo (1985) studied the comparative tolerance to two antibiotics, oxytetracycline and chloramphenicol (chloromycetin) by M. rosenbergii PL and found that the tolerance limit to chloromycetin was much higher than that to oxytetracycline. In the present observations also chloromycetin gave a higher value for LC_{50} for different stages compared to oxytetracycline. The 24 hr. LC_{50} values of 112.25 ppm chloromycetin observed for first stage M. rosenbergii and the 22.08 ppm oxytetracycline also agrees with Liao and Guo (1985).

Regarding the tolerance to nitrogenous waste, the highest degree of tolerance in terms of LC_{50} was shown to nitrates followed by NH_3 and then to nitrite by the larvae. As seen in the other case, the tolerance of the larvae increased as the size

increased. Observations in the nitrite tolerance of Penaeus monodon (Ctiokeluechai and Duangswasdi, 1981) and NH_3-N, NO_3-N and NO_2-N tolerance of P. indicus (Jayasankar and Muthu, 1984) have shown an increase in the nitrogenous waste tolerance as the larvae advanced from nauplii to mysis. Jayasankar and Muthu (1984), have also presented the relative tolerance of the larvae to the three nitrogenous wastes. The lowest tolerance was shown to NH_3-N followed by NO_2-N and then to NO_3-N. In the present observations also the tolerances of the larvae increased as the larvae advanced from Z-1 to Z-X stage. Wickins (1976) observed similar tolerances to ammonia of both M. rosenbergii and penaeid shrimps but the former species exhibits less tolerance to nitrite and nitrate when compared to the latter. According to New and Singholka (1985) the intake water for M. rosenbergii larval rearing in the hatchery should not have levels of nitrite and nitrate higher than 0.1 ppm and 20 ppm respectively. The 20 ppm NO_3-N, recommended by them has been found to be much higher than the 72 hour LC_{50} of 11.16 obtained for first stage zoea in the present study.

M. equidens was seen to be more tolerant to pH than M. rosenbergii. The 12 hr. LC_{50} for first stage larvae was 4.74 and 10.517 in M. equidens, but in M. rosenbergii it was 5.22 and 10.14. But Natividad (1982) reported the occurrence of M. rosenbergii in some Philippine river systems in which the pH fluctuated between 3.0 and 9.0. Some workers reported 80% post-

larval mortality at a pH level Of 9.5 (Sarver et al., 1979, 1982; Malecha et al., 1980). However, Sandifer and Smith (1985) reported the culture of prawns in South Carolina in which the pH ranged from 6.0 to 10.5 with no apparant adverse effect.

5.3.5 Oxygen consumption and Ammonia excretion

It can be seen from Table 12 that the oxygen consumption and the ammonia excretion of the larvae increases as the stage (size) advances. At 12 ppt salinity, in M. rosenbergii an amount of 0.0001755 ml/l/hr oxygen was consumed by the first stage and 0.00665535 ml/l/hr by the 10th stage larvae. The PL and juveniles had a much higher consumption of 0.139526 ml/l/hr and 0.0860124 ml/l/hr respectively. The consumption of oxygen by egg was only 0.0002604 ml/l/hr. The ammonia excretion also showed a similar pattern of increase. The excretion by egg was 0.0001755 ml/l/hr and that by the first stage larvae was 0.0044267 ml/l/hr and by 10th stage was 0.05531167 ml/l/hr. The ammonia excretion by the PL was 0.02471069 and that by juveniles was 0.10344 ml/l/hr.

In M. equidens O_2 consumption and NH_3 excretion followed the same patterns as in M. rosenbergii. At 18 ppt the O_2 consumption by egg was 0.0003518 ml/l/hr and that by the first stage was 0.00478244 ml/l/hr. The ammonia excretion of egg was 0.0003518 ml/l/hr, by first stage larvae was 0.002309 ml/l/hr and that of 10th stage larvae was 0.043100 ml/l/hr. The O_2 consumption and NH_3

excretion of PL and juveniles were 0.132366 ml/l/hr O_2 (0.080600 ml/l/hr NH_3) and 0.0692079 ml/l/hr O_2 (0.12068 ml/l/hr NH_3) respectively.

Stephenson and Knight (1980) observed that oxygen consumption of M. rosenbergii larvae is proportionate to body size (dry wt.) and they worked out a relationship of log O_2 = 1.54 + 0.904 log wt. Yagi et al. (1990) also observed that the O_2 consumption of the larvae of Palaemon serratus increased with advancement in larval stages, but they could not find any relationship between the O_2 consumed and the dry weight of the successive larval stages. The present observations also agree with the findings of Yagi et al. (1990). In Macrobrachium vollenhoveni, Udo and Taege (1989) observed that the metabolic rates decreased with increasing body size while O_2 consumption per unit time increased with wet weight.

When the larvae were subjected to a hyperosmotic transfer from 12 ppt to 15 ppt for M. rosenbergii and 18 ppt to 21 ppt for M. equidens, the ammonia concentration in the exposure water declined for the first one hour. Armstrong et al. (1981) also observed that when adult Macrobrachium rosenbergii was subjected to a hyperosmotic transfer from 0 ppt to 24 ppt salinity, ammonia concentration in exposure water declined for 24 hrs. They opined that the reduction in ammonia might be due to the uptake of ammonia by the organism. They also indicated a reversal of normal Na^+/NH_4^+ exchange following a hyperosmotic shock, such that blood

Na^+ is hyporegulated using exogenous NH_4^+ as a counter-ion. The net ammonia acquired by uptake could be used to increase intracellular ammonia concentration as a preclude to increased synthesis of free amino acids (FAA). Reports by other investigators (Magnum and Towle, 1977; Gylles, 1979) also show that crustaceans subjected to hypersaline stress regulate the cell volume by an increase in intracellular FAA by the greater use of endogenous ammonia (by reduced excretion) to synthesise amino acids. Taylor et al. (1987) also observed an intake of ammonia when the Palaemon elegans was transferred to higher saline conditions containing stable isotope 15_N. They also observed that P. elegans showed a pronounced increase in the rates of ammonia excretion during the first two hours after transfer to lower salinities. Similarly Regnault (1987) also reported that in Crustacea an increase in ammonia excretion rate was usually observed as salinity decreased.

CHAPTER VI

MASS LARVAL REARING

VI. MASS LARVAL REARING

6.1 INTRODUCTION

The main handicap in the development of commercial farming of Macrobrachium is the lack of availability of seed in required quantities in right time. Since the seed collection from natural sources is not commercially feasible the mass production of Macrobrachium seed by setting up hatcheries is the only method to meet the growing demand for the seed. With a view to develop an indegenous technology for the hatchery production of Macrobrachium seed a series of experiments were conducted.

For successful larval rearing, salinity of about 12 ppt is required for M. rosenbergii and 18 ppt for M. equidens. The non-availability of water having the desired salinity poses a real problem in hatchery production of seed round the year. Further more, it also acts as the major constraint in setting up hatcheries in inland region where large culture operations of M. rosenbergii are carried out in fresh water. These problems can be solved by using artificial brackish water for larviculture. Experiments were conducted to study the possibility of using suitable artificial brackish water for larval rearing of Macrobrachium.

6.2 MATERIALS AND METHODS

Two sets of experiments were conducted. The first set was for developing a package of practice for mass rearing of Macrobrachium larvae and the second set for using artificial brackish water for the larval rearing.

The experiments were conducted using wild berry obtained from Murinjapuzha area of the Vembanad lake. After bringing them to the hatchery at Panangad, the berried females were transferred directly to fresh water. It took 1-18 days for M. rosenbergii and 1-16 days for M. equidens to hatch, depending on the stage of development of the eggs. The newly hatched larvae were collected using bolting silk and transferred to 1.2 tonne capacity oval FRP tanks (175 cm length, 95 cm width and 95 cm depth with an effective volume of 1 tonne water when filled to 65 cm height) having saline water of optimum salinity 12 ppt water for M. rosenbergii (New, 1988) and optimum salinity of 18 ppt for M. equidens (Pillai, 1990). The larval density was maintained at 75 nos/litre for M. rosenbergii and 100 nos/litre for M. equidens.

The saline water was pumped from the nearby backwater through an'insitu' bed filter. According to the salinity regime this was either diluted by adding filtered freshwater taken from an open well or the salinity was increased by adding filtered sea water. Thelly (Metapenaeus dobsoni) meat with hen's egg (1 kg meat plus

10 eggs) were blended, coagulated and particulated by passing through standard stainless steel test sieves of appropriate mesh size. For the first IV Stages the particle size of 300-400 microns, V to VIII Stages, 400-500 microns and IX to XI stages, 600-1000 microns were used and fed at 3 hourly intervals during day time, ad libitum. Newly hatched brine shrimp nauplii were fed at the rate of 3 nos/ml, of water, only once in the evening. The bottom of the tanks were cleaned daily in the evening before feeding with artemia nauplii and 50% of the water was exchanged soon after cleaning. Aeration was provided from a 145 cfm roots blower or a 5 HP autostop air compressor, channeled through P.V.C. pipes and diffusion stones. A series of 5 experiments was conducted for each species.

The second set of experiments have been conducted to know the feasibility of using artificial sea water for larval rearing. Synthetic sea water was prepared by mixing the ingredients in required quantities as per Table 13. The common salt used for the experiments was brought from the salt pans of Tuticorin. A control was set using natural saline water which was procured as in the first set of experiments. A bio filter was set up in a 300 litre capacity concrete tank of 90 cm height and 32 cm diameter and the same was connected to each of the larval rearing tanks. Pebbles of 3 to 5 mm size stacked to a height of 40 cm was used as the substrate for the biofilter. The filtered water from the bottom of the biofilter was taken to the larval rearing tank with

the help of an air lift pump and water from there was returned by siphonic force. The rate of flow was regulated in such a way as to enable the entire water to pass through the filter bed at least 5 times a day. The inlet of the siphon was covered with a 200 micron filter cloth with a view to prevent the larvae and food particles from being siphoned into the filter. A series of 10 experiments were conducted for both species in natural and artificial water and the results were statistically compared.

In the third set of experiments the artificial water used for the first set of larval rearing was reused for the second set of rearing and it was again used for the third set of rearing without any treatment in between the cultures. A series of 5 experiments were conducted in each case. The percentage of production obtained was transferred using angular transformation

$\theta = \text{Sin}^{-1}\sqrt{P/100}$ where the P is the percentage survival, and the result compared using Students 't' test (Snedecor and Cochran, 1967)

In the fourth set of experiments relative importance of each of the six ingredients in the synthetic brackish water for Macrobrachium larval rearing was studied and the results compared using Student 't' test. Five replicates were maintained for each treatment. The third and fourth set of experiments were conducted in small 100 litre capacity cylindroconical tanks with biofilter arrangement set up in another 100 litre capacity tank. A stocking density of 50/litre was used.

6.3 RESULTS AND DISCUSSION

In mass larval rearing an average survival of 49.96% with a production 39 PL/litre was obtained for M. rosenbergii, and for M. equidens a survival of 70.08% and a production of 70 PL/litre was obtained (Table 14). On an average the first settlement started on the 25th day in M. rosenbergii and 21st day in M. equidens. Of the experiments conducted the maximum survival obtained was 64.8% with a production of 56 PL/litre for M. rosenbergii and 74.4% with 74 PL/litre production for M. equidens (Table 14).

The average survival and production registered from the synthetic and natural water in closed recirculatory system using biofilters was 51% (23 nos/l) and 47.27% (24 nos/l) respectively for M. rosenbergii and 58.01% (29 nos/l) and 71.36% (36 nos/l) respectively for M. equidens. Pairwise comparison of the two types of water using 't' test showed no significant differences between then in M. rosenbergii but in the M. equidens the increase of production was significant at 1% level (the calculated value of t was 5.944 while the table value, t_{18} 0.01 is 3.922), suggesting that the larval rearing of M. equidens gives better performance in synthetic brackish water than in natural sea water (Table 15). This may be due to low load of bacteria and other pollutants in the synthetic brackish water compared to the natural one.

The results on survival rate when water was repeatedly used

revealed that there is a significant decrease in the percentage of survival in subsequent rearing for M. rosenbergii (Fig. 53) and M. equidens (Fig. 54). The average percentage of survival registered in I, II and III set of rearing experiments using the same water medium was 51.49, 39.93 and 13.69 respectively for M. rosenbergii and for M. equidens it was 82.96, 40.24 and 13.66 respectively. These values were tested statistically using Analysis of Variance and the results shown in Table 16. The values of 'F' were found to be highly significant at 5% level. Pairwise comparison of the data using Students 't' test also revealed that the 't' values computed for the different pairs showed significant differences at 1% level. Hence it is concluded that it may not be worthwhile to use the synthetic water, once used, repeatedly for subsequent rearing purposes.

In the fourth set of experiments when all 6 ingredients of the synthetic brackish water (Table 13) were used, a survival of 63.12% was received for M. rosenbergii and 82.96% for M. equidens. When all ingredients other than potassium bromide was used the larvae died within 48 hours. When potassium bromide alone was used along with common salt a percentage survival of 28 and 29.1 were obtained respectively for M. rosenbergii and M. equidens. An analysis of the percentage of survival in larval rearing experiments in water medium containing 6 ingredients (Table 13) and only common salt and KBr showed significant differences at 1% level. The calculated value of 't' for M. rosenbergii was 3.82 and

for M. equidens, 12.63 and the table value for t_8 0.05 is 2.306 and t_8 0.01 is 3.355.

The reports of larval rearing of M. rosenbergii on a mass scale from India and abroad are available but no work on the mass rearing of M. equidens is seen reported. The Hawaiian hatcheries get a production of 30/l (New, 1990). Thai hatchery management produces from a stocking density of 30-50/l, a production of 10-20/l (New, 1988). An average production of 50/l from antibiotic aided highly intensive system was reported by AQUACOP (1977). These production rates of the commercial hatcheries are comparable to the average production of 39/l obtained in the present mass rearing experiments. In contrast to the present survival of 49.96%, Adisukresno et al. (1982) reported a high survival of 72% with a stocking density of 15/l. It may be pointed out that a percentage survival expressed on the basis of different stocking densities, does not give a concrete index for comparison and evaluation of the efficiency of the rearing system. On the other hand, the post larval production per unit volume of water gives a better index of the efficiency of the system. Whereas Adisukresno's production can be worked out only as 7 PL/l, in the present study the average production was 39/l. Alikunhi et al. (1980) reported a high survival of 90% by feeding with moina and thelly meat. However, their results are based on an isolated experiment without replications and statistical design.

The time taken for the first and final settlement of the larvae is an indication of the degree of success of a rearing system. Lee (1982) reported that the first post larval settlement was seen after 35 days and on an average all the larvae metamorphosed by the end of 60 days at a temperature ranging from 23.5 to 27.0°C. In the present experiments the larval rearing was done at a temperature range of 29-30°C. The first post larvae settled on an average on the 25th day and completed the settlement on the 35th day. Temperature of rearing medium may be the most important factor in this regard as reported by Suharto et al. (1982) who found that better results were obtained when temperature was maintained without wide fluctuations at 28-31°C.

On statistical analysis the average production of 23/l obtained with natural sea water did not show any significant difference with the 24/l got for the synthetic brackish water. The duration taken for the first as well as final settlement in synthetic brackish water was 24 and 56 respectively while those in natural brackish water was 25 and 51 days respectively. This showed that there was no significant variation in this between the two media, leading to the conclusion that the moulting frequency in both the media were similar. The average production of 26/l obtained here is almost similar to that obtained by Malecha (1983) in Hawaii (30/l) and much more than the 10-20/l obtained in Thai hatcheries (New & Singholka, 1985) using natural sea water. The mean production of 26/l obtained with synthetic brackish water is

comparable to the production figures furnished by Sandifer and Smith (1975) as 6.6-40.6/1 when Instant Ocean brand of artificial sea salts (Aquarium Inc., U.S.A) was dissolved and used for rearing in closed recirculation system with gravel biofilter. Sandifer and Smith (1975) reported the first and final settlement days as 20-24 and 34-35 days respectively whereas in the present study the first and final settlement days were 24-30 and 36-51 respectively. This may be due to the strain difference of the species used as suggested Alikunhi et al. (1980). Rock salt, sea salt, Rock salt-brine and salt stock solution were used with appropriate dilution in experimental larviculture by different workers (Tunsutapanich, 1980; Prakash, 1988; Prakash, 992). Prakash (1992) reported 74-80% survival when rock salt and liquid residual waste were used for rearing. But in terms of production it was only 14-15/1 and this was a lower rate when compared to the present production of 26/1. The high percentage of survival reported by Prakash may be due to the low stocking density. Yambol and Cruz (1986) reported the survival rate as 6.71% when the rearing was done with the help of a combination of sea salt, deionized fresh water and green water. The present survival rate is far above that of Yambol and Cruz and this may be due to the use of additional ingredients.

CHAPTER VII

UNIFORMITY IN LARVAL STAGES AS AN INDEX OF POST-LARVAL PRODUCTION

VII. UNIFORMITY IN LARVAL STAGES AS AN INDEX OF POST-LARVAL PRODUCTION

7.1 INTRODUCTION

The larvae of Macrobrachium species have the property of moulting during its growth. M. rosenbergii and M. equidens pass through eleven stages decided by moulting (Uno & Soo, 1969) before attaining the post-larval stage. In a single culture the moulting of individual larvae is to take place simultaneously, provided there is uniformity as far as health is concerned. But the moulting time generally vary from individual to individual. Unhealthy conditions and genetical causes contribute towards this variation in moulting time. Both the reasons may be considered as an incidence of abnormality and hence can be expected to affect the post-larval production. Hence a measurement of the variability of moulting stages helps in forecasting the production potential of the post larvae of Macrobrachium.

7.2 MATERIALS & METHODS

Culture of M. rosenbergii was carried out in 35 tanks (same as used for mass larval rearing in Chapter VI) under uniform conditions. Random samples of same day aged larvae were taken from each of these tanks. The samples were taken on every 5th day until post-larvae appeared in the tanks.

In the case of M. equidens 13 culture tanks were maintained from which the random samples were taken. The number was less owing to the non-availability of the species.

The samples thus collected were observed for the various stages and the number of occurrences of each stage was noted. Since growth stages decided by moulting showed the variation in every 5th day sample, the stages were treated as variables and the mean and standard deviation of them within a sample were worked out. Coefficients of variation defined as the ratio of standard deviation to the mean expressed as percentages (Mode, 1971), are used as a relative measure of spread of stages within samples. The post-larval production figures in each culture tank were also noted down. In the case of M. rosenbergii these figures are correlated with the coefficients of variation values of the 15th day's observations. Also linear regression between them was worked out following Sankaran & Nair (1992). But in the case of M. equidens the 10th day's observations were used for the above computations, since the post-larval settlements were comparatively earlier in this case.

7.3 RESULTS & DISCUSSION

The frequencies, expressed as percentages, of each moulting stages on the basis of the pooled samples of M. rosenbergii observed on every 5th day are given in Table 17. Similar

representation of M. equidens for the 5th & 10th day are given in Table 18.

For easy comparison the frequency distributions of the growth stages of M. rosenbergii are plotted in graph (Fig. 55). The frequency polygons thus obtained show that the uniformity in moulting stages is more prominent at the initial stages. Comparatively very small values of standard deviation both for the observations on 5th and 10th days point towards the fact that most of the values are clustering around the mean stages. But the numbers of stages represented in the latter samples are more, thereby leading to higher values of standard deviation. This indicates that all the larvae do not attain the required maturity as the days pass. In the 15th day sample while a very few of them (not even 0.10%) have reached advanced stages like 9th and 10th the majority come under 6th, 7th and 8th stage. About 17.5% could not reach even these stages. Their health condition must definitely be poor compared to the others which have reached advanced stages. Quantification of these variations in stages based on the pooled samples, obtained in every 5th day is done by finding the values of mean, standard deviation and co-efficient of variation. These are incorporated in Table 17. Coefficients of variation are worked out, since they give a relative index of spread of the stages as percentages of mean values.

The post larvae of M. rosenbergii appeared in one culture tank on the 20th day. On the 25th day, the larvae of 10 culture tanks out of the 35 had attained the post-larval stage, while on 30th day this number became 23, and on the 35th day 29. On the 40th day there was only one tank containing a few animals yet to reach the post-larval stage.

In the case of M. equidens the post-larval settlement was seen to be taking place between 15th and 25th days. On the 10th day itself the highest frequencies were observed for the 8th stage.

In M. rosenbergii the spread of stages was more prominent from 15th day onwards. Also 15th day may be taken as half the period of culture. Because of these reasons the frequency data for that day were further analysed. The co-efficients of variation of the growth stages for each sample were worked out. The correlation coefficient between these values and the post-larval production figures (in percentages) of each culture tank was calculated. It gave a high negative value ($r = -0.9418$). Since this value is an indication of a linear relationship between the two variables, a linear regression model was fitted for them, which gave the relation as $y = 94.4952 - 6.0834\ x$, where y being the post-larval production percentage and x, the coefficient of variation of growth stages decided by moulting. This line is plotted in Fig. 56 together with the observed values. This linear

model helps to forecast the post-larval production percentage on the basis of the spread of the growth stages in the 15th day sample. Thus from this a co-efficient of variation of the order of 7.3% or more implies a post-larval production of 50% or less.

In the case of M. equidens the 10th day's data were analysed in a similar manner in order to get a relationship between the spread of the stages and the post-larval production. The correlation co-efficient between the post-larval production and co-efficients of variation was seen to be -0.9478 and the linear relationship as $y = 95.6299 - 4.5114 x$. This model helps to predict the production of post-larvae. Accordingly if the co-efficient of variation value is more than or equal to 10.1% the post-larval production will be less than or equal to 50%.

CHAPTER VIII

NURSERY REARING

VIII. NURSERY REARING

8.1 INTRODUCTION

Stocking of post larvae directly into grow out ponds results in lower production and loss of valuable seed as compared to stocking of juveniles (Eble et al., 1979; Smith et al., 1983; Smith and Sandifer, 1982). The aggressive and cannibalistic nature of M. rosenbergii under crowded conditions is a major problem encountered in attempts to rear post larvae in tanks at high stocking densities. Hence there is a need for perfecting the nursery rearing techniques of Macrobrachium spp. Experiments were conducted to study the best feed, salinity, stocking density and water management techniques suitable for the nursery phase of both M. rosenbergii and M. equidens.

8.2 MATERIALS AND METHODS

8.2.1 The experiments to study the effect of salinity were conducted in circular fibre glass tanks having 52 cm diameter and 33 cm height, filled with water to a height of 20 cm. A stocking density of 100 nos/tank (470 nos/m^2) was used in all the tanks. Hatchery produced post larvae with an average initial length of 13.5 mm and 10 mg weight were used for the experiments in M. rosenbergii and for M. equidens, with a length of 12 mm and weight of 10.54 mg. The experiments were conducted for a period of 25 days and 7 different

salinity levels of 0, 3, 6, 9, 12, 15 and 18 ppt were maintained for M. rosenbergii and 0, 6, 12, 18, 24, 30 and 38 ppt for M. equidens. Additional substratum was provided using polypropylene webbing in all the tanks and the larvae were fed ad libitum with clam meat. 50% water exchange was made once in two days and continuous gentle aeration was provided from an air compressor.

Hatchery produced post larvae of average size 10 mg and 13.5 mm were used in the case of M. rosenbergii and 10.54 mg and 12 mm in the case of M. equidens.

Continuous mild aeration was provided in all the tanks. The water quality parameters were monitored regularly.

Using the results of first set of experiments, the percentage survival and the increment in weight obtained under different salinity conditions were compared. Student's 't' test was used for this purpose.

In the case of second set of experiments, the percentage of survival and production per litre were analysed for knowing the difference among the various stocking densities and also among different feed cum water management. The analysis was carried out using the method of two-way Analysis of Variance. Before analysis the percentage production values were transformed using angular transformation,

$$\theta = \text{Sin}^{-1} \sqrt{\frac{P}{100}}$$

where P is the percentage value expressed as probability value (Snedecor and Cochran, 1967). Wherever the difference showed significance, the treatments were compared pairwise, using 't' test, finding critical differences. The formula for critical difference (C.D) is given by C.D = Standard error of the difference of means x 't' value at 1% level for the degrees of freedom of the error mean square ie.,

$$C.D = t \times \sqrt{\frac{2 \times \text{Error Mean Square}}{\text{no. of replications}}}$$

The production figures per litre for each feed were plotted separately against the stocking density values. A second degree curve $y = a+bx+cx^2$ was fitted for these plots, taking 'x' as the stocking density and 'y' as the production per litre. The optimum stocking density in each case was obtained from this fitted model using the principle of finding maxima and minima in Differential Calculus (Vance, 1966).

8.2.2 The experiments to study the best stocking density, feed and water management were conducted in circular cement cisterns having 90 cm diameter, 60 cm height and 0.64 m^2 floor area and the inside coated with epoxy resin. The experiments were conducted in freshwater for M. rosenbergii and 18‰ saline water for M. equidens.

Five different stocking densities of 500, 1000, 2000 and 2500 nos/m^2 were tried with the following four treatments.

8.2.2.1 Fed with dried broken clam meat ad libitum and provided with additional substrata comprising 500g seasoned cashew twigs. A biological filter system, made up of pebbles was fitted to the nursery tanks to maintain the water quality.

8.2.2.2 Fed with dried broken clam meat ad libitum and additional substrata provided with 500g seasoned cashew twigs. No biological filter system was provided, but 50% of the total water in the tank was exchanged daily.

8.2.2.3 Fed with dried broken clam meat ad libitum and no additional substrata and biological filter system was provided. 50% of water was exchanged daily.

8.2.2.4 Fed with a commercial prawn feed; no additional substrata and biological filter system: 50% exchange of water done daily.

The cisterns were assigned randomly to five stocking densities for each of the treatments in the case of the two species. Three replicates were maintained in each case. The whole experiment was planned using Randomized Block Design technique.

The proximate composition of the clam meat used was protein 53.45%, fat 6.82%, carbohydrate 16.99%, moisture 9.92% and ash 12.82%. The commercial prawn feed had protein 31.2%, fat 7.28%, carbohydrate 28.22%, moisture 7.45% and ash 25.85%.

8.3 RESULTS AND DISCUSSION

In the first set of experiments, to study the effect of salinity, the percentage survival increased as salinity increased upto a level and afterwards started decreasing. In the case of M. rosenbergii, the maximum survival of 89% was obtained at 12 ppt (Table 19) which showed no significant difference with the 80% survival obtained for the 9 ppt salinity and 85.67% obtained for the 15 ppt salinity (Table 21); but with all other treatments on pairwise comparison using 't' test (Snedecor and Cochran, 1967), the lowest percentage of survival of 22.33 was obtained at 0 ppt. The maximum increment in weight of 0.0506 g obtained at 3 ppt showed no difference with those obtained at any other treatments. But there was significant differences in the increment in weight between treatments 6-12, 6-18 and 12-18 ppt on pairwise comparison using 't' test. The maximum increment in length of 18.75 mm obtained at 3 ppt showed no significant difference with any of the treatments except 0 ppt and 6 ppt. There was also significant differences in length gain between 0-9, 0-15, 0-18 and 6-18 ppt.

In the case of M. equidens, the percentage of survival varied

from 2.5 to 84.33. The maximum survival was obtained at 18 ppt and the minimum at 0 ppt (Table 20). The maximum survival of 84.33% obtained at 18 ppt showed statistical differences with those obtained at each other treatments except the one at 24 ppt. The maximum increase in weight and length of 0.0041 g and 18.61 mm respectively obtained at 12 ppt showed no significant difference with those at any other treatment in the case of weight gain but not with 6 and 30 ppt in respect of increase in length (Table 22).

Wickins (1972) got better growth of Macrobrachium rosenbergii post larvae at 2 ppt in comparison to that obtained at 12 ppt which is in confirmation with the present results of maximum increment in length obtained at 3 ppt. Goodwin and Hanson (1975) also found that Macrobrachium juveniles grow more rapidly in freshwater or slight brackishwater when compared with more brackishwater. Here the maximum survival was obtained at 12 ppt and 18 ppt, but the maximum growth was at 3 ppt and 12 ppt respectively for M. rosenbergii and M. equidens. Thus at farmers' level, where survival and growth are equally important, it is ideal to grow M. rosenbergii at salinity in between 3 and 12 ppt and M. equidens at 12 and 18 ppt.

The results of the second series of experiments are presented in Table 23 & 24.

The water quality parameters in the experimental cisterns were temperature 28-31°C, pH 7.8-8.8, alkalinity 70-95 ppm ($CaCO_3$), DO 10.42-14 ppm, Ammonia 0.01 - 0.1 ppm in cisterns with biofilter attached and 0.21 - 0.38 ppm in those without biofilter.

In tanks wherein the Macrobrachium rosenbergii PL were fed with clam meat and provided with additional substrata and biological filter the percentage of survival remained almost uniform at around 75% for stocking densities ranging from 500/m^2 to 2000/m^2. The maximum survival of 76.04% was obtained at a stocking density of 1500/m^2, and at 2000/m^2 it was 75.9% which then suddenly dropped to 56.65% as the stocking density was further increased to 2500/m^2. From Fig. 60 it is seen that the production per m^2 increased with the stocking density upto 2000/m^2 and then decreased. A production of 1518.00 nos/m^2 was obtained by stocking 2000/m^2 and then it dropped to 1417 nos/m^2 by stocking 2500/m^2. From Fig. 60 it is seen that the calculated production/m^2 increased upto a stocking density of 2609/m^2 and then started decreasing. The second degree model fitted for the data gave the equation, $y = -296.8407857 + 1.361422857\ x - 0.000260914\ x^2$, where x indicates the stocking density and y, the production/m^2.

In the case of M. equidens, the survival rate almost remained at around 90% for a stocking density upto 3000/m^2 and then dropped to 59.1% as the stocking density increased to 3500/m^2. It can be

seen from Fig. 64 that the production/m^2 increased with the stocking density upto 3000/m^2 and then decreased. A production of 2706 was obtained for 3000/m^2 which then dropped to 2070 for 3500 nos/m^2. The calculated maximum production/m^2 was obtained as 2500 at a stocking density 3000/m^2.

The corresponding data of stocking density and production gave the equation as

$$y = -308.396 + 1.752\ x\ -0.0002727x^2$$

The optimum density as per the fitted model is found to be 3212/m^2.

In those tanks where the M. rosenbergii PL were fed with clam meat and provided with additional substrata, but no biological filter, the percentage of survival of M. rosenbergii remained almost uniform upto 2000/m^2 and then decreased with increase in stocking density. A survival of 74.85% was obtained for a stocking density of 2000 nos/m^2. The production/m^2 steadily increased from 365.63 for 500/m^2 to a maximum of 1497 for 2500/m^2. From the Fig. 59 it can be seen that calculated maximum production/m^2 of 1404 was obtained at a stocking density of 2587 nos/m^2. The model fitted indicating the relation between production and stocking density was obtained as

$$y = -255.49875 + 1.283395\ x\ -0.000248085x^2$$

In the case of M. equidens, the survival remained almost uniform upto 3000/m^2. A survival of 89.2% was obtained at 3000/m^2 which then decreased to 58% as stocking density increased to 3500/m^2. The production/m^2 steadily increased upto a stocking density of 3000/m^2 and then decreased. The production increased from 454/m^2 to 2677/m^2 as the stocking density increased to 3500 nos. A calculated maximum production (Fig. 63) of 2315 nos was obtained at a stocking density of 3230 nos/m^2. The model $y = -470.7755 + 1.725 x - 0.000267x^2$ is fitted to represent the relationship between production and stocking density.

In M. rosenbergii where the post larvae were provided with no additional substrata and biofilter, the percentage of survival remained almost the same upto 1500/m^2 and thereafter reduced. A survival of 53.18% was obtained at 1500/m^2, 42.5% at 2000/m^2 and 32.19% at 2500/m^2. The calculated maximum production/m^2 (Fig.58) of 836 nos was obtained at a stocking density of 1806/m^2.

The corresponding model is fitted as follows.

$y = -161.8 + 1.105 x - 0.000306x^2$

In M. equidens the percentage of survival remained almost uniform upto 2000/m^2 and then decreased as the stocking density increased to 2500/m^2. The production increased from 500 to 2000/m^2 and then it started decreasing to 1408 nos for 2500/m^2 and reached 746 nos for 3500/m^2 stocking densities. The

model fitted was as follows:

$$y = -336.4605 + 1.509\ x - 0.0003318x^2$$

When the clam meat was substituted by a commercial prawn feed, the maximum survival and production/m^2 were only 21.4% and 2646 obtained for a stocking density of 500/m^2 and 1500/m^2 respectively, for M. rosenbergii. The calculated maximum production was 306 nos at an optimum stocking density of 1824 nos/m^2 (Fig. 61). The model fitted for estimating optimum stocking density was as

$$y = 36.38196428 + 0.295117142\ x - 0.000080885x^2$$

In M. equidens the survival percentage remained uniform at around 60% upto a stocking density of 2000/m^2 and then decreased as the stocking density increased. A survival of 46.4% was obtained at 2500/m^2 and 16.6% at 3500/m^2 stocking densities. The production/m^2 increased from 321 to 1253 /m^2 as the stocking density increased from 500 to 2000.m^2 and then decreased. It can be seen from Fig. 65 that the calculated maximum production of 1866 was obtained at a stocking density of 2146/m^2. The model fitted was as follows:

$$y = 365.5607134 + 1.398728577\ x - 0.000325885x^2$$

The results of comparison between treatments using Student's 't' test showed that the difference is not significant between the treatments with clam meat + additional substrate + biofilter and

clam meat + additional substrata + no biofilter; showing that the biofilter has not helped in increasing the survival rate in M. rosenbergii but M. equidens (Table 23 & 24).

In all other cases the differences were found to be significant, showing that clam meat as feed and twigs as additional substrata are two important factors influencing the survival of Macrobrachium post larvae in nursery rearing.

A comparison of the results obtained under different densities feeds and water management techniques shows that upto a certain level, the percentage of survival has no relation to the stocking density when other conditions are optimum. For the different treatments of survival was uniform upto 1500/m^2 and 2000/m^2 for M. rosenbergii and M. equidens respectively (Table 23 & 24) and after that there was a drastic reduction. The reduction in survival beyond certain stocking density shows that the rate of survival after a level will be density dependent as suggested by Smith and Sandifer (1979). Their suggestion of stocking M. rosenbergii post larvae at a rate of 2000/m^2 as reasonable is holding good here also. The survival rate of 76.04% obtained in the present experiment wherein biofilter + artificial substrata + clam meat were provided is quite appreciable, but not quite high as the 84.92% survival obtained by Smith and Sandifer (1979) wherein they have used stocking densities of 1000 and 1500/m^2 in 2 to 6 m diameter circular tanks provided with biological filter, water circulation,

formulated feed and an elaborate additional habitat constructed out of wood, PVC pipes and plastic meshes, meant to provide edges.

The decrease in the survival rate in those tanks, where additional substrata were not provided in the present experiment, points to the fact that cannibalism is serious at the PL stages and that provision of additional substrate is able to help in boosting the chances of survival of post larvae. At a stocking density of 2000/m^2 the rate of survival of M. rosenbergii is only 42.5% in the tanks without additional substrata, in comparison to the 74.85% obtained in those with additional substrata and in the case of M. equidens at a stocking density of 3000 nos/m^2, the survival was 89.2% and 37% for treatments with additional substrata and without additional substrata respectively. Sandifer and Smith (1975) also have found the provision of additional substrate as helping to increase the stocking density. They found that the survival rate of 90% which was obtained with a stocking density of 100-200/m^2 could be maintained at a higher stocking density of 500/m^2 also, when artificial substrates were added. But Mulla and Rouse (1985) obtained no significant difference in the survival rate when additional substrates were used. It may be due to the very low stocking density of 40/m^2, they have used in their experiments.

The drastic reduction in the survival rate of post larvae fed with the commercial prawn feed, shows that clam meat is a better

alternative to the commercial prawn feed tried, as higher survival rate of 32.19% to 63.76% and 26.1% to 73.8% was obtained with clam meat compared to 7.41% to 21.41% and 16.6% to 63.3% with commercial prawn feed for M. rosenbergii and M. equidens, respectively. Sherief (1989) also found that a formulated feed, containing 40% dry clam meat together with 25% rice bran, 25% ground nut oil cake and 10% tapioca powder, gives better survival and growth for M. rosenbergii post larvae in comparison to the one wherein fish meat was used instead of clam meat.

Thus it can be seen that provision of additional substratum is of much importance in boosting the survival rate of the post larvae. The rate of survival remains almost uniform upto 2000/m^2 and 3000/m^2 for M. rosenbergii and M. equidens respectively.

CHAPTER IX

SUMMARY AND CONCLUSIONS

IX. SUMMARY AND CONCLUSION

The present study is an investigation into the different aspects of reproductive biology and mass larval rearing of two commercially important species of Macrobrachium namely M. rosenbergii and M. equidens.

The landing data continuously collected for one year from two major landing centres in Vembanad lake revealed that the berried females (egg carrying) of M. rosenbergii are available from June to April with peak in November/December months and those of M. equidens from November to May with peak in April/May. Salinity changes of the backwater influence the fishery of both species. M. rosenbergii has been observed in the catches when the salinity ranges between 0 and 18 ppt, and M. equidens when salinity is above 15 ppt only. The fecundity was estimated by collecting the final stage eggs from more than 200 specimens in each species. The mean fecundity of M. rosenbergii has been found to be 50799 eggs (598 eggs/g body weight) and that of M. equidens is 4698 eggs (987 eggs/g body weight). Significant linear relationships exist between fecundity and morphometric characters namely total length, rostral length, claw length and body weight in M. rosenbergii, the r^2 being 0.642665, 0.600976, 0.605570 and 0.627336 respectively. However such relationships have not been observed in M. equidens.

The effect of eyestalk ablation, exogenous glands and the

influence of parameters like light, salinity, age and season, and also the presence of males in inducing ovary development in brood stock prawns have been studied. In both M. rosenbergii and M. equidens bilateral ablation does not make effective berries whereas unilateral ablation produces a significant increase in the berries. Out of the 25 prawns ablated and observed for a period of 45 days, 22 berries were obtained in M. rosenbergii and 33 in M. equidens whereas in the control it was only 16 and 25 respectively.

Out of the 3 exogenous glands examined namely HCG, progesterone and ovaprim, crude HCG injected at a rate of 0.01 µg/g body weight once in 15 days showed a significant increase ($P<0.05$) in the development of ovary.

Both salinity and the presence of males do not have influence in inducing ovary developments. On the other hand, maturation of the ovary has been found to be fast in prawns kept in darkness than those kept in 12D and 12L photoperiod. From the 30 number of specimens kept in darkness for 30 days, 33 berries were obtained for M. rosenbergii and 50 for M. equidens, whereas in the control it was respectively 19 and 13 only.

Both age and season influence the ovary development. During the summer months prawns of only less than one year of age became berried but during winter all the age groups responded and produced eggs.

In the larval rearing, after sreening 16 combinations of feed, it has been found that a combination of live and prepared feed gives better results than the live or prepared feed alone. A wet feed prepared by blending thelly (Metapenaeus dobsoni) meat with chicken egg in the ratio 100 g meat: 1 egg, fed along with Brine Shrimp Nauplii has given better larval survival. A survival of 67% and 84% have been obtained for M. rosenbergii and M. equidens respectively.

The results of the larval rearing experiments revealed that an initial stocking density of 75 nos/litre and 100 nos/litre is optimum for M. rosenbergii and M. equidens respectively. The tanks exposed to direct sunlight as well as those kept under transparent roofing sheets, permitting approximately 50% day light, are found to increase the larval survival.

The tolerance of the larvae to nitrogenous wastes, therapeutic chemicals, antibiotics and pH have been found to increase as the size of the larvae advances. However salinity tolerance is seen more in young larvae.

The Oxygen consumption and ammonia excretion of the larvae increases with the progress of larval stages. In the normal physiology of the larvae ammonia is excreted as a nitrogenous waste by the larvae. However at the time of hyperosmotic transfer, the ammonia is absorbed from the external environment.

A survival of 50% with a production of 39 nos/litre for M. rosenbergii and a survival of 70% with a production 70 nos/litre for M. equidens have been obtained in the mass larval rearing experiments. In a completely closed circulatory system incorporating biofilters, commercial rearing of Macrobrachium larvae in artificial brackishwater gives as good a result as in natural brackishwater. The performance of M. equidens in artificial brackishwater is found to be better than that in the natural brackishwater. In the commercial rearing using artificial brackishwater, potassium bromide has been found to be a vital ingredient which influences the survival rate than the other ingredients do. The possibility of reusing the water medium used once in the closed recirculating system, for a second and third culture has been tried in a statistically designed experiment with 5 replicates. It was seen that the percentages of survival registered in 1st, 2nd and 3rd set of rearing were respectively 51, 39 and 14 in M. rosenbergii, and 83, 40 and 14 in M. equidens. The sharp decline in the percentage of survival shows that it is not worthwhile to use the same water repeatedly for subsequent rearing.

Observations made in the number of culture experiments in M. rosenbergii and in M. equidens show that the uniformity of larval stages taken at a defenite interval during the larval rearing period can be utilized for predicting the final survival in a particular culture. A linear regression model fitted to establish the relationship between the coefficient of variation in the larval stages and the production has given a relation $y = 94.4952 - 6.0834x$ for

M. rosenbergii and y = 95.6299 - 4.5114x for M. equidens, where 'y' is the post larval production and 'x' is the coefficient of variation.

In the nursery rearing, seasoned twigs used as additional substratum and clam meat as feed gave a high survival of 76% at a stocking density of 1500 nos/m^2 for M. rosenbergii. For M. equidens a survival of 90% was obtained at a stocking density of 3000 nos/m^2. Using the second degree model fitted for the data, a stocking density of 1800 nos/m^2 and 2125 nos/m^2 have been calculated as the maximum survival rate in M. rosenbergii and M. equidens respectively.

The experimental study to find optimum salinity for nursery rearing has given the maximum survival of 89% at 12 ppt for M. rosenbergii and 84% at 18 ppt for M. equidens. Based on the results of the above experiments it can be concluded that in the nursery rearing, a salinity of 12 ppt and stocking density of 1800 nos/m^2 for M. rosenbergii, and 18 ppt and 2125 nos/m^2 for M. equidens, with seasoned twigs used as additional substraum and clam meat as feed can be recommended.

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APPENDIX

TABLE - 1

SEX RATIO AND OCCURRENCE OF BERRIED FEMALES

Month	Total No. obtained	Percentage		Male-Female Ratio	Chi.square value	Probability	Total berried femeles	Total spent females	Percentage		Salinity (ppt)
		Male	Female						berried in female Population	Spent in female Population	
1	2	3	4	5	6	7	8	9	10	11	12
Murinjapuzha											
June	300	71	29	1:0.408	17.64	<0.001	22	-	25	-	2-0
July	480	58	42	1:0.724	2.56	0.1<P<0.2	88	-	44	-	0
Aug.	514	43	57	1:1.326	1.96	0.1<P<0.2	218	-	74	-	0
Sep.	780	30	70	1:2.333	16.00	<0.001	499	2	91	0.3	0-3
Oct.	1470	27	73	1:2.704	21.16	<0.001	878	16	81	1.5	3-0
Nov.	2220	26	74	1:2.846	23.04	<0.001	1201	390	73	24.0	0-4
Dec.	1650	18	82	1:4.556	40.96	<0.001	612	708	45	52.0	6-9
Jan.	1380	15	85	1:5.667	49.00	<0.001	320	826	27	70.0	9-11
Feb.	770	11	89	1:8.091	60.84	<0.001	180	550	26	80.0	11-15
March	280	8	92	1:11.5	70.56	<0.001	42	206	16	80.0	15-17
April	98	4	96	1:24	84.64	<0.001	8	80	8	85.0	17-18
May	-	-	-	-	-	-	-	-	-	-	18-20
Total	9942	25.51	74.49	1:2.92	23.99	<0.001	4068	2778	55	38	-

Contd...A-2

1	2	3	4	5	6	7	8	9	10	11	12
Panangad											
June	28	61	39	1:0.639	4.84	0.02<P<0.05	4	-	40	-	2-0
July	356	52	49	1:0.961	0.04	0.08<P<0.90	116	-	66	-	0
Aug.	860	36	64	1:1.778	7.84	0.001<P<0.01	389	-	70	-	0-3
Sept.	471	35	65	1:1.857	9.00	0.001<P<0.01	278	-	90	-	3-16
Oct.	1264	32	68	1:2.125	12.96	<0.001	764	94	89	11	17-0
Nov.	1008	32	68	1:2.125	12.96	<0.001	601	80	88	12	0-13
Dec.	416	30	70	1:2.333	16.00	<0.001	221	60	75	21	13-18
Jan.	-	-	-				-	-	-	-	-
Feb.	-	-	-	-	-		-	-	-	-	-
March	-	-	-	-	-		-	-	-	-	-
April	-	-	-	-	-		-	-	-	-	-
May	-	-	-	-	-		-	-	-	-	-
Total	4403	34	66	1.941	10.24	<0.001	2373	234	81.13	8	
Grand Total	14345	28	72	1:2.571	19.36	<0.001	6441	3012	62.35	29.16	

Contd...A-3

M. equidens

1	2	3	4	5	6	7	8	9	10	11	12
Murinjapuzha											
June											2-0
July											0
Aug.											0
Sept.											0-3
Oct.											3-0
Nov.											0-4
Dec.											6-9
Jan.											9-11
Feb.	278	63	37	1:0.587	6.76	0.001<P<0.01	76	-	43	-	11-15
Mar.	393	52	48	1:0.923	0.16	0.80<P<0.90	142	22	70	11	15-17
April	576	41	59	1:1.439	3.24	0.05<P<0.10	162	41	69	17	17-18
May	618	40	60	1:1.5	4.00	0.02<P<0.05	191	47	61	19	18-20
Total	1865	46.2	53.8	1:1.165	0.578		531	110	52.9	10.97	

Contd...A-4

1	2	3	4	5	6	7	8	9	10	11	12
Panangad											
June											2-0
July											0
Aug.											0-3
Sept.											3-16
oct.											17-0
Nov.											0-13
Dec.	194	68	32	1:0.471	59.04	<0.001	67	-	51	-	13-18
Jan.	271	51	49	1:0.961	0.04	0.80<P<0.90	82	14	59	10	21-26.2
Feb.	371	45	55	1:1.222	1.00	0.30<P<0.50	102	19	61	11	21-28.7
March	402	44	56	1:1.273	1.44	0.20<P<0.30	114	30	65	17	26.8-31.6
April	526	44	56	1:1.273	1.44	0.20<P<0.30	139	37	60	16	29-32
May	514	42	58	1:1.381	2.56	0.10<P<0.20	122	48	49	19	30-32
Total	2278	46.66	53.34	1:1.143	0.45		626	148	51.52	12.18	
Grand Total	4143	46.46	53.54	1:1.152	0.50		1157	258	52.16	11.63	

Concld.

TABLE - 2

REGRESSION ANALYSES OF FECUNDITY-MORPHOMETRIC RELATIONSHIPS

a. M. rosenbergii

FECUNDITY - TOTAL LENGTH

Constant	-1.60269
Std Error of Y est	0.184640
r^2	0.642665
No. of Observations	34
Degrees of Freedom	32
X Coefficient	2.719879
Std Error of Coef.	0.358524

FECUNDITY-RCSTRAL LENGTH

Constant	-0.30881
Std Error of Y Est	0.195114
r^2	0.600976
No. of Observations	34
Degrees of Freedom	32
X Coefficient	2.654414
Std Error of Coef.	0.382352

FECUNDITY-CLAW LENGTH

Constant	2.777517
Std Error of Y Est	0.193987
r^2	0.605570
No. of Observations	34
Degrees of Freedom	32
X Coefficient	1.338241
Std Error of Coef.	0.190924

FECUNDITY - BODY WEIGHT

Constant	3.170644
Std Error of Y Est	0.188559
r^2	0.627336
No. of Observations	34
Degrees of Freedom	32
X Coefficient	0.764466
Std Error of Coef.	0.104157

Contd..A-6

b. M. equidens

FECUNDITY - TOTAL LENGTH

Constant		-3.29915
Std Error of Y Est		0.263882
r^2		0.323465
No. of Observations		63
Degrees of freedom		61
X Coefficient	3.790813	
Std Error of Coef.	1.196337	

FECUNDITY - ROSTRAL LENGTH

Constant		-3.27145
Std Error of Y Est		0.293311
r^2		0.281437
No. of Observations		63
Degrees of Freedom		61
X Coefficient	3.801191	
Std Error of Coef.	1.684571	

FECUNDITY - CLAW LENGTH:

Constant		2.835891
Std Error of Y Est		0.265048
r^2		0.317472
No. of Observations		63
Degrees of freedom		61
X Coefficient	1.112380	
Std Error of Coef.	0.355918	

FECUNDITY - BODY WEIGHT

Constant		0.580849
Std Error of Y Est		0.211370
r^2		0.088354
No. of Observations		63
Degrees of Freedom		61
X Coefficient	1.568629	
Std Error of Coef.	1.397487	

Concld.

TABLE - 3

EFFECT OF EYE STALK ABLATION IN MACROBRACHIUM ON OVARY DEVELOPMENT

a. Cumulative number of berries obtained in 40 days

Day	Unilateral ablation				Bilateral ablation				No ablation			
	R1	R2	R3	R4	R1	R2	R3	R4	R1	R2	R3	R4
M. rosenbergii												
15th Day	1	3	-	3	-	-	-	-	-	-	1	1
30th Day	3	4	2	4	-	-	-	-	2	2	2	2
40th Day	5	6	7	4	-	-	-	-	4	4	4	4
Total		22								16		
Average		6								4		
Percentage		110%								80%		
M. equidens												
15th day	4	5	4	4	5	5	5	5	4	4	3	4
30th day	5	5	5	5	15	15	15	15	5	5	5	5
45th day	8	10	7	8	28	30	16	27	6	6	6	6
Total		33				116				25		
Average		8								6		
Percentage		165%								125%		

All bilaterally ablated M. rosenbergii died before 15th day.

All berries in the bilaterally ablated M. equidens were shed and new ones extruded in the next day.

Contd..A-8

b. Pairwise comparison using 't' test.

	Pairs	't' value	Significance
1.	Between unilaterally ablated and unablated *M. rosenbergii* at df 6.	2.32379	0.025<P<.05
2.	Between unilaterally ablated and unablated *M. equidens* at df 6	2.954196	0.01<P<.025

Concld.

TABLE - 4

EFFECT OF EXOGENOUS GLANDS ON OVARY MATURATION

a. Analysis of Variane

Source	SS	DF	MS	F	Table Value
Between samples	4.00	4	1.00	2.500	3.48 (at 5%)
Within Samples	4.00	10	0.40		
Total	8.00	14			

b. Pairwise comparison using 't' test

Pairs	't' value	Significance
1. 17,∝hydroxyprogesterone and HCG	1.732051	0.05<P<0.10
2. 17,∝hydroxyprogesterone and Ovaprim at df 4	0.500	0.20<P<0.35
3. 17,∝hydroxyprogesterone and control I at df 4	0.500	0.20<P<0.35
4. 17,∝hydroxyprogesteron and Control II at df 4	0.500	0.20<P<0.35
5. HCG and Ovaprim at df 4	4.000	0.005<P<0.01
6. HCG and Control I at df 4	4.000	0.005<P<0.01
7. HCG and Control II at df 4	4.000	0.005<P<0.01
8. Ovaprim and Control I at df 4	0.000	N.S.
9. Ovaprim and Control Ii at df 4	0.000	N.S.
10. Control I and Control Ii at df 4	0.000	N.S.

N.S. - NOT SIGNIFICANT.

TABLE - 5

EFFECT OF LIGHT ON OVARY MATURATION IN MACROBRACHIUM

a. Cumulative number of berries obtained in 30 days

Date	Dark shed						Open shed					
	R1	R2	R3	R4	R5	R6	R1	R2	R3	R4	R5	R6
M. rosenbergii												
1.10.90	0	0	0	0	0	0	0	0	0	0	0	0
15.10.90	4	5	4	5	5	4	3	4	3	3	2	1
30.10.90	5	7	5	6	5	5	3	4	3	3	3	3
(Cumulative number)												
Total		33						19				
M.equidens												
1.10.90	0	0	0	0	0	0	0	0	0	0	0	0
15.10.90	5	5	5	5	5	5	4	3	3	4	4	4
30.10.90	8	8	9	9	8	8	6	5	5	6	7	6
Total		50						33				

b. Pairwise comparison using 't' test

Pairs	't' value	Significance
1. Between dark shed and open shed in M.rosenbergii at df 10.	6.139406	P<0.005
2. Between dark shed and open shed in M.equidens at df 10	6.708203	P<0.005

TABLE - 6

INFLUENCE OF AGE AND SEASON ON THE OVARY DEVELOPMENT OF M.ROSENBERGII

a. Pairwise comparison of different age groups in summer using 't' test

t-value between 5-6 month and 10-11 month age group in summer season at df 6	't' value	Significance
	11.597155	P<0.005

b. Analysis of variance of Ovary maturation in different age groups of Macrobrachium rosenbergii in winter months.

Sources	SS	DF	MS	F	Table value
Between samples	163.25	3	54.42	76.824	3.49(at5%)
Within samples	8.50	12	0.71		

Pairwise comparison of different age groups in winter using 't' test

	Pairs	df	't' value	Significance
1.	Between 5-6 and 10-11 month age group	6	7.602631	P<0.001
2.	Bwtween 5-6 and 17-18 month age groups	6	14.10029	P<0.001
3.	Between 5-6 and wild groups	6	15.788942	P<0.001
4.	Between 10-11 and 17-18 month age groups	6	3.872983	P<0.001
5.	Between 10-11 month and wild group	6	6.139678	P<0.001
6.	Between 17-18 month and wild group	6	2.781518	0.01< P<0.025

TABLE - 7

PROXIMATE COMPOSITION OF THE LARVAL FEEDS

Sl. No.	Feed	Crude protein	Fat	Ash	Carbohydrate (by difference)
1.	Thelly meat+egg	69.12	7.18	4.26	19.44
2.	Clam meat+egg	56.69	13.21	4.58	25.52
3.	Squilla + egg	57.26	21.03	11.01	10.7
4.	Squid + egg	62.97	22.91	1.41	12.71
5.	Egg custard	52.77	40.64	3.66	2.93
6.	Fish roe	49.11	17.70	2.31	30.88
7.	Carp meat + egg	56.61	10.20	3.32	29.87
8.	Soyabean + egg	45.92	16.68	2.00	35.40
9.	Moina	58.50	21.42	10.45	7.75
10.	Artemia	60.12	20.20	8.06	9.81
11.	Artificial feed	56.4	19.4	10.4	13.8

TABLE - 8

INGREDIENTS USED FOR PREPARING ARTIFICIAL FEED

	Ingredients	Quantity
1.	Wheat flour	- 50g
2.	*Macrobrachium* egg	- 500g
3.	Non-fat dry milk	- 100g
4.	Vitamin & Mineral mix	- 2g
5.	Antibiotics	- 500mg
6.	Jelly	- 20g
7.	Agar	- 2g

TABLE - 9

PAIR-WISE COMPARISON OF THE PERCENTAGE OF SURVIVAL USING DIFFERENT FEEDS IN LARVAL REARING OF MACRBRACHIUM USING 't' TEST (P=0.05)

M.rosenbergii

Feed No. 13 12 4 16 14 5 9 11 6 7 3 10 15 8 2 1

M. equidens

Feed No. 13 16 14 4 5 11 10 9 6 7 3 8 15 2 12 1

Feed M. rosenbergii				M. equidens			
1.	Thelly meat + BSN	9.	Egg Custard + BSN	1.	Thelly meat + BSN	9.	Egg custard + BSN
2.	M.idella + BSN	10.	Moina + thelly meat	2.	M.idella + BSN	10.	Artemia alone
3.	Squid + BSN	11.	Artemia alone	3.	Squid + BSN	11.	Moina alone
4.	Squilla + BSN	12.	Egg Custard alone	4.	Squilla + BSN	12.	Moina + Thelly meat
5.	Clam + BSN	13.	Carp roe alone	5.	Clam + BSN	13.	Egg custard alone
6.	Carp meat + BSN	14.	Moina alone	6.	Carp meat + BSN	14.	Carp roe alone
7.	Soyabean + BSN	15.	Artificial feed + BSN	7.	Soyabean + BSN	15.	Artificial feed+BSN
8.	Carp roe + BSN	16.	Artificial feed alone	8.	Carp roe + BSN	16.	Artificial feed alone

TABLE - 10

EFFECT OF STOCKING DENSITY ON LARVAL REARING

Pair-wise comparison of production per litre using 't' test

M. rosenbergii

10/1 25/1 50/1 75/1 100/1 150/1 175/1 200/1

M. equidens

10/1 25/1 50/1 75/1 100/1 150/1 175/1 200/1 225/1 250/1

TABLE 11

VALUE OF LC50 OF MACROBRACHIUM LARVAE AT 95% CONFIDENCE LEVEL TO NITROGENOUS WASTES, THERAPEUTIC CHEMICALS, ANTIBIOTICS AND PHYSICOCHEMICAL PARAMETERS

Treatment	Duration	M. rosenbergii				M. equidens
		Ist stage	5th Stage	10th Stage	PL	5th Stage
Nitrogenous Wastes NH_3-N_3	12hr	33.20 < 33.25 > 33.30	38.48 < 38.52 > 38.56	38.58 < 38.59 > 38.61	63.92 < 63.94 > 63.95	63.98 < 64.00 > 64.02
	24hr	32.84 < 32.95 > 33.01	33.64 < 33.69 > 33.74	34.64 < 34.68 > 34.72	62.29 < 62.31 > 62.32	27.64 < 27.67 > 27.69
	36hr	30.90 < 30.95 > 31.00	33.29 < 33.35 > 33.41	34.01 < 34.06 > 34.11	57.83 < 57.83 > 57.85	26.52 < 26.55 > 26.58
	48hr	30.39 < 30.45 > 30.52	32.89 < 32.95 > 33.01	30.49 < 30.57 > 30.65	57.23 < 57.25 > 57.27	21.96 < 22.04 > 22.11
	60hr	29.24 < 29.31 > 29.38	30.70 < 30.75 > 30.80	27.56 < 27.64 > 27.71	54.65 < 54.67 > 54.68	14.76 < 14.84 > 14.92
	72hr	21.70 < 21.79 > 21.87	26.59 < 26.63 > 26.68	26.33 < 26.41 > 26.48	52.36 < 52.37 > 52.38	6.89 < 7.14 > 7.40
NO_3-N	12hr	157.82<158.18>158.53	150.20<150.65>151.97	299.29<299.33>299.37	292.65<292.69>292.73	170.06<170.17>170.28
	24hr	108.65<108.87>109.10	117.35<117.71>118.08	246.87<246.95>247.04	285.18<185.21>285.24	158.70<158.80>158.91
	36hr	24.62< 24.77> 24.92	27.99 < 28.12 > 28.25	170.10<170.16> 170.21	203.57<203.63>203.69	16.45 < 16.58 > 16.72
	48hr	19.88 < 20.01 > 20.13	24.45 <24.58 > 24.70	130.74<130.82> 130.20	171.10<171.16>171.22	13.99 < 14.11 > 14.24
	60hr	12.25 < 12.41 > 12.56	15.35 <15.47 > 15.58	130.01<130.12> 130.19	143.47<143.53>143.59	10.91 <11.01 > 11.12
	72hr	11.00 < 11.16 > 11.32	11.91 <12.04> 12.17	126.95<126.98 > 127.02	136.21<136.27>136.32	6.62 < 6.80 > 6.98
NO_2-N	12hr	134.34<134.62 >134.92	139.77<140.05>140.32	194.99<195.27 > 195.56	217.04<217.34>217.63	38.33 <88.38 > 88.42
	24hr	95.86 < 96.08 > 96.30	71.36 < 71.52 > 71.69	182.56<182.80> 183.05	111.91<112.22>112.53	37.17 < 37.20 > 37.23
	36hr	23.63 < 23.70 > 23.78	12.54 <12.79 > 12.87	10.79 < 10.93 > 11.08	28.26 < 28.41 > 28.56	23.61 < 23.73 > 23.84
	48hr	17.06 < 17.14 > 17.22	11.69 <11.79 > 11.89	8.16 < 8.41 > 8.66	20.78 <20.94 > 21.11	15.24 <15.48 > 15.71
	60hr	12.69 < 12.87 > 13.05	8.12 < 8.22 > 8.33	7.23 < 7.49 > 7.75	16.03 <16.19 > 16.34	10.86 <11.02 > 11.18
	72hr	9.95 <10.09> 10.23	6.72 < 6.85 > 6.97	6.67 < 6.87 > 7.07	12.35 <12.46>120.58	8.79 < 8.79 > 9.15

Contd... A-17

Treatment	Duration	M.rosenbergii				M.equidens
		Ist stage	5th Stage	10th stage	PL	5th stage
Antibiotics						
Chloromycetin	12hr	183.48<183.57>183.65	216.59<216.65>216.71	195.54<195.58 > 195.62	962.35<962.38>962.42	417.09<417.16>417.23
	24hr	112.19<112.25 >112.32	188.20<188.29>188.37	126.72<126.80 >126.89	908.41<908.46>908.50	355.42<355.48>355.55
	36hr	78.29 < 78.37 > 78.45	140.10<140.16>140.21	58.66 < 58.85 > 59.05	775.51<775.58>775.65	264.58<264.69>264.80
	48hr	67.03 < 67.12 > 67.21	130.20<130.26> 130.32	49.81 < 50.03 > 50.24	545.38<545.80>546.02	168.87<168.99>169.10
	60hr	44.41 < 44.60 > 44.79	97.15 < 97.29> 97.44	45.88 <46.12 > 46.34	322.30<322.51>322.72	136.43<136.55>136.68
	72hr	42.25 < 42.39 > 42.54	61.34 < 61.53> 61.73	43.09 < 43.33 > 43.56	178.11<178.46>178.80	118.91<119.05>119.19
Oxytetra-cylin	12hr	150.77<150.83>150.88	154.71<154.76>154.81	153.14<153.21 > 153.29	409.87<409.91>409.95	125.41<125.51>125.61
	24hr	22.06 < 22.08> 22.09	20.475<20.478>20.48	34.85 < 35.01 > 35.17	379.38<379.43>379.49	22.79 < 22.85 > 22.91
	36hr	17.86 < 17.90 > 17.94	16.32 < 16.36 > 16.40	21.37 < 21.42 > 21.48	247.59<247.85>248.11	18.64 < 18.75 > 18.86
	48hr	13.50 < 13.60 > 13.70	14.10 < 14.21 >14.31	34.85 <35.01 > 35.17	230.66<230.78>230.90	17.24 < 17.32 > 17.40
	60hr	10.20 < 10.42 > 10.63	10.28 < 10.45 > 10.63	17.96 < 18.05 > 18.14	158.55<158.79 >159.03	10.41 < 10.61 > 10.81
	72hr	9.06 < 9.24 > 9.43	9.33 < 9.44 > 9.66	15.97 < 16.10 > 16.23	86.01< 86.31 > 86.61	9.72 < 9.01 > 10.11
Therapeutic	12hr	260.66<260.75 > 20.84	213.10<213.21>213.33	161.18 <161.22> 161.25	322.79<322.80 >322.81	387.17 <387.22 >387.26
	24hr	198.12<198.19>198.27	128.68<128.82>128.96	111.42<111.48 >111.54	257.93<257.95>257.97	347.11<347.18>347.26
	36hr	131.41<131.46>131.50	95.47 < 95.63 > 95.79	66.42 < 66.51 > 66.60	154.86<154.90>154.95	188.58<188.66>188.75
	48hr	116.45<116.49>116.53	77.76 < 78.00> 78.24	45.12 < 45.25 > 45.38	134.35<134.39>134.43	133.04<133.12>133.20
	60hr	78.05 <78.21> 78.37	71.22 <71.41 > 71.60	39.27 < 39.44 > 39.61	112.0 <112.07> 112.14	116.82<116.90>117.0
	72hr	73.59 < 73.75> 73.72	50.17 <50.43 > 50.68	37.68 < 37.88 > 38.08	84.01 <84.09 > 84.12	106.72<106.81>106.90

Contd... A-18

Treatment	Duration Hrs	*M. rosenbergii* (larvae - 5th stage)		Post larvae	
		Lower limit	Upper limit	Lower limit	Upper limit
Physicochemical Parameters	12	5.19<5.22>5.25	10.12<10.14>10.15	5.20<5.21>5.23	10.32 <10.33 > 10.34
	24	5.38<5.39>5.41	9.83 < 9.84 > 9.85	5.42 <5.43>5.544	10.19 < 10.20 > 10.21
	36	5.61<5.63 >5.64	9.77 < 9.78 > 9.79	5.42<5.43>5.544	9.98 < 9.99 > 10.00
	48	5.63<5.65>5.67	9.54 < 9.55 > 9.56	5.42<5.43>5.444	9.57 < 9.58 > 9.59
	60	5.67<5.70>5.72	9.51 < 9.52 > 9.53	5.42 <5.43>5.544	9.532 < 9.535 > 9.538
pH	72	5.74<5.76>5.79	9.48 < 9.49 > 9.50	5.42<5.43>5.544	9.52 < 9.525> 9.529
		Ist stage			
Salinity	12	0 < 0 > 0	35.65<35.68 > 35.71	0 < 0 > 0	37.7547<37.76 > 36.77
	24	0 < 0 > 0	33.41<33.46 > 33.51	0 < 0 > 0	37.5168<37.5246>36.77
	36	0 < 0 > 0	31.78<31.85 > 31.95	0 < 0 > 0	36.248<36.26 > 36.27
	48	0 < 0 > 0	27.25<27.35 > 27.45	0 < 0 > 0	36.02 <36.03 > 36.04
	60	0 < 0 > 0	21.27<21.31 > 21.36	0 < 0 > 0	35.698<35.70 > 35.71
	72	0 < 0 > 0	21.27<21.31 > 21.36	0 < 0 > 0	35.27 <35.28 >35.29
		5th Stage			
	12	0 < 0 > 0	29.01<29.07 > 29.13		
	24	0 < 0.069>1.05	28.3 <28.36 > 28.41		
	36	0 < 0.46>1.45	27.7 < 27.78 > 27.83		
	48	4.04<4.09>4.14	27.7 < 27.78 > 27.83		
	60	4.4 <4.49>4.59	27.7 < 27.78 > 27.83		
	72	5.16<5.38>5.6	27.7 < 27.78 > 27.83		

Contd..A-19

Treatment	Duration Hrs	*M. equidens* (larvae - 5th stage)		Post larvae	
		Lower limit	Upper limit	Lower limit	Upper limit
Physicochemical Parameters	12	5.11<5.12>5.13	10.280<10.285>10.29	4.72 < 4.74 >4.77	10.514 < 10.517 >10.521
	24	5.1665.1675.168	10.049<20.050>10.0518	5.06 < 5.08 > 5.10	10.514 < 10.517 > 10.52
	36	5.56<5.58>5.60	10.049<10.050>10.0518	5.16 < 5.18 > 5.20	10.289 < 10.296 > 10.302
	48	5.68<5.69>5.70	10.04 <10.045>10.048	5.33 < 5.34 >5.36	10.066 <10.075 > 10.085
pH	60	5.8965.8985.899	10.027<10.031>10.034	5.65 < 5.66 > 5.67	9.841 < 9.847 > 9.852
	72	5.8965.8985.899	10.027<10.031>10.034	5.65 < 5.66 >5.67	9.841 < 9.847 > 9.852
		Ist stage			
Salinity	12	0.0 < 0.0 >0.0	37.58 <37.59 > 37.60	0 < 0 > 0	35.53 < 35.53 > 35.54
	24	3.16<3.21>3.26	36.86 <36.87 > 36.88	0 < 0 > 0	35.02 < 35.03 > 35.04
	36	6.85<6.90>6.95	36.14 <36.15 > 16.16	0 < 0 > 0	34.58 < 34.59 > 34.60
	48	8.35<8.40>8.45	35.43 < 35.44 > 35.45	0 < 0 > 0	34.02 < 34.03 > 34.04
	60	8.56<8.61 >8.66	35.43 <35.44 > 35.45	0 < 0 > 0	33.17 < 33.18 > 33.19
	72	8.95<8.98 >8.73	35.26 <34.26 >34.27	0 < 0 > 0	32.83 < 32.84 > 33.85
		5th Stage			
	12	6.94<6.98>7.03	36.94 < 36.95 > 36.95		
	24	7.67<7.71>7.74	36.27 < 36.29 >36.31		
	36	8.56<8.61>8.65	35.22 < 35.24 > 35.25		
	48	8.94<8.98>9.02	34.66 < 34.67 >34.68		
	60	9.04<9.06>9.09	34.26 <34.27 >34.28		
	72	9.58<9.59>9.60	33.62 <33.63 >33.63		

Concld.

TABLE 12

OXYGEN CONSUMPTION AND AMMONIA EXCRETION

M . rosenbergii

Stages	O_2 Consumption in 12ppt	Ammonia Excretion in 12 ppt	O_2 consumption imemdiately after change of salinity from 12 to 18ppt	NH_3 excretion immediately after change of salinity from 12 to 15ppt (Quick transfer)
Egg	0.0002604	0.0001755	0.00036058	-0.0003518
I stage	0.00061995	0.0044267	0.00067864	-0.001326
V Stage	0.002475	0.006034	0.003469326	-0.028015
X stage	0.00665535	0.05531167	0.0075264	-0.05172
PL	0.0139526	0.02471069	0.017954725	-0.0863955
Juvenile	0.0860124	0.10344	0.10605	-0.1034400

M.equidens

Stages	O_2 Consumption in 18 ppt	Ammonia excretion in 18 ppt	O_2 consumption immediately after quick transfer to 21 ppt	NH_3 excretion immediately after transfer to 21 ppt
Egg	0.0003518	0.0003518	0.0005914	-0.0008796
I Stage	0.0012549	0.002309	0.0016128	-0.00383
V Stage	0.002309	0.0402266	0.0028672	-0.03448
X stage	0.00478244	0.043100	0.0049920	-0.05172
PL	0.0132366	0.080600	0.0189017	-0.087008
Juvenile	0.0692079	0.12068	0.07758	-0.13002

TABLE - 13

INGREDIENTS USED FOR PREPARING ONE LITRE OF SYNTHETIC BRACKISHWATER

Ingredients	Quantity (g)	
	12 ppt	18 ppt
Common Salt	9.4	14.1
Magnesium chloride	1.9	2.85
Sodium sulphate	1.56	2.34
Calcium Chloride	0.44	0.66
Potassium chloride	0.26	0.39
Sodium bicarbonate	0.08	0.12
Potassium bromide	0.04	0.06
Boric Acid	0.001	0.0015

TABLE 14

MASS LARVAL REARING WITH WET FORMULATED FEED PREPARED FROM PRAWN MEAT AND EGG AND ONE TIME FEEDING WITH ARTEMIA NAUPLII IN DAILY WATER EXCHANGE SYSTEM.

Expt No.	Larval density & total no.of larvae	Day of Ist settle-ment	Day of final settling/ disconding	Percentage survival	Production PL/1	Temp.°C Range	Temp.°C mean	pH Range	pH mean	NH_3 (PPm) Range	NH_3 (PPm) mean
M.rosenbergii											
1.	75/1 75,000	24	34	42.2	36	29-30	29.5	7.5-8.5	8.3	1.3-1.6	1.4
2.	75/1 7500	26	36	48.3	36	29-30	29.5	7.8-8-5	8.2	1.0-1.2	1.1
3.	75/1 7500	24	34	53.3	40	29-30	29.5	7.6-8.4	8.1	1.2-1.3	1.2
4.	75/1 75000	25	35	64.8	56	29-30	29.5	7.4-8.6	8.2	1.1-1.3	1.1
5.	75/1 75000	24	34	41.2	31	29-30	29.5	7.6-8.7	8.3	1.0-1.2	1.1
Aver-age	75/1 75000	25	35	49.96	39		29.5		8.2		1.2
M . equidens											
1)	100/1 100,000	21	31	68.1	68	30-31	30	7.6-8.2	8.1	1.2-1.5	1.3
2)	100/1 10,00,000	22	32	64.3	64	29-30	30	7.2-7.8	7.6	1.2-1.4	1.3

Contd..A-23

Expt. No.	Larval density& total no. of larvae	Day of Ist settle-ment	Day of final settling/ disconding	Percentage survival	Production PL/1	Temp.°C Range	Temp.°C Mean	pH Range	pH Mean	NH_3 (PPM) Range	NH_3 (PPM) Mean
3)	100/1 10,00,000	20	30	72.4	72	29-30	29	76-83	7.8	1.2-1.3	1.3
4)	100/1 10,00,000	19	29	74.4	74	29-31	30	7.5-8.4	8.2	1.3-1.4	1.3
5)	100/1 10,00,000	22	32	71.2	71	30-31	30	7.5-8.4	8.3	1.3-1.4	1.3
Average	100/1 100,000	21	31	70.08	70		30		8		1.3

Concld.

TABLE - 15

PAIRWISE COMPARISON OF THE PERCENTAGE OF SURVIVAL

IN LARVAL REARING USING NATURAL AND SYNTHETIC BRACKISH WATER

't' TEST

Species	't' value	Table value t_{18} 0.05	significance
M. rosenbergii	0.6977	3.2	N.S.
M equidens	5.944	3.2	S

NS - Not significant

S - Significant.

TABLE 16

ANALYSIS OF VARIANCE OF PERCENTAGE OF SURVIVAL WHEN THE WATER WAS REUSED (Ist, 2nd, 3rd)

M. rosenbergii

Source of Variation	df	Sum of square due to error	Mean sum of squares	Mean sum of squares due to error	Calcu-ated F value	Table value
Between treatments	2	0.00058307	0.00002812	0.0048589	57.8805	2,8=4.459 (at 5% level)8.65 (at 1% level)

Significant at 5% and 1% levels.

Pairwise comparison applying 't' test

Pairs	't' value	df	table value
1. Ist use and 2nd use	4.8541	8	3.355 (1% level) 2.306 (5% level)
2. Ist use and 3rd use	10.7426	8	"
3. 2nd use and 3rd use	5.8885	8	"

M . equidens

Between treatments	2	148.06	2467.61	18.51	133.33	2,8=4.459 (at 5% level) 8.65 (at 1% level)

Significant at 5% and 1% level

Pairwise camparison applying 't' test

Pairs	't' value	df	Table value
1. Ist use and 2nd use	3.0524	8	3.555 (1% level) 2.306 (5% level)
2. Ist use and 3rd use	5.4467	8	"
3. 2nd use and 3rd use	22.357	8	"

TABLE - 17

FREQUENCIES IN PERCENTAGES OF GROWTH STAGES DECIDED BY MOULTING OF M . ROSENBERGII, BASED ON THE POOLED SAMPLES OBSERVED ON EVERY 5TH DAY TOGETHER WITH THE CALCULATED VALUES OF MEAN, S.D. AND C.V.

Stage	Day 5	10	15	20	25	30	35	40
1.								
2.	6.3							
3.	35.0	4.4	0.3					
4.	58.7	19.1	3.5	0.3				
5.		23.1	13.7	3.9	1.5	1.8	1.7	1.0
6.		53.4	22.6	10.0	7.3	5.2	5.0	3.0
7.		35.6	35.6	15.2	17.3	13.3	13.3	9.0
8.			24.3	20.2	16.2	27.6	18.2	22.0
9.			0.06	26.8	18.6	26.3	28.5	22.0
10.			0.03	20.7	25.3	16.9	22.7	25.0
11.				3.7	11.3	4.8	10.5	18.0
12.				0.06	4.5	0.1	0.2	-
Mean Stage	3.52	5.26	6.63	8.32	8.87	8.42	8.78	9.08
S.D.	0.6127	0.9143	1.1162	1.5089	1.6621	1.3249	1.4315	1.4048
C.V.	17.41	17.38	16.83	18.13	18.74	15.74	16.30	15.47

TABLE 18

FREQUENCIES IN PERCENTAGES OF GROWTH STAGES DECIDED BY MOULTING OF M. EQUIDENS LARVAE, BASED ON THE POOLED SAMPLES, OBSERVED ON 5TH AND 10TH DAYS, TOGETHER WITH THE SDs AND CVs.

Stage	Day 5	Day 10
1.		
2.	1.4	0.7
3.	31.1	0.7
4.	67.5	4.6
5.		12.9
6.		20.9
7.		20.4
8.		31.6
9.		8.2
mean stage	3.66	6.81
SD	0.5021	1.4264
CV	13.72	20.95

TABLE - 19

M . ROSENBERGII

GROWTH AND SURVIVAL AT DIFFERENT SALINITY LEVELS

IN NURSERY REARING

Salinity (ppt)	Survival (%)	Increment in wt. (g)	Increment in length (mm)
0	23	0.042	17.80
	21	0.0675	19.00
	23	0.0402	17.25
	Average 22.33	0.0499	18.02
3	70	0.0425	18.00
	71	0.0392	18.50
	76	0.0700	19.75
	Average 76.33	0.0506	18.75
6	75	0.0460	17.80
	85	0.0428	17.60
	62	0.0340	16.60
	Average 74	0.0295	17.33
9	73	0.0240	15.80
	83	0.0348	17.00
	84	0.0248	16.20
	Average 80	0.0295	16.33
12	85	0.0304	16.80
	93	0.0302	16.40
	89	0.0304	17.40
	Average 89	0.0303	16.87
15	86	0.0302	16.39
	81	0.0340	16.60
	90	0.0301	16.20
	Average 85.67	0.0314	16.40
18	74	0.0295	16.20
	72	0.0289	16.00
	69	0.0299	16.30
	Average 71.67	0.0294	16.17

TABLE - 20

M. EQUIDENS

GROWTH AND SURVIVAL AT DIFFERENT SALINITY LEVELS IN NURSERY REARING

Salinity(ppt)		Survival (%)	Increment in wt. (g)	Increment in length (mm)
0		2.0	0.01880	14.80
		2.8	0.01900	14.75
		2.6	0.01831	14.50
	Average	2.5	0.0187033	14.68
6		46	0.03102	17.65
		49	0.03401	18.20
		51	0.03123	18.00
	Average	48.67	0.0320866	17.95
12		56	0.04246	19.20
		60	0.03982	18.63
		62	0.04020	18.00
	Average	59.33	0.0408266	18.61
18		84	0.02923	16.84
		80	0.02810	16.76
		89	0.02348	16.71
	Average	84.33	0.0269366	16.77
24		80	0.02320	15.98
		79	0.021120	16.00
		81	0.02002	15.72
	Average	80	0.0214733	15.9
30		72	0.02461	16.20
		76	0.02672	18.81
		70	0.02881	16.98
	Average	72.67	0.0267133	17.33
36		14	0.02021	15.62
		20	0.01920	15.31
		12	0.01776	15.42
	Average	15.33	0.0190556	15.45

TABLE - 21

M. ROSENBERGII

RESULTS OF PAIR-WISE COMPARISON OF SURVIVAL RATE (%) INCREMENT IN WEIGHT (G) AND LENGTH (mm) AT DIFFERENT SALINITY LEVELS IN NURSERY REARING

Between pairs (salinity levels)	Survival rate	Increment in wt.	Increment in length
0 - 3	**	N.S.	N.S.
0 - 6	**	N.S	N.S
0 - 9	**	N.S	*
0 - 12	**	N.S	N.S
0 - 15	**	N.S	*
0 - 18	**	N.S	*
3 - 6	N.S	N.S	N.S
3 - 9	N.S	N.S	*
3 - 12	**	N.S	*
3 - 15	*	N.S	*
3 - 18	N.S	N.S	*
6 - 9	N.S	N.S	N.S
6 - 12	**	*	N.S
6 - 15	N.S	N.S	N.S
6 - 18	N.S	*	*
9 - 12	N.S	N.S	N.S
9 - 15	N.S	N.S	N.S
9 - 18	N.S	N.S	N.S
12 - 15	N.S	N.S	N.S
12 - 18	**	*	N.S
15 - 18	**	N.S	N.S

** Significant at 1% level

* Significant at 5% level

N.S Not significant

TABLE - 22

M . EQUIDENS

RESULTS OF PAIR-WISE COMPARISON OF SURVIVAL RATE (%) INCREMENT IN WEIGHT (G) AND LENGTH (mm) AT DIFFERENT SALINITY LEVELS IN NURSERY REARING

Between pairs (salinity levels)	Survival rate	Increment in wt.	Increment in length
0 - 6	**	**	**
0 - 12	**	**	**
0 - 18	**	**	**
0 - 24	**	*	**
0 - 30	**	**	*
0 - 36	**	N.S	**
6 - 12	**	**	N.S
6 - 18	**	N.S	**
6 - 24	**	**	**
6 - 30	**	*	N.S
6 - 36	**	**	**
12 - 18	**	**	**
12 - 24	**	**	**
12 - 30	**	**	N.S
12 - 36	**	**	**
18 - 24	N.S	N.S	**
18 - 30	*	N.S	N.S
18 - 36	**	*	**
24 - 30	*	*	N.S
24 - 36	**	N.S	*
30 - 36	**	**	N.S

** Significant at 1% level

* Significant at 5% level

N.S Not significant

TABLE - 23

ANALYSIS OF VARIANCE GIVING DIFFERENCES AMONG FEEDS AND STOCKING DENSITIES OF M. ROSENBERGII IN NURSERY REARING

Source of variation	Degrees of freedom	SVM of squared	Mean SVM of squares	F	F (5%)
Between feeds	3	1206902	4623.01	*1872.69	2.84
Between densities	4	1402.82	350.71	* 163.25	2.61
Interaction	12	453.1	37.76	* 17.58	2.00
Between cells	19	13924.94			
Error	40	85.93	2.15		
Total	59	14010.87			

* Significant

PAIRWISE COMPARISON - M. ROSENBERGII

MEANS OF DENSITIES

1 (500/m^2)	2(1000/m^2)	3(1500/m^2)	4(2000/m^2)	5.(2500/m^2)
48.2283	47.5883	46.7358	45.1117	35.7408

Densities compared	Numerical difference of the means				C.D. at 5%
(1) - (5)	48.2283	- 35.7408	=	12.4875	1.6186
(1) - (4)	48.2283	- 45.1117	=	3.1166	1.6186
(1) - (3)	48.2283	- 46.7358	=	* 1.4925	1.6186
(1) - (2)	48.2283	- 47.5883	=	* 0.64	1.6186
(2) - (5)	47.5883	- 35.7408	=	11.8475	1.6186
(2) - (4)	47.5883	- 45.1117	=	2.4766	1.6186
(2) - (3)	47.5883	- 46.7358	=	*0.8525	1.6186
(3) - (5)	46.7358	- 35.7408	=	10.9950	1.6186
(3) - (40	46.7358	- 45.1117	=	1.6241	1.6186
(4) - (5)	45.1117	- 35.7408	=	9.3709	1.6186

* Difference between the densities in (1) and (2), (1) and (3), and (2) and (3) is not significant, i.e., between 500/m and 1000/m^2, 500/m^2 and 1500/m^2, and 1000/m^2 and 1500/m^2. The densities upto 1500/m^2 have no effect on survival rate.

Contd..A-33

MEANS OF FEEDS

Clam meat + additional substrate + Bio filter (1)	Clam meat + additional substrate (2)	Clam meat (3)	Commercial prawn feed (4)
57.238	55.918	44.8307	21.8573

Feeds compared	Numerical difference of the means	C.D. at 5%
(1) - (4)	57.238 - 21.8573 = 35.3807	1.4478
(1) - (20	57.238 - 44.8307 = 12.4073	1.4478
(1) - (20	57.238 - 55.918 = *1.38	1.4478
(2) - (4)	55.918 - 21.8573 = 34.0607	1.4478
(2) - (3)	55.918 - 44.8307 = 11.0873	1.4478
(3) - (4)	44.8307 - 21.8573 = 22.9734	1.4478

* The difference between clam meat + additional substrate + Bio filter and clam meat + additional substrate is not significant.

Concld.

TABLE - 24

ANALYSIS OF VARIANCE GIVING DIFFERENCES AMONG FEEDS AND STOCKING DENSITIES OF M. EQUIDENS IN NURSERY REARING

Source of Variation	Degrees of freedom	Sum of squares	Mean sum of squares	F
Between feeds	3	11100.76	3700.25	*1051.5808
Between desities	6	7031.87	1171.98	*333.0671
Interaction	18	1129.48	62.75	*17.833
Between cells	27	19262.11		
Error	56	197.05	3.5188	
Total	83	19459.16		

$F_{3,56} = 3.68$; $F_{6,56} = 3.15$; $F_{18,56} = 2.29$

* Differences are highly significant.

Contd..A-35

PAIRWISE COMPARISON-M. EQUIDENS

MEANS OF DENSITIES

1(500/m^2)	2(1000/m^2)	3(1500/m^2)	4(2000/m^2)	5(2500/m^2)	6(3000/m^2)	7(3500/m^2)
65.2742	65.2283	64.8375	63.5617	59.5617	51.0933	38.6725

Densities compared	Numerical differences of the means				CD at 5%
(1) - (7)	65.2742	- 38.6725	=	26.6017	2.0439
(1) - (6)	65.2742	- 51.0933	=	14.1809	2.0439
(1) - (5)	65.2742	- 59.4475	=	5.8266	2.0439
(1) - (4)	65.2742	- 63.5617	=	*1.7125	2.0439
(1) - (3)	65.2742	- 64.8475	=	*0.4367	2.0439
(1) - (2)	65.2742	- 65.2283	=	*0.0459	2.0439
(2) - (7)	65.2283	- 38.6725	=	26.5558	2.0439
(2) - (6)	65.2283	- 51.0933	=	14.1350	2.0439
(2) - (5)	65.2283	- 59.4475	=	5.7808	2.0439
(2) - (4)	65.2283	- 63.5617	=	*1.6666	2.0439
(2) - (3)	65.2283	- 64.8375	=	*0.3908	2.0439
(3) - (7)	64.8375	. 38.6725	=	26.1650	2.0439
(3) - (6)	64.8375	- 51.0933	=	13.7442	2.0439
(3) - (5)	64.8375	- 59.4475		5.39	2.0439
(3) - (4)	64.8375	- 63.8375	=	*1.02758	2.0439
(4) - (7)	63.8375	- 38.6725	=	24.8892	2.0439
(4) - (6)	63.8375	- 51.0933	=	12.4684	2.0439
(4) - (5)	63.8375	- 59.4475	=	4.1142	2.0439
(5) - (7)	59.4475	- 38.6725	=	20.7750	2.0439
(5) - (6)	59.4475	- 51.0933	=	8.3542	2.0439
(6) - (7)	51.0933	- 38.6725	=	12.4208	2.0439

The differences between (1) & (4); (1) & (3); (1) & (2); (2) & (4), (2) & (3); and (3) & (4) are found to be not significant. At levels of stocking densities 500-2000, not much effect on survival rate.

MEANS OF FEEDS

Clam meat	Clam meat + additional substrate	Clam meat + additional substrate Bio filter	Commercial prawn feed
1	2	3	4

Feeds compared	Numerical diffrences of means				C.D
(1) - (4)	70.5576	- 43.6705	=	26.8871	1.5451
(1) - (3)	70.5576	- 50.9962	=	19.5614	1.5451
(1) - (2)	70.5576	- 68.8414	=	1.7162	1.5451
(2) - (4)	68.8414	- 43.6705	=	25.1710	1.5451
(2) - (3)	68.8414	- 50.9962	=	17.8452	1.5451
(3) - (4)	50.9962	- 43.6705	=	7.3257	1.5451

The feed effects are significant.

Concld.

MAP OF VEMBANAD LAKE SHOWING COLLECTION CENTRES

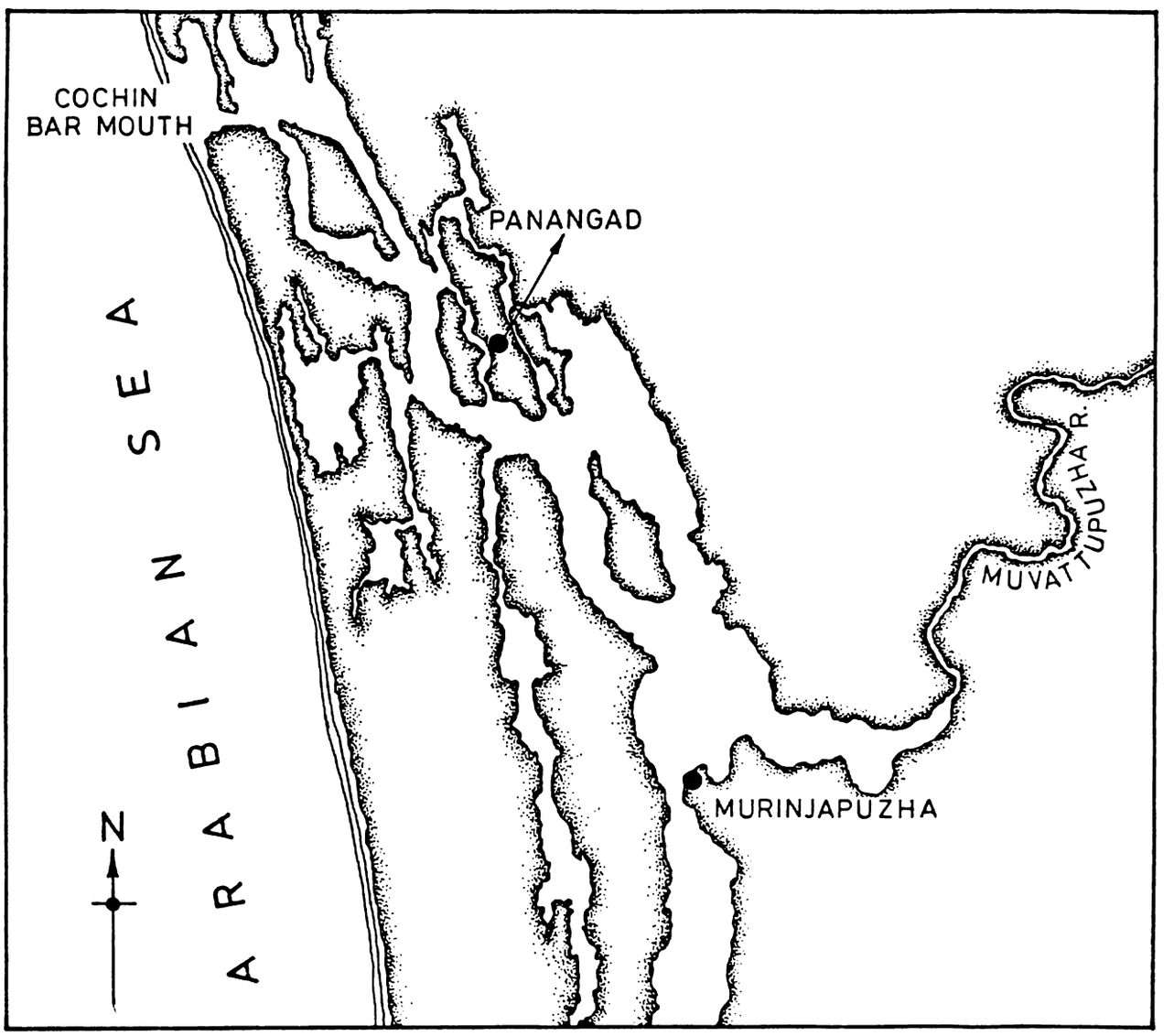


FIG: 1

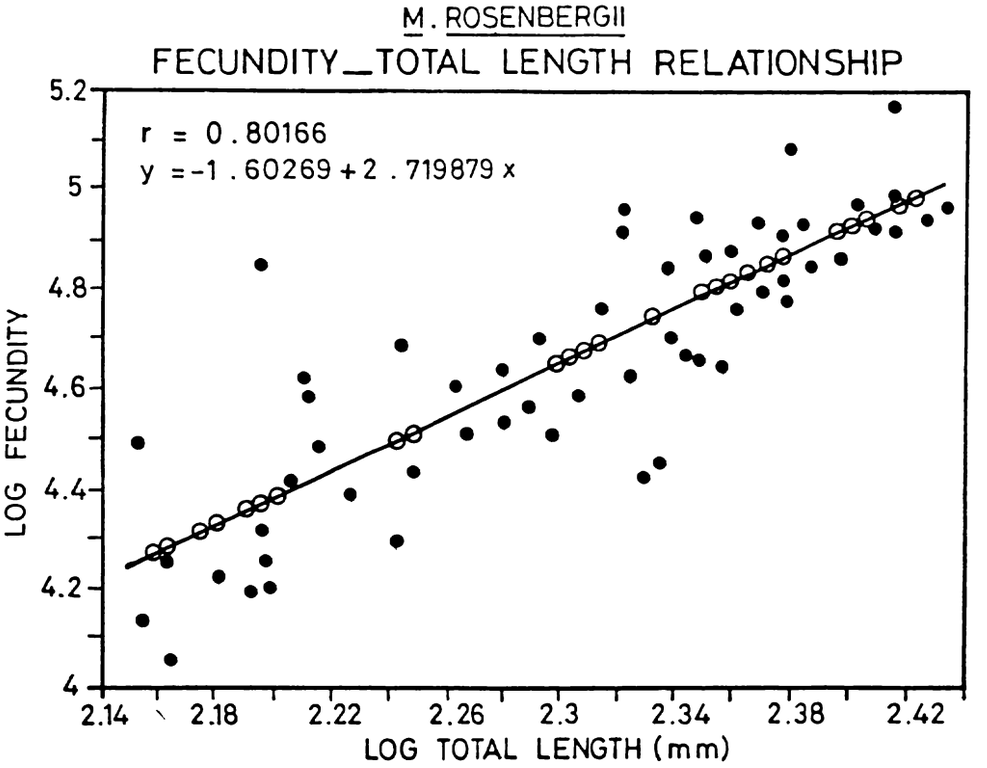

M. ROSENBERGII
FECUNDITY_TOTAL LENGTH RELATIONSHIP
r = 0.80166
y = -1.60269 + 2.719879 x
LOG FECUNDITY
5.2
5
4.8
4.6
4.4
4.2
4
2.14
2.18
2.22
2.26
2.3
2.34
2.38
2.42
LOG TOTAL LENGTH (mm)

FIG: 2

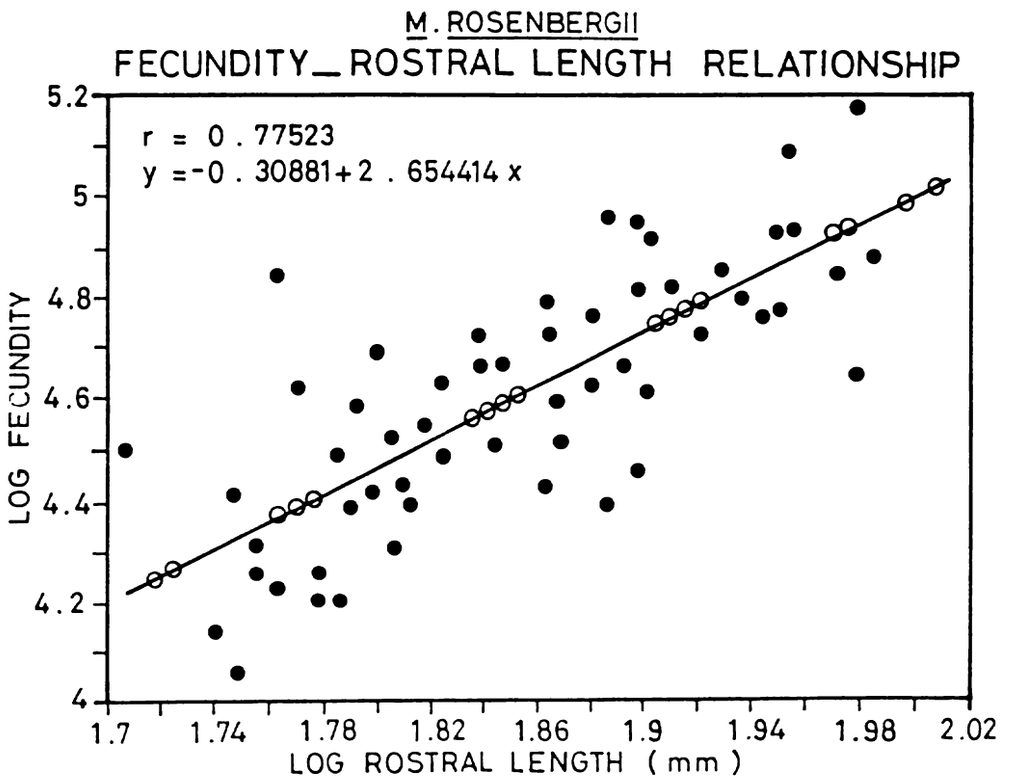

M. ROSENBERGII
FECUNDITY_ROSTRAL LENGTH RELATIONSHIP
r = 0.77523
y = -0.30881 + 2.654414 x
LOG FECUNDITY
5.2
5
4.8
4.6
4.4
4.2
4
1.7
1.74
1.78
1.82
1.86
1.9
1.94
1.98
2.02
LOG ROSTRAL LENGTH (mm)

FIG: 3

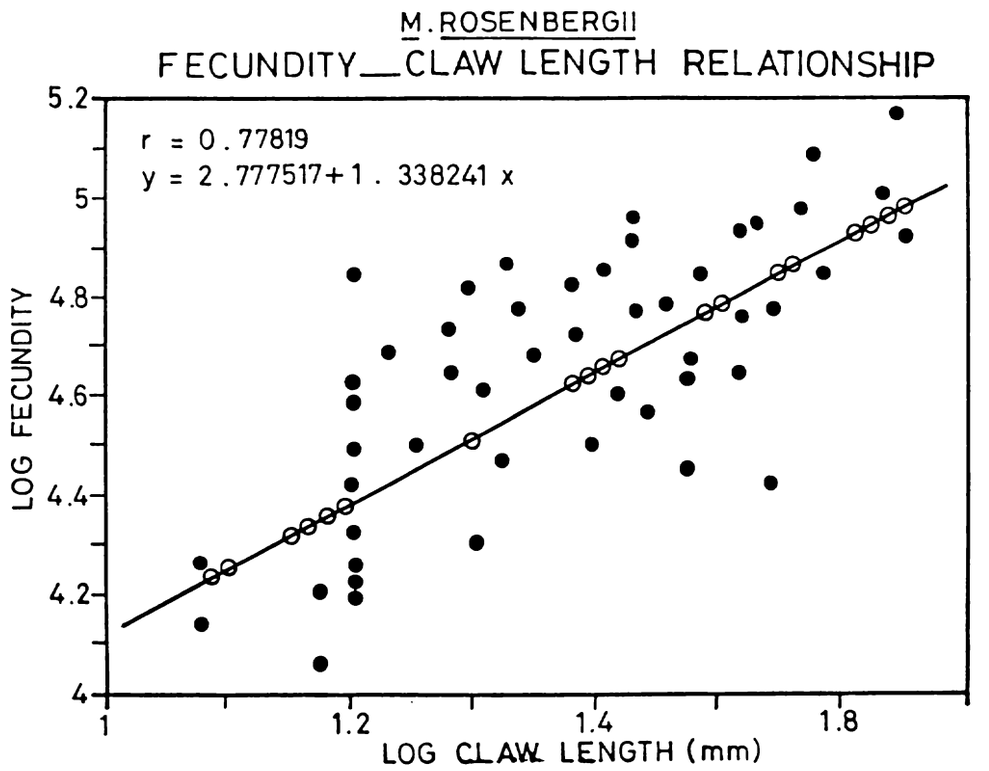

M. ROSENBERGII
FECUNDITY__CLAW LENGTH RELATIONSHIP
r = 0.77819
y = 2.777517+1.338241 x
LOG FECUNDITY
LOG CLAW LENGTH (mm)
5.2
5
4.8
4.6
4.4
4.2
4
1
1.2
1.4
1.8

FIG: 4

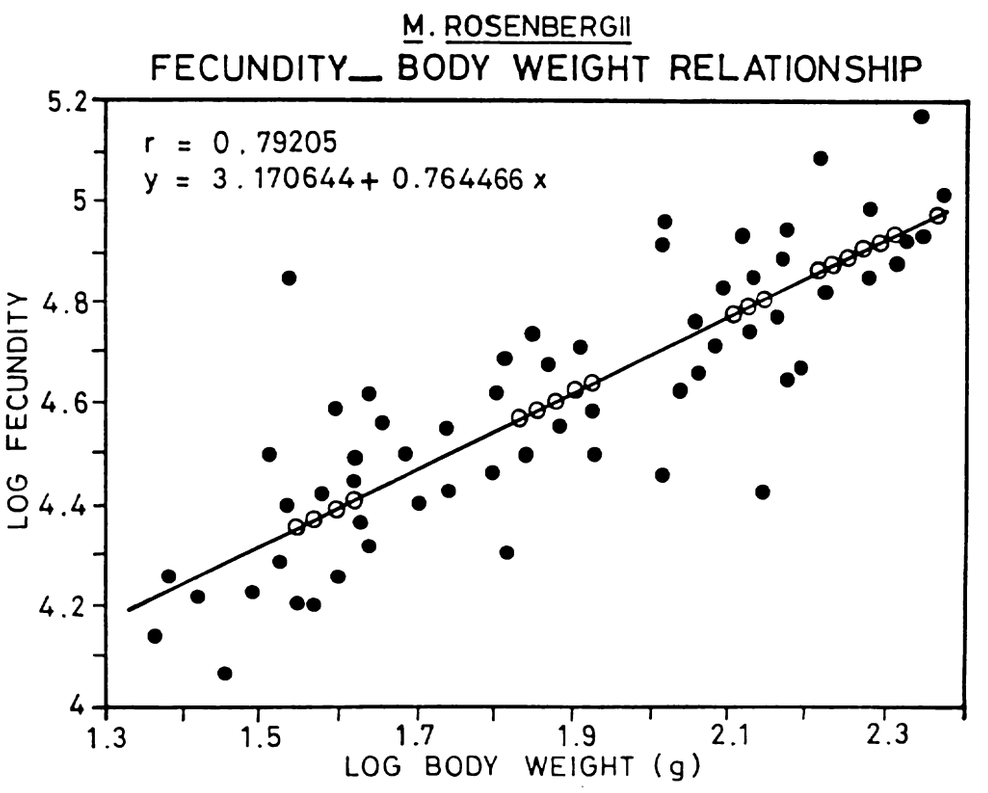

M. ROSENBERGII
FECUNDITY_ BODY WEIGHT RELATIONSHIP
r = 0.79205
y = 3.170644+ 0.764466 x
LOG FECUNDITY
LOG BODY WEIGHT (g)
5.2
5
4.8
4.6
4.4
4.2
4
1.3
1.5
1.7
1.9
2.1
2.3

FIG: 5

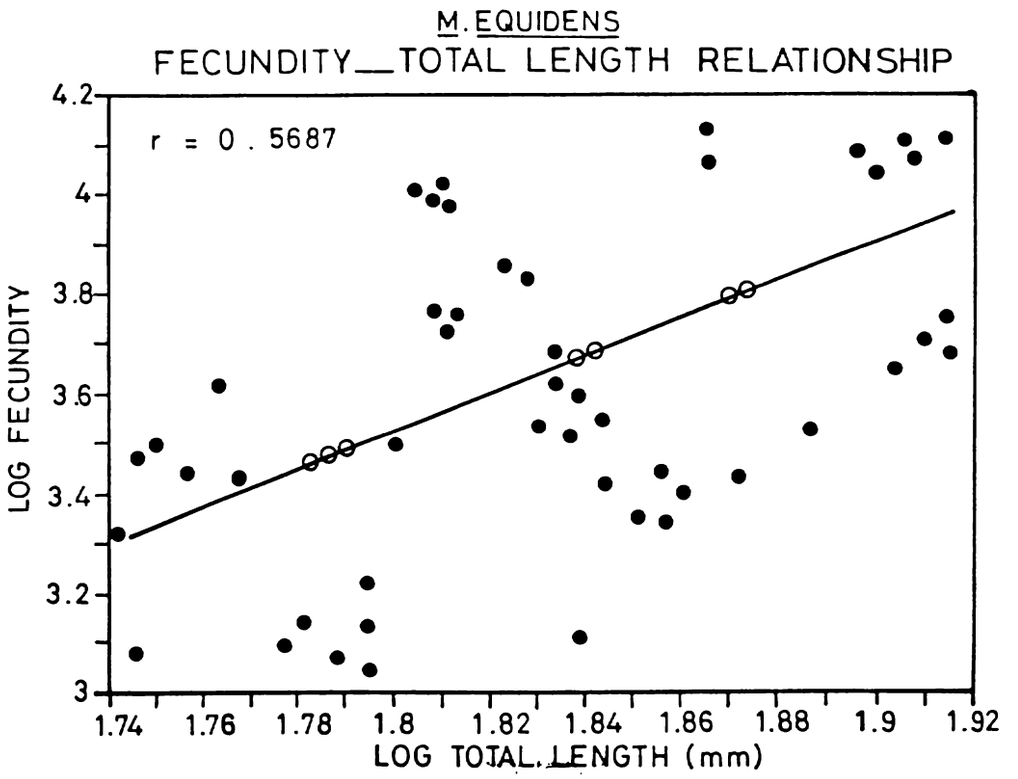

M. EQUIDENS
FECUNDITY__TOTAL LENGTH RELATIONSHIP
r = 0 . 5687
LOG FECUNDITY
4.2
4
3.8
3.6
3.4
3.2
3
1.74
1.76
1.78
1.8
1.82
1.84
1.86
1.88
1.9
1.92
LOG TOTAL LENGTH (mm)

FIG: 6

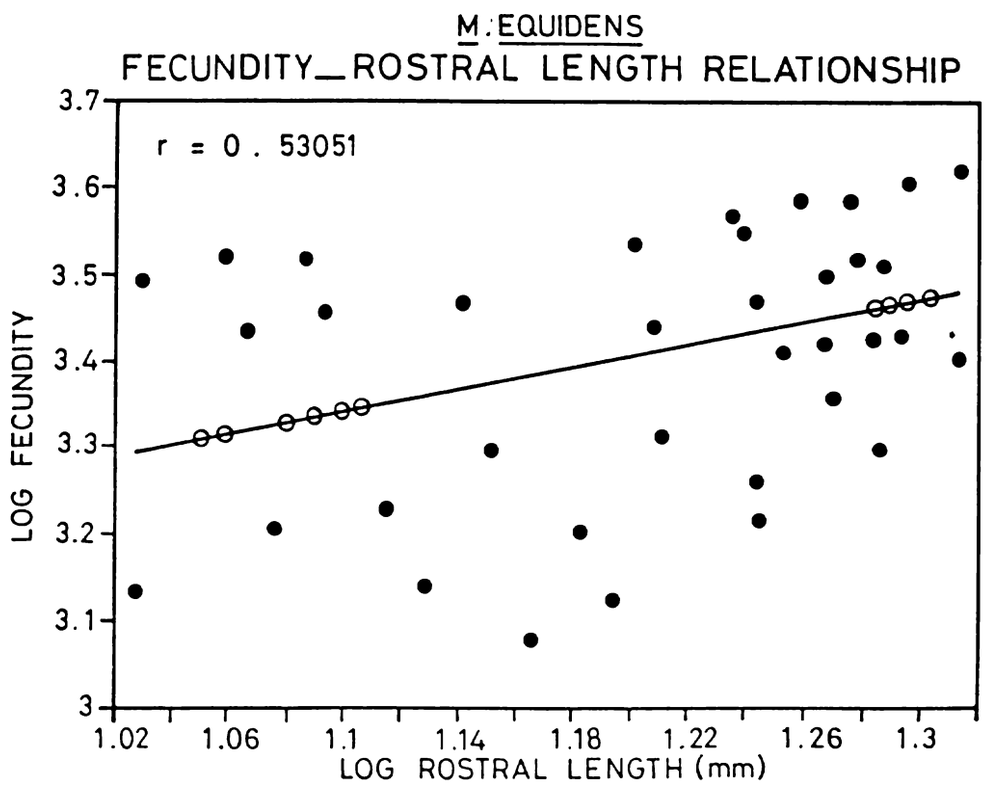

M. EQUIDENS
FECUNDITY__ROSTRAL LENGTH RELATIONSHIP
r = 0 . 53051
LOG FECUNDITY
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3
1.02
1.06
1.1
1.14
1.18
1.22
1.26
1.3
LOG ROSTRAL LENGTH (mm)

FIG: 7

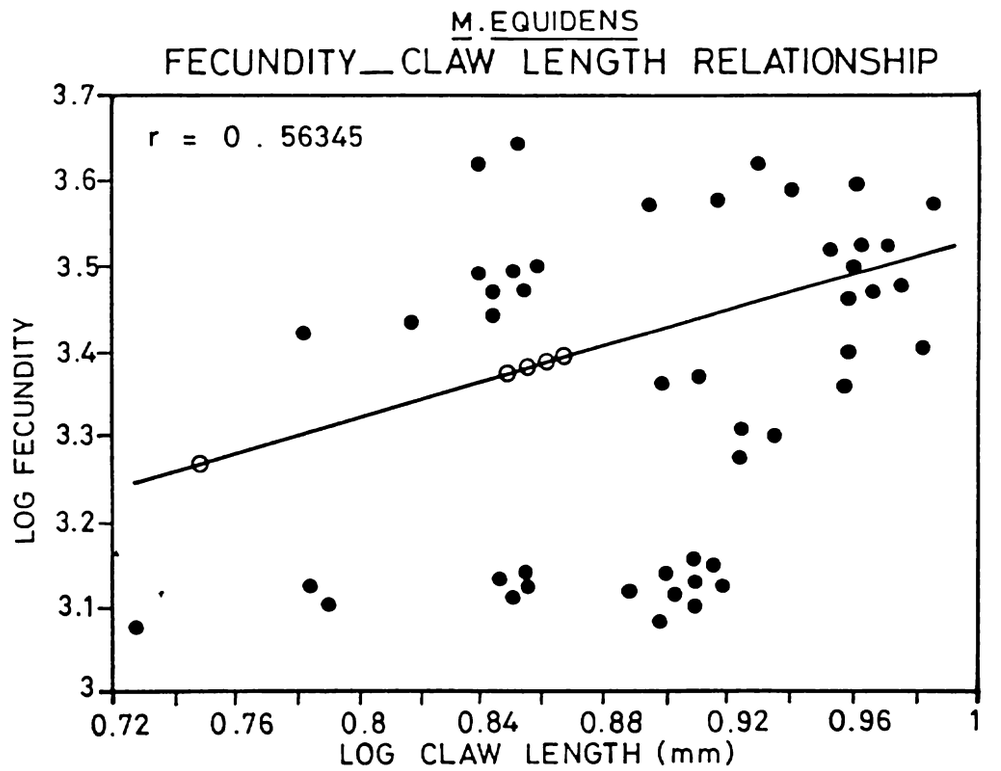

M. EQUIDENS
FECUNDITY_CLAW LENGTH RELATIONSHIP
r = 0 . 56345
LOG FECUNDITY
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3
0.72
0.76
0.8
0.84
0.88
0.92
0.96
1
LOG CLAW LENGTH (mm)

FIG: 8

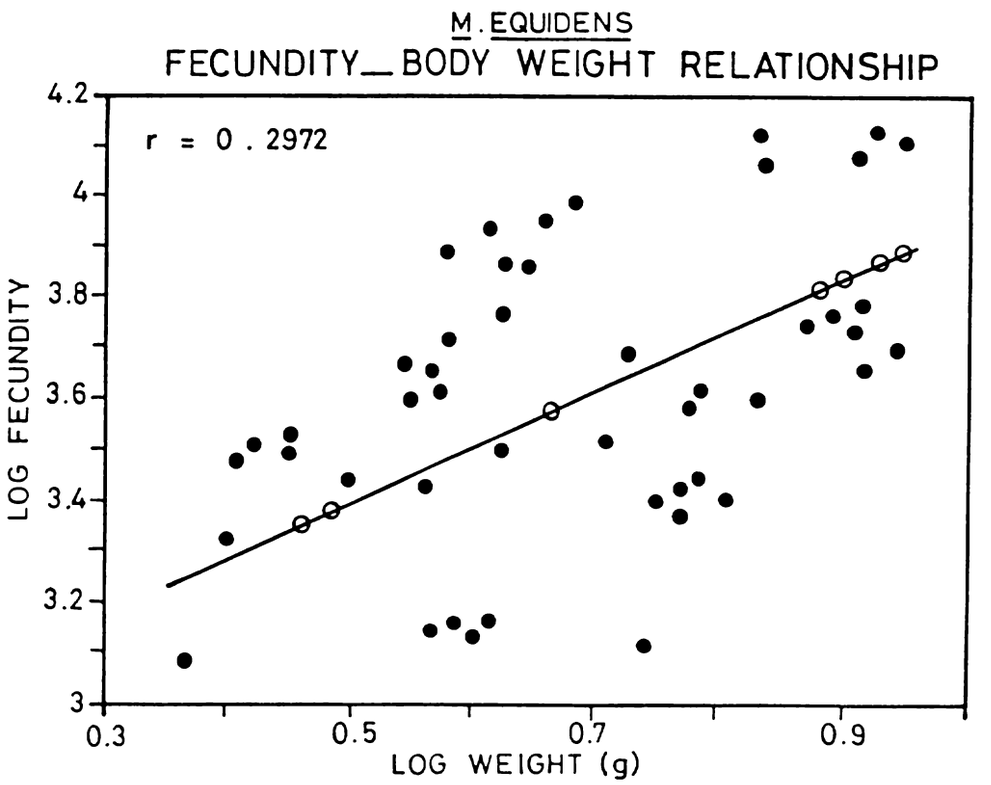

M. EQUIDENS
FECUNDITY_BODY WEIGHT RELATIONSHIP
r = 0 . 2972
LOG FECUNDITY
4.2
4
3.8
3.6
3.4
3.2
3
0.3
0.5
0.7
0.9
LOG WEIGHT (g)

FIG: 9

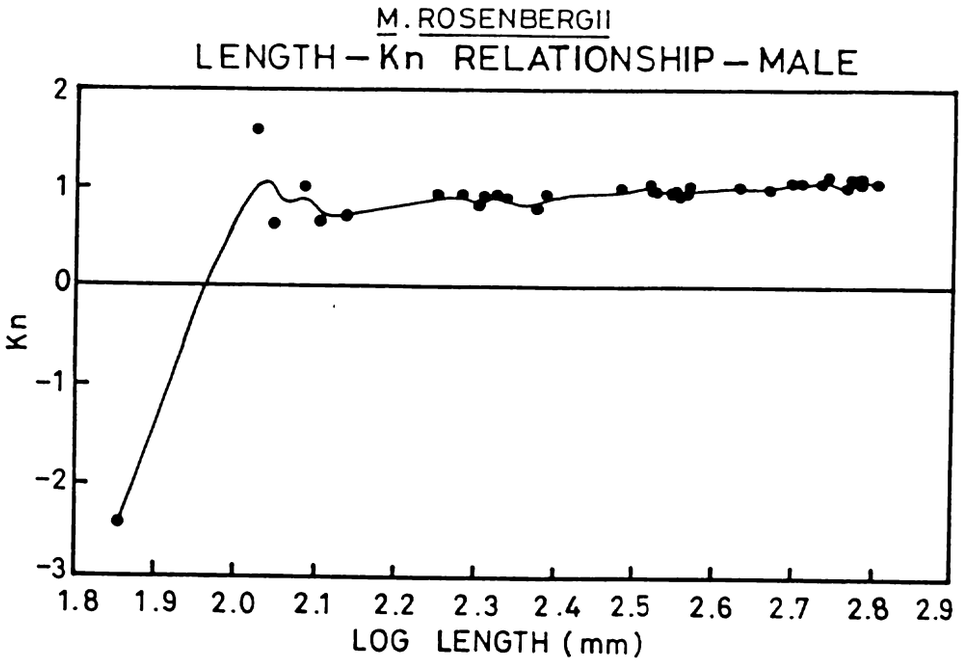

M. ROSENBERGII
LENGTH – Kn RELATIONSHIP – MALE
Kn
2
1
0
-1
-2
-3
1.8
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
LOG LENGTH (mm)

FIG: 10

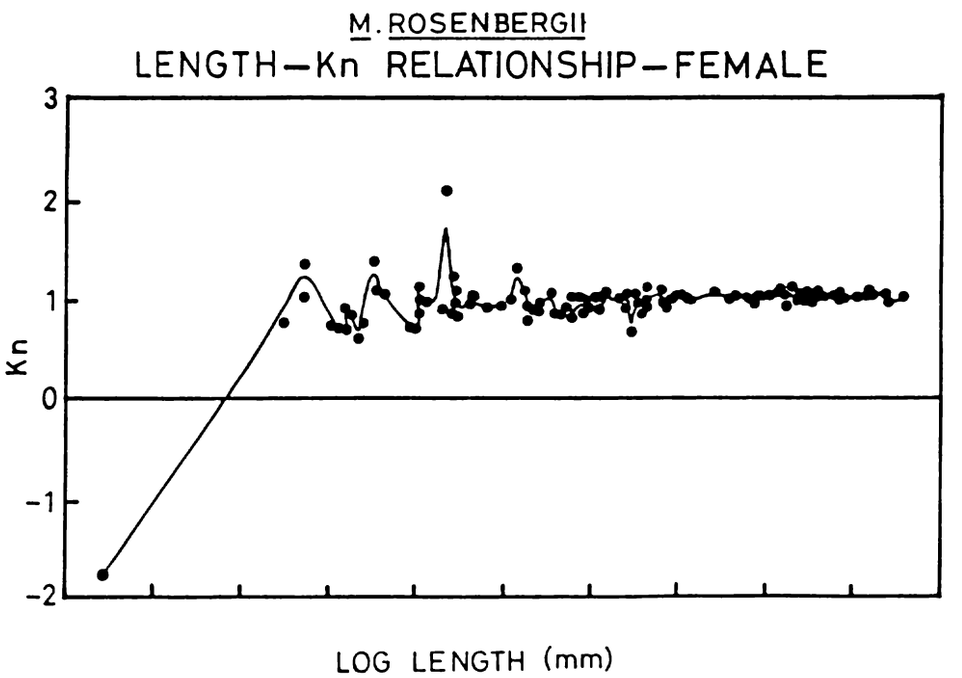

M. ROSENBERGII
LENGTH–Kn RELATIONSHIP–FEMALE
Kn
3
2
1
0
-1
-2
LOG LENGTH (mm)

FIG: 11

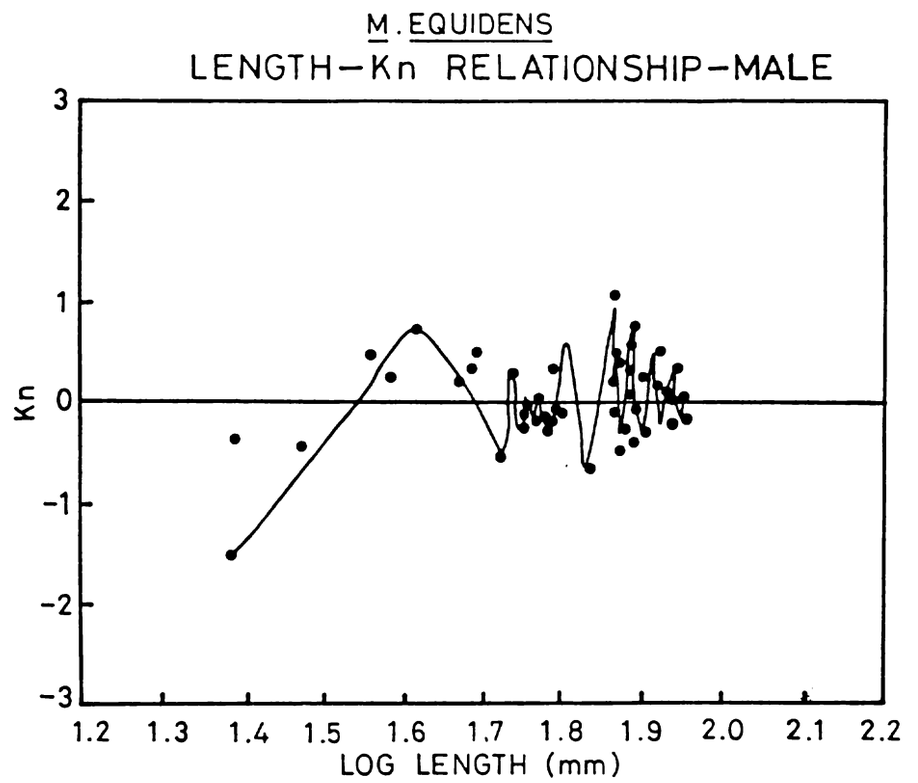
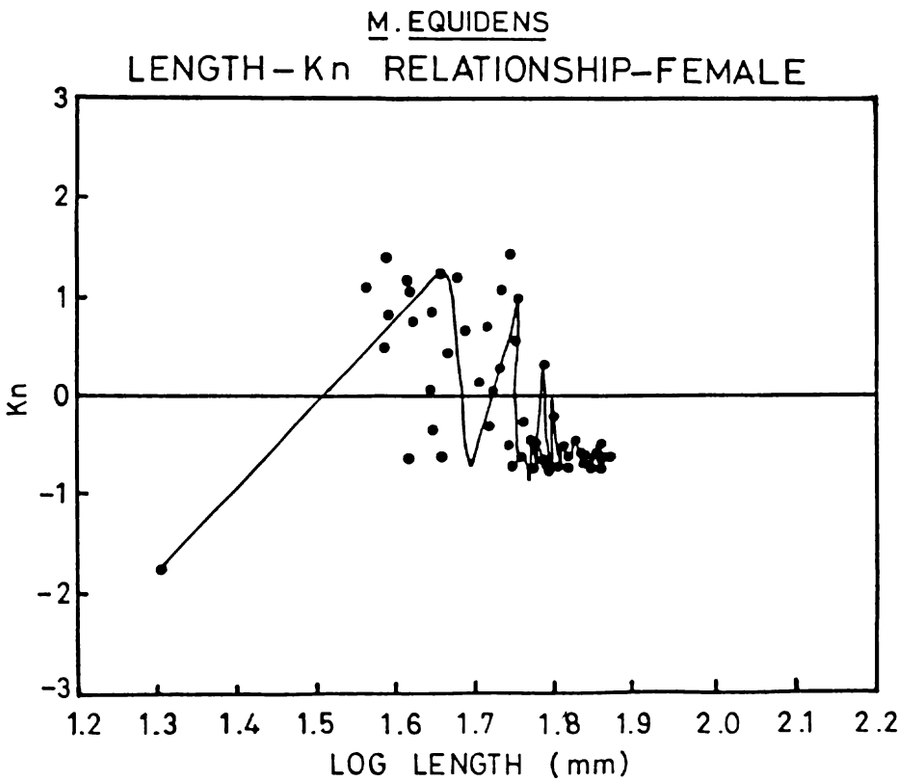

M. EQUIDENS
LENGTH–Kn RELATIONSHIP–MALE
Kn
3
2
1
0
-1
-2
-3
1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2
LOG LENGTH (mm)

FIG: 12


M. EQUIDENS
LENGTH–Kn RELATIONSHIP–FEMALE
Kn
3
2
1
0
-1
-2
-3
1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.1 2.2
LOG LENGTH (mm)

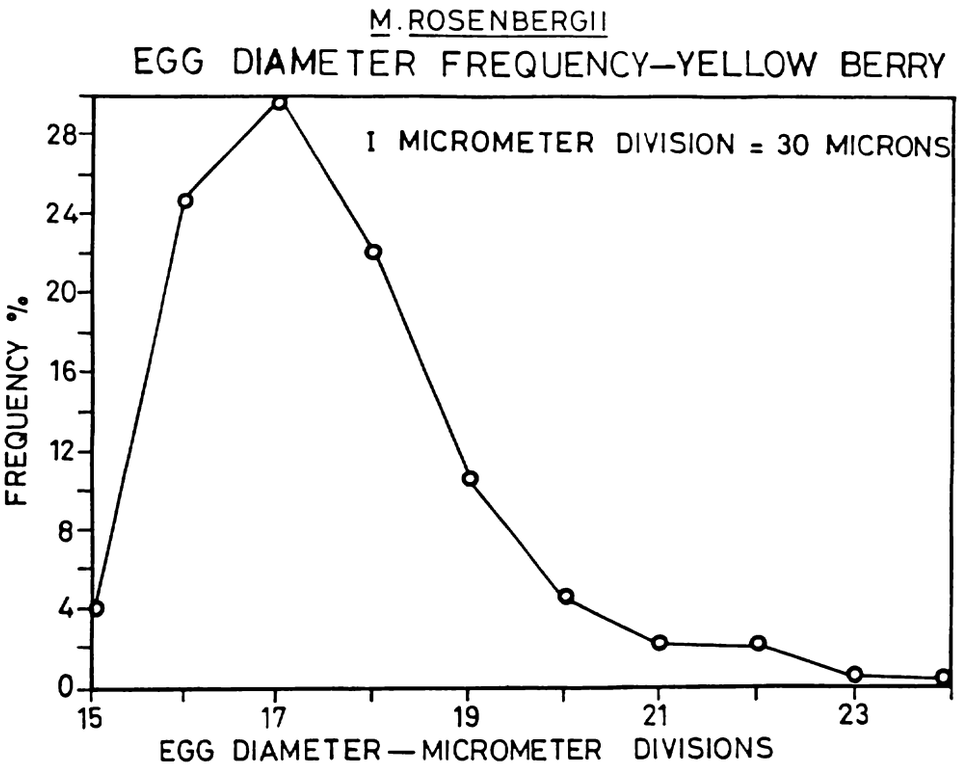

M. ROSENBERGII
EGG DIAMETER FREQUENCY—YELLOW BERRY
I MICROMETER DIVISION = 30 MICRONS
FREQUENCY %
0
4
8
12
16
20
24
28
15
17
19
21
23
EGG DIAMETER — MICROMETER DIVISIONS

FIG: 14

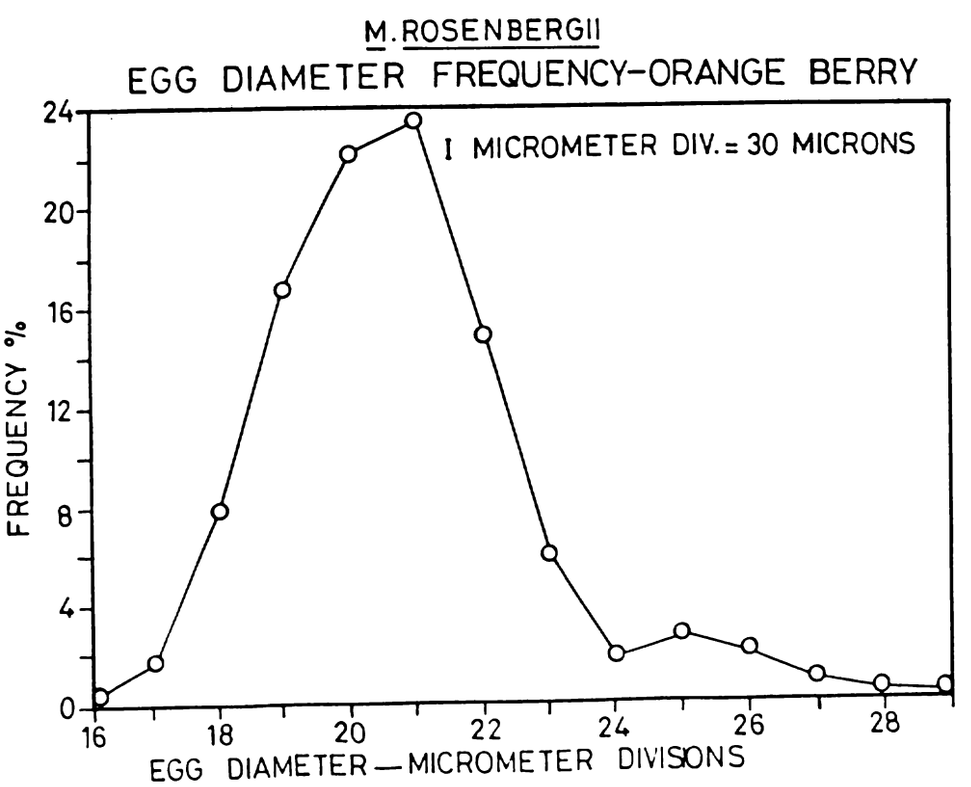

M. ROSENBERGII
EGG DIAMETER FREQUENCY-ORANGE BERRY
I MICROMETER DIV. = 30 MICRONS
FREQUENCY %
0
4
8
12
16
20
24
16
18
20
22
24
26
28
EGG DIAMETER — MICROMETER DIVISONS

FIG: 15

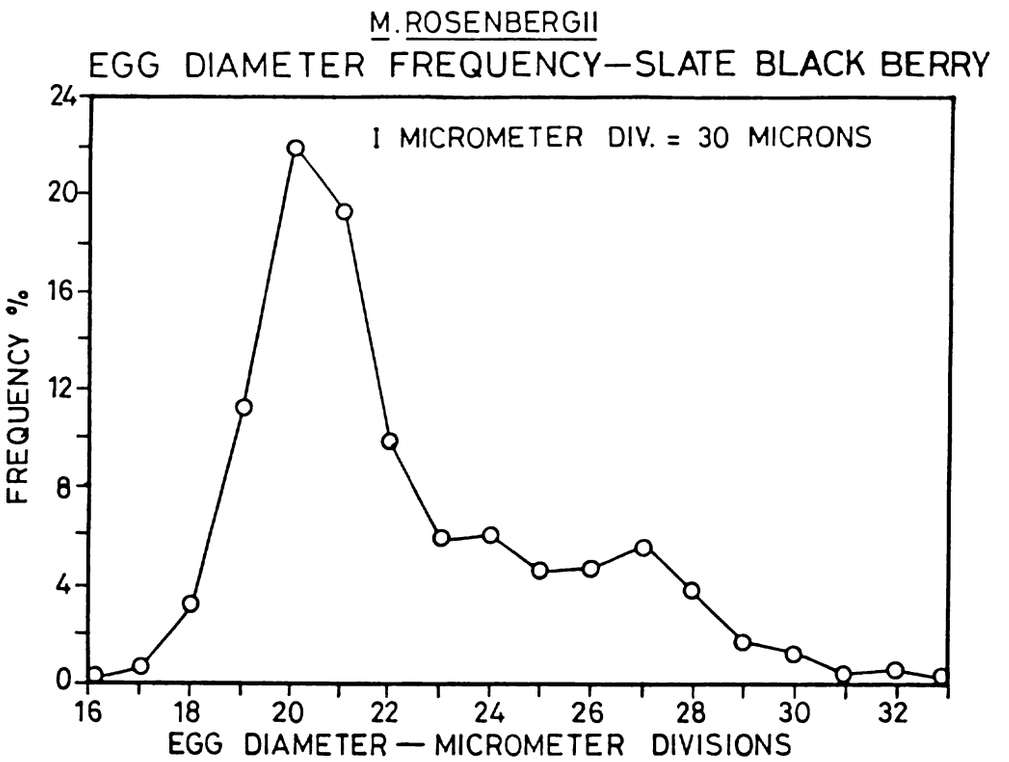

M. ROSENBERGII
EGG DIAMETER FREQUENCY—SLATE BLACK BERRY
1 MICROMETER DIV. = 30 MICRONS
FREQUENCY %
0
4
8
12
16
20
24
16
18
20
22
24
26
28
30
32
EGG DIAMETER — MICROMETER DIVISIONS

FIG:16

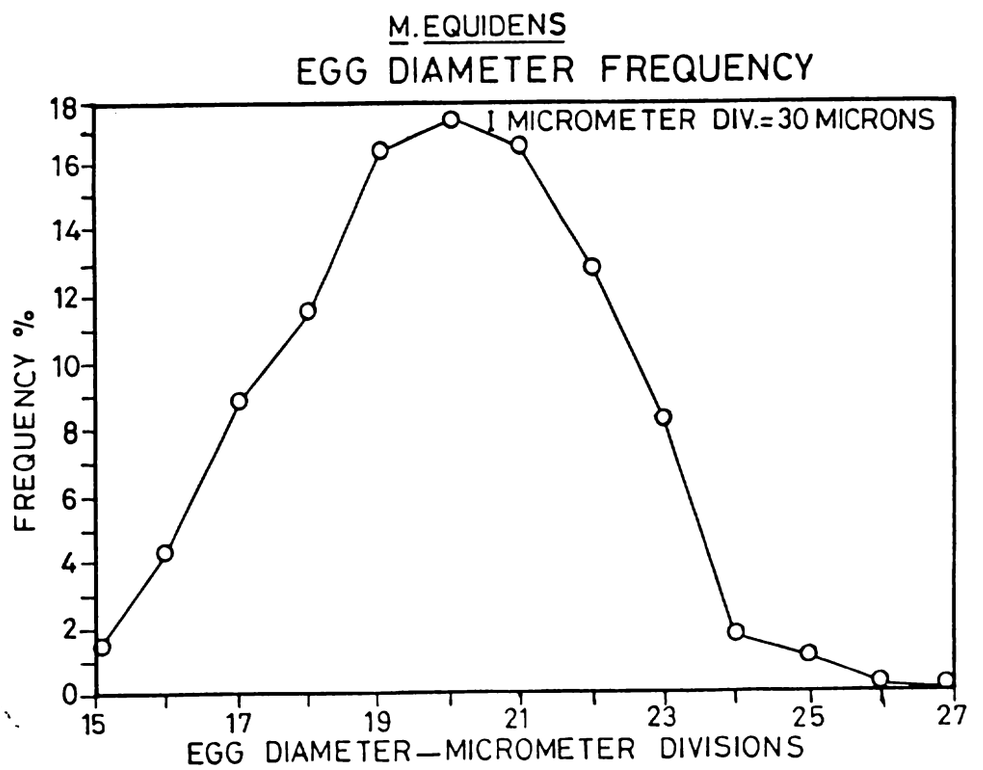
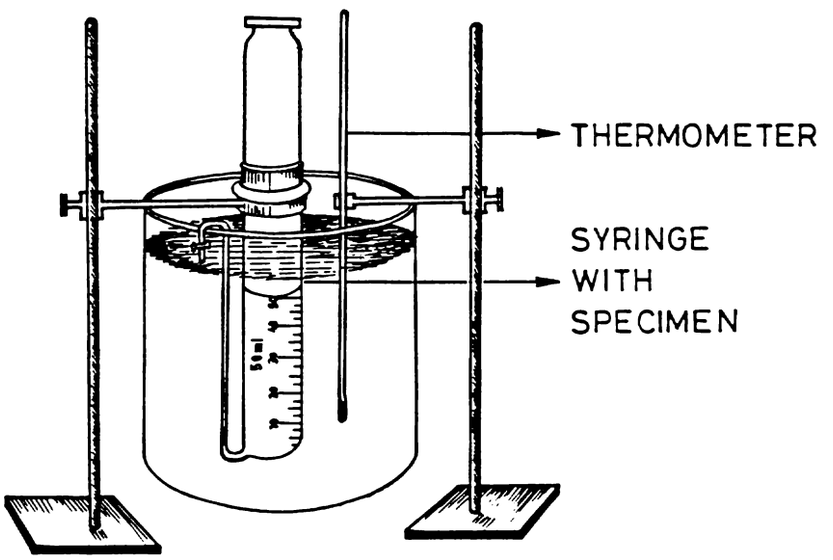

M. EQUIDENS
EGG DIAMETER FREQUENCY
1 MICROMETER DIV.=30 MICRONS
FREQUENCY %
0
2
4
6
8
10
12
14
16
18
15
17
19
21
23
25
27
EGG DIAMETER—MICROMETER DIVISIONS

FIG:17

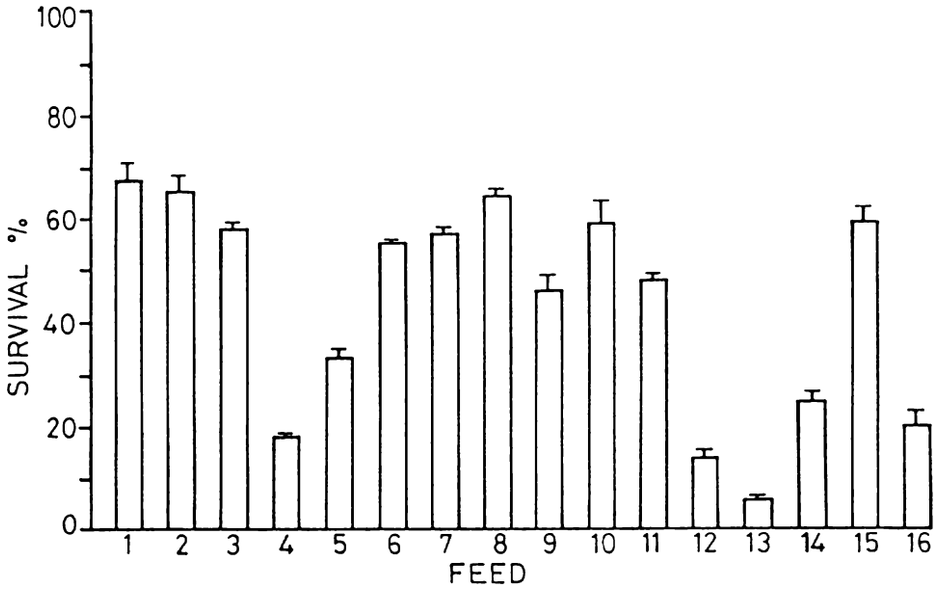


THE RESPIRATORY APPARATUS USED IN O_2 UPTAKE EXPERIMENTS. (AFTER CHERIYAN, 1973).

FIG:18

M. ROSENBERGII

EFFICACY OF SELECTED FEED AS LARVAL FEED



FIG; 19

M. EQUIDENS

EFFICACY OF SELECTED FEED AS LARVAL FEED

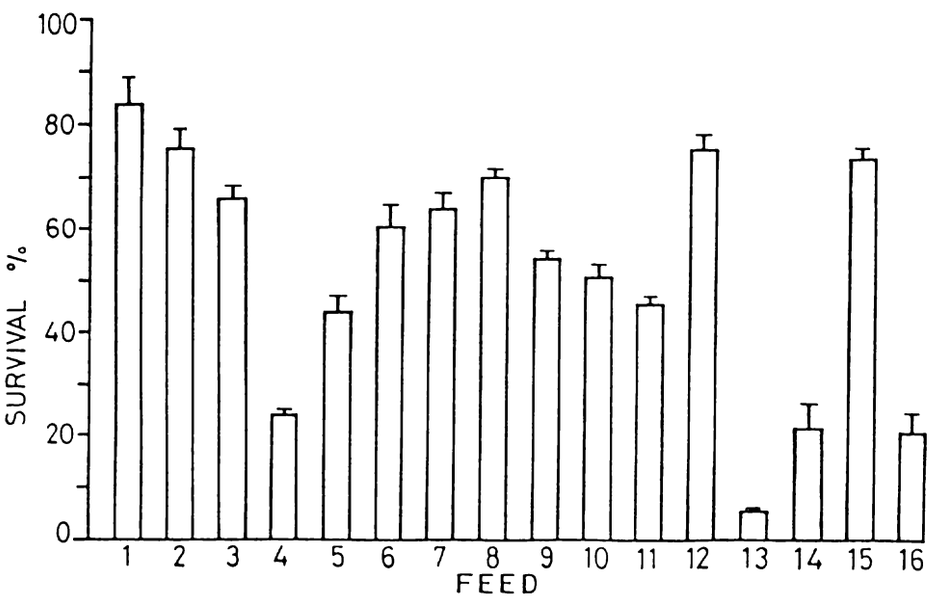


FIG: 20

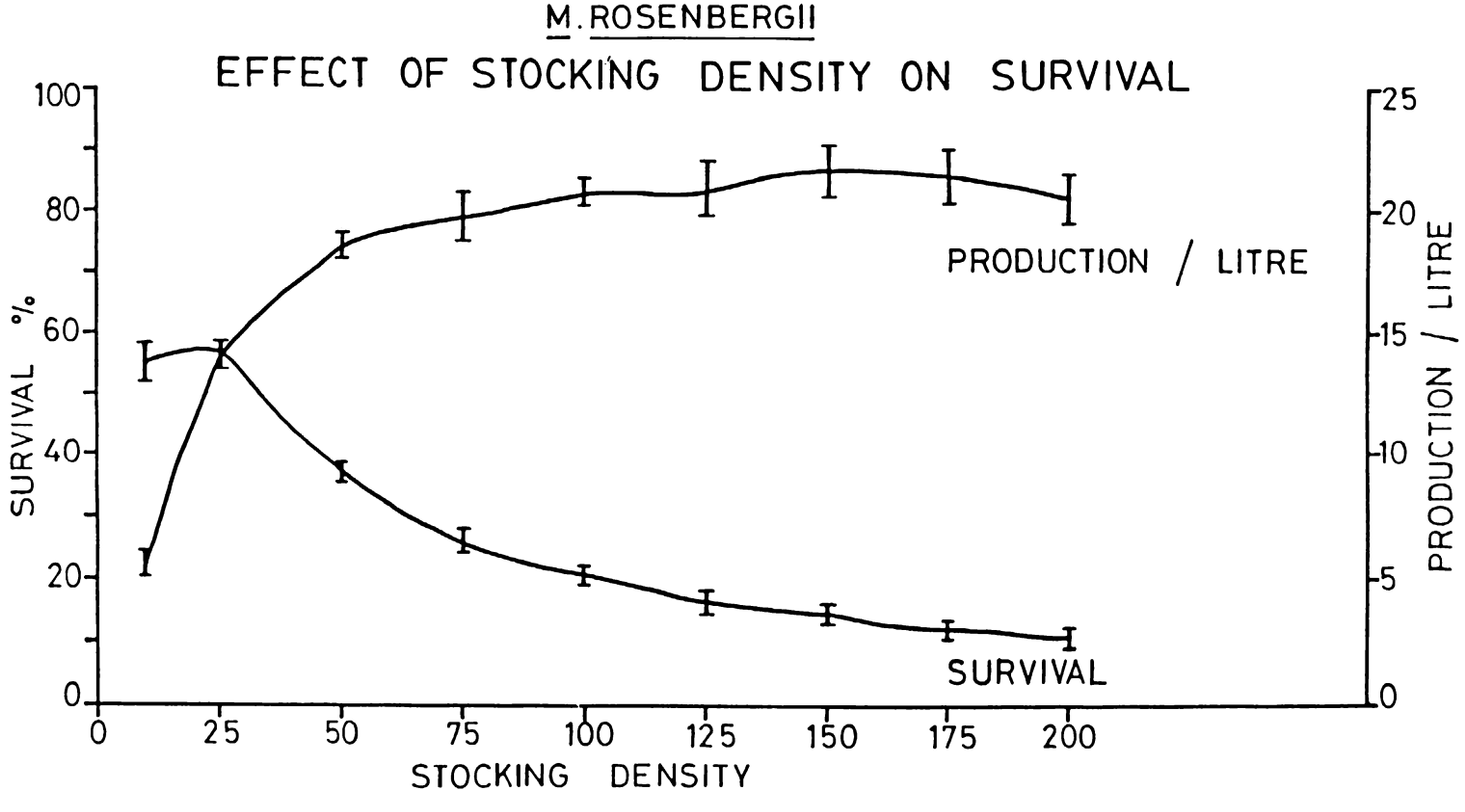

M. ROSENBERGII
EFFECT OF STOCKING DENSITY ON SURVIVAL
SURVIVAL %
PRODUCTION / LITRE
PRODUCTION / LITRE
SURVIVAL
STOCKING DENSITY
0
20
40
60
80
100
0
5
10
15
20
25
0
25
50
75
100
125
150
175
200

FIG: 21

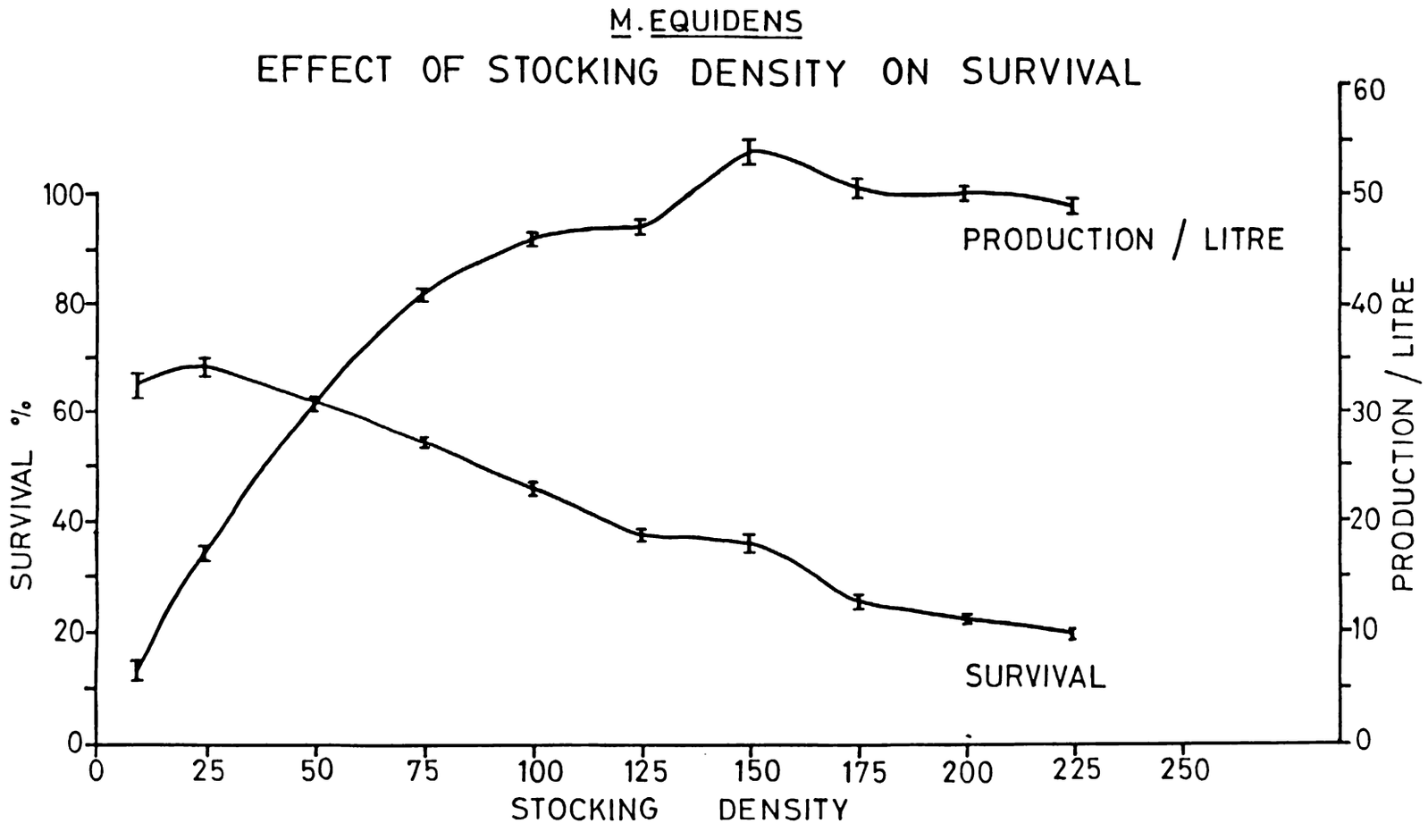

M. EQUIDENS
EFFECT OF STOCKING DENSITY ON SURVIVAL
SURVIVAL %
PRODUCTION / LITRE
PRODUCTION / LITRE
SURVIVAL
STOCKING DENSITY
0
20
40
60
80
100
0
10
20
30
40
50
60
0
25
50
75
100
125
150
175
200
225
250

FIG: 22

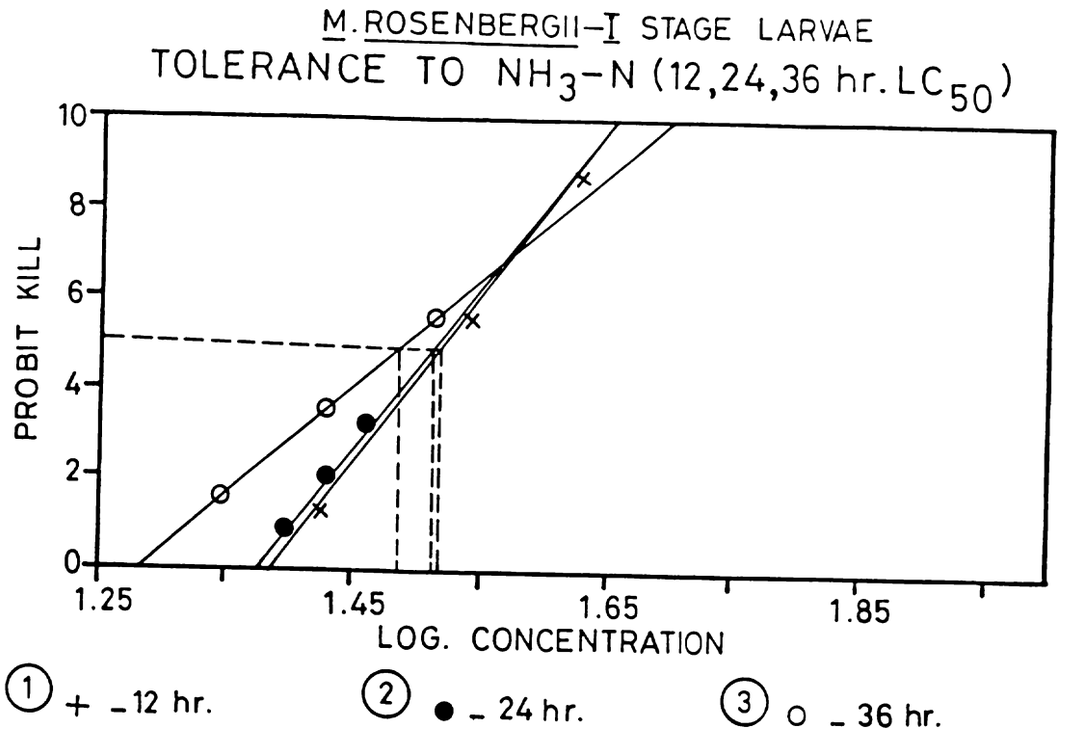

M.ROSENBERGII–I STAGE LARVAE
TOLERANCE TO NH3–N (12,24,36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.25
1.45
1.65
1.85
LOG. CONCENTRATION
① + – 12 hr.
② ● – 24 hr.
③ o – 36 hr.

FIG: 23

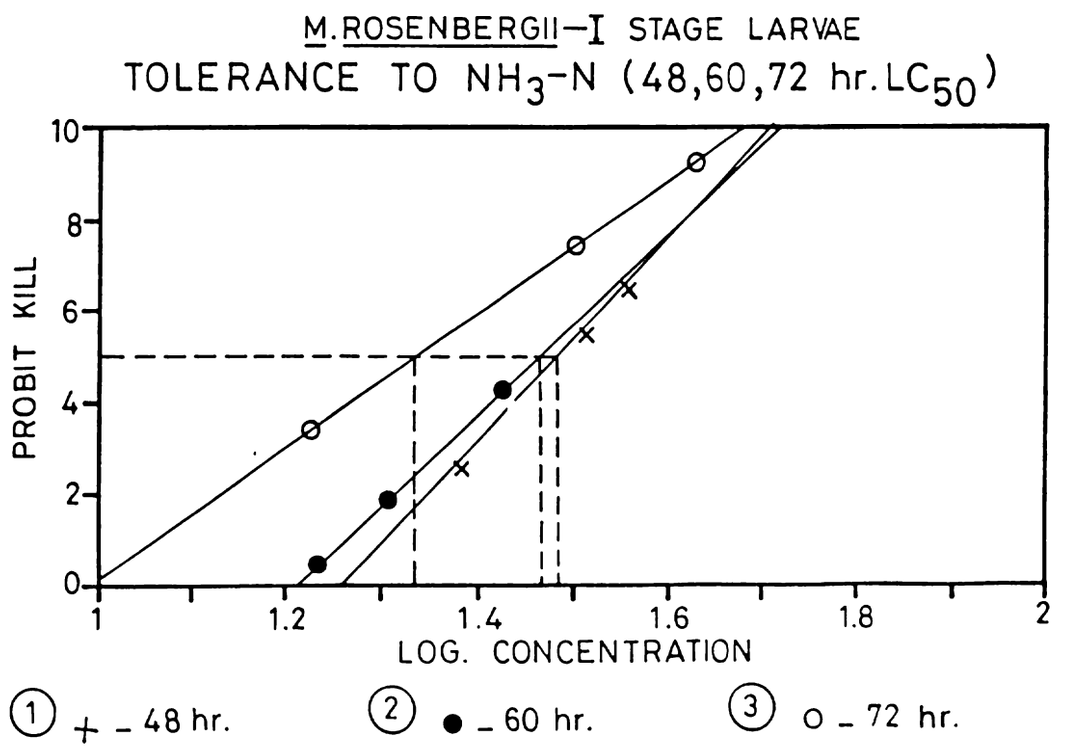

M.ROSENBERGII–I STAGE LARVAE
TOLERANCE TO NH3–N (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.2
1.4
1.6
1.8
2
LOG. CONCENTRATION
① + – 48 hr.
② ● – 60 hr.
③ o – 72 hr.

FIG: 24

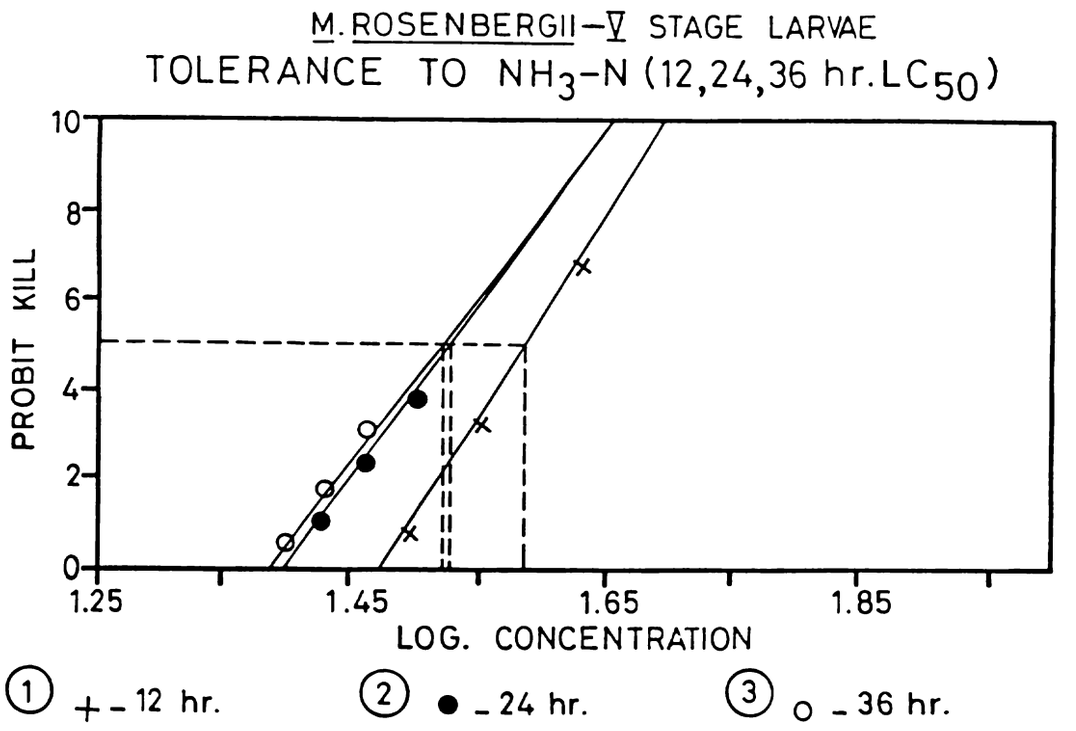

M. ROSENBERGII – V STAGE LARVAE
TOLERANCE TO NH3-N (12,24,36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.25
1.45
1.65
1.85
LOG. CONCENTRATION
① + – 12 hr.
② ● – 24 hr.
③ o – 36 hr.

FIG: 25

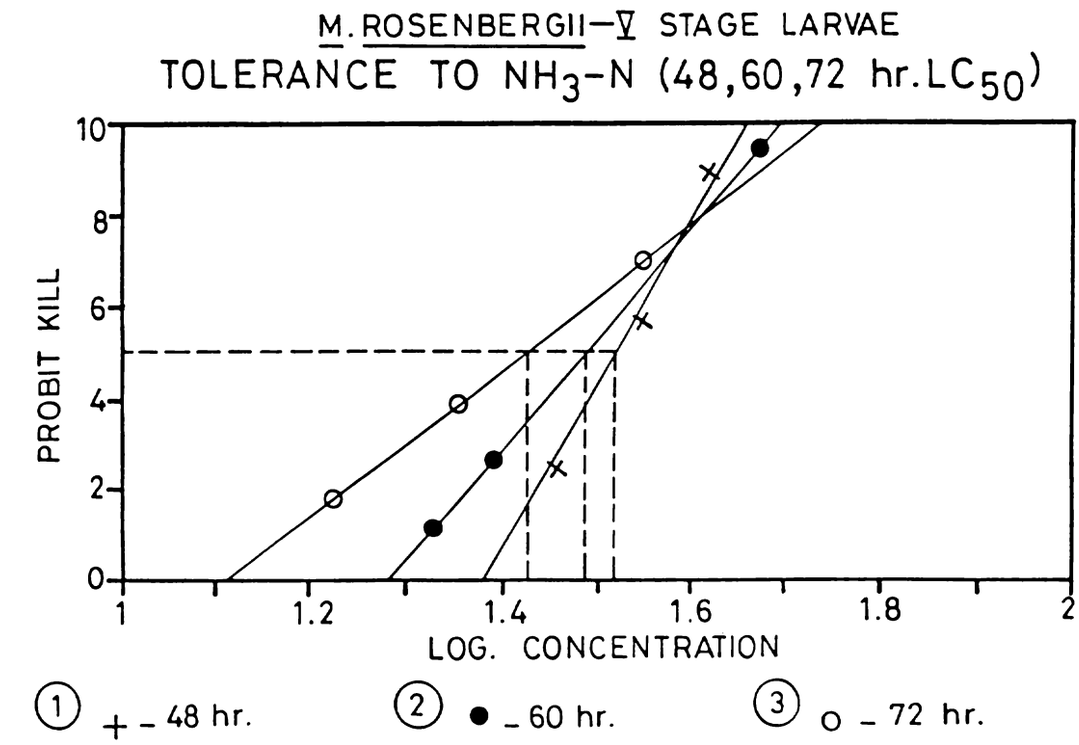

M. ROSENBERGII – V STAGE LARVAE
TOLERANCE TO NH3-N (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.2
1.4
1.6
1.8
2
LOG. CONCENTRATION
① + – 48 hr.
② ● – 60 hr.
③ o – 72 hr.

FIG: 26

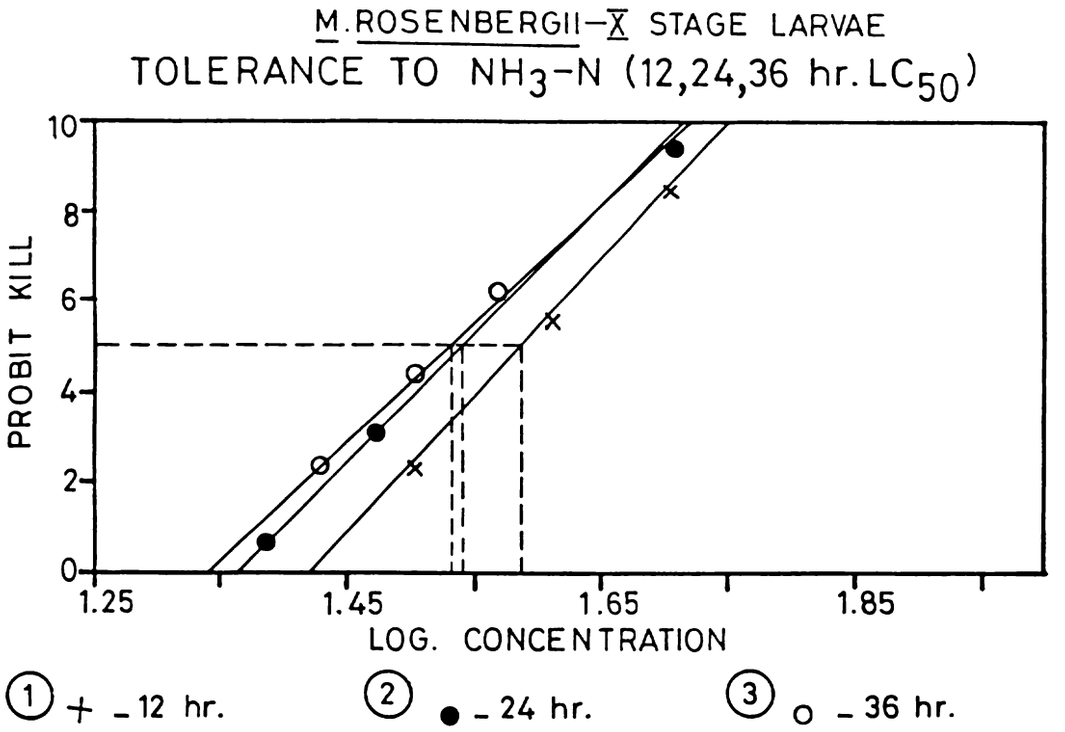

M.ROSENBERGII–X STAGE LARVAE
TOLERANCE TO NH3–N (12,24,36 hr.LC50)
PROBIT KILL
LOG. CONCENTRATION
1.25
1.45
1.65
1.85
0
2
4
6
8
10
① + – 12 hr.
② ● – 24 hr.
③ o – 36 hr.

FIG: 27

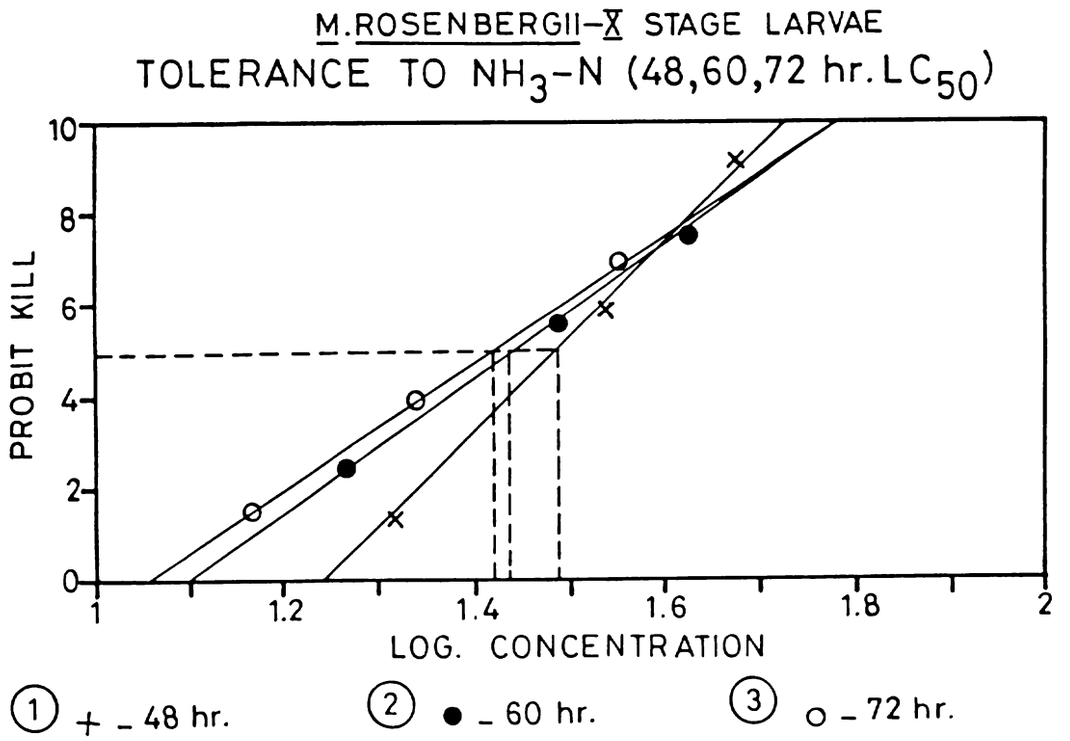

M.ROSENBERGII–X STAGE LARVAE
TOLERANCE TO NH3–N (48,60,72 hr.LC50)
PROBIT KILL
LOG. CONCENTRATION
1
1.2
1.4
1.6
1.8
2
0
2
4
6
8
10
① + – 48 hr.
② ● – 60 hr.
③ o – 72 hr.

FIG: 28

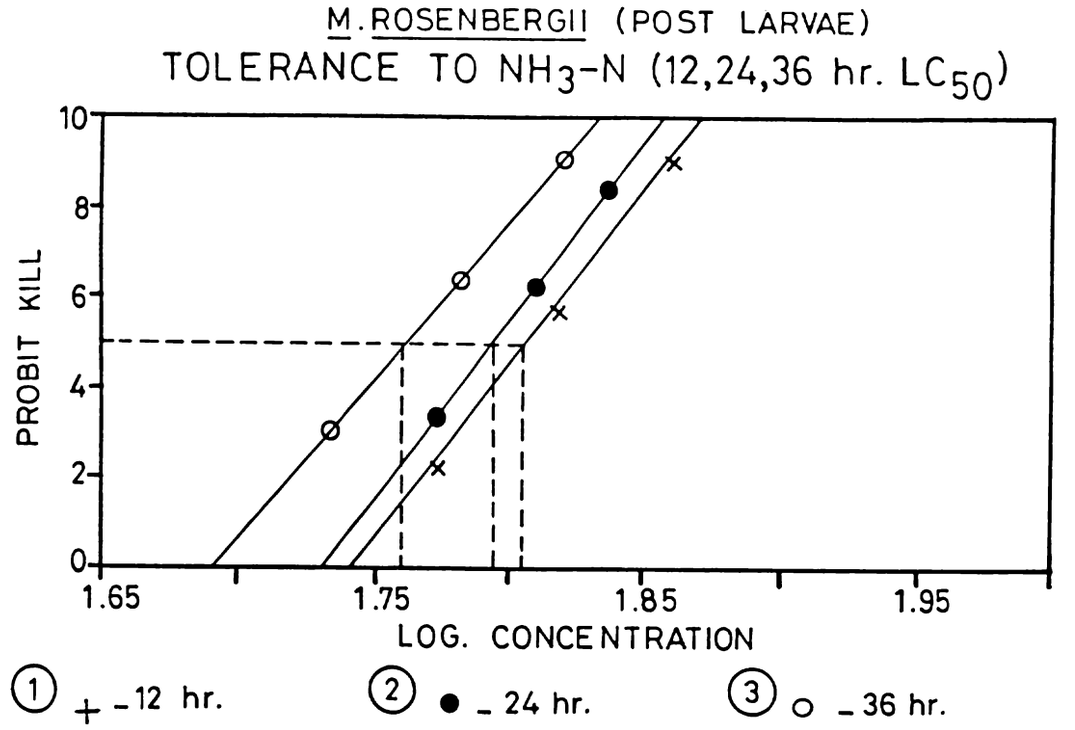

M.ROSENBERGII (POST LARVAE)
TOLERANCE TO NH3-N (12,24,36 hr. LC50)
PROBIT KILL
10
8
6
4
2
0
1.65
1.75
1.85
1.95
LOG. CONCENTRATION
1 + - 12 hr.
2 ● - 24 hr.
3 o - 36 hr.

FIG: 29

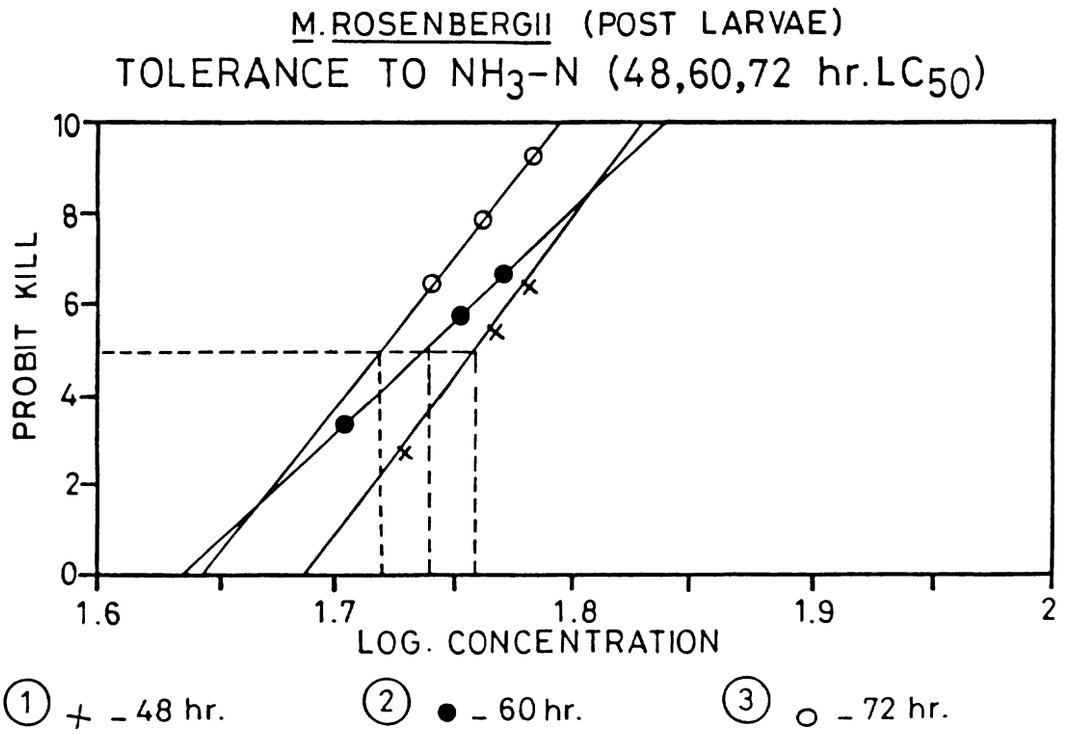

M.ROSENBERGII (POST LARVAE)
TOLERANCE TO NH3-N (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.6
1.7
1.8
1.9
2
LOG. CONCENTRATION
1 + - 48 hr.
2 ● - 60 hr.
3 o - 72 hr.

FIG: 30

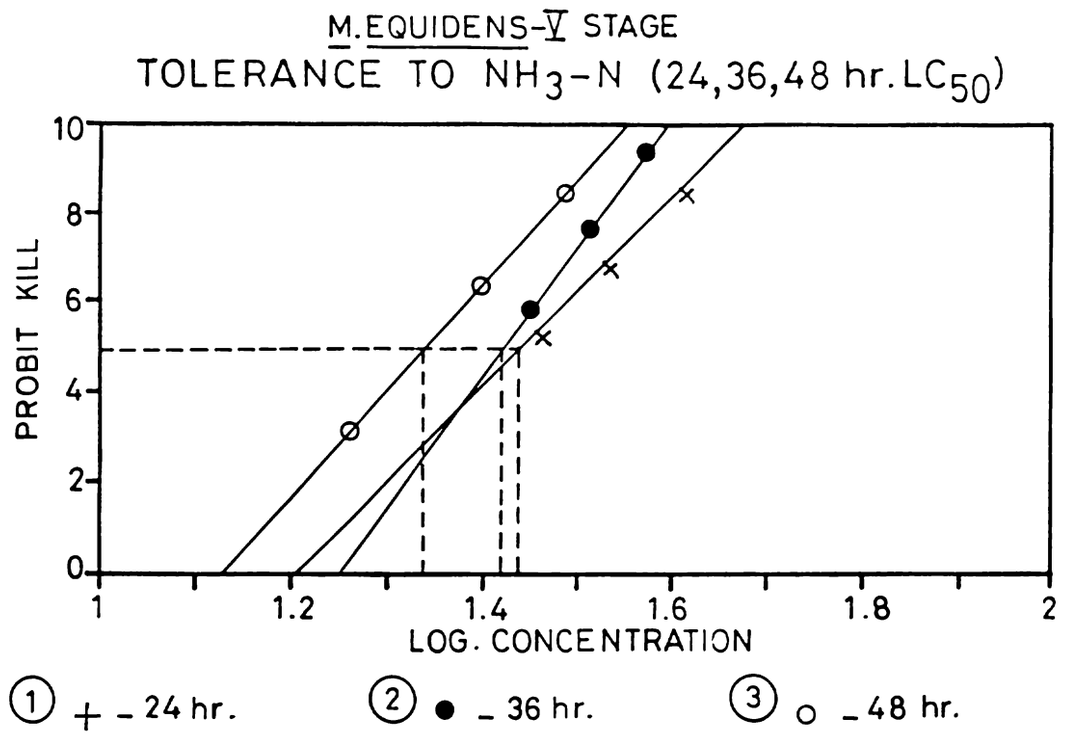

M.EQUIDENS-V STAGE
TOLERANCE TO NH3-N (24,36,48 hr.LC50)
PROBIT KILL
LOG. CONCENTRATION
1 + - 24 hr.
2 ● - 36 hr.
3 o - 48 hr.

FIG: 31

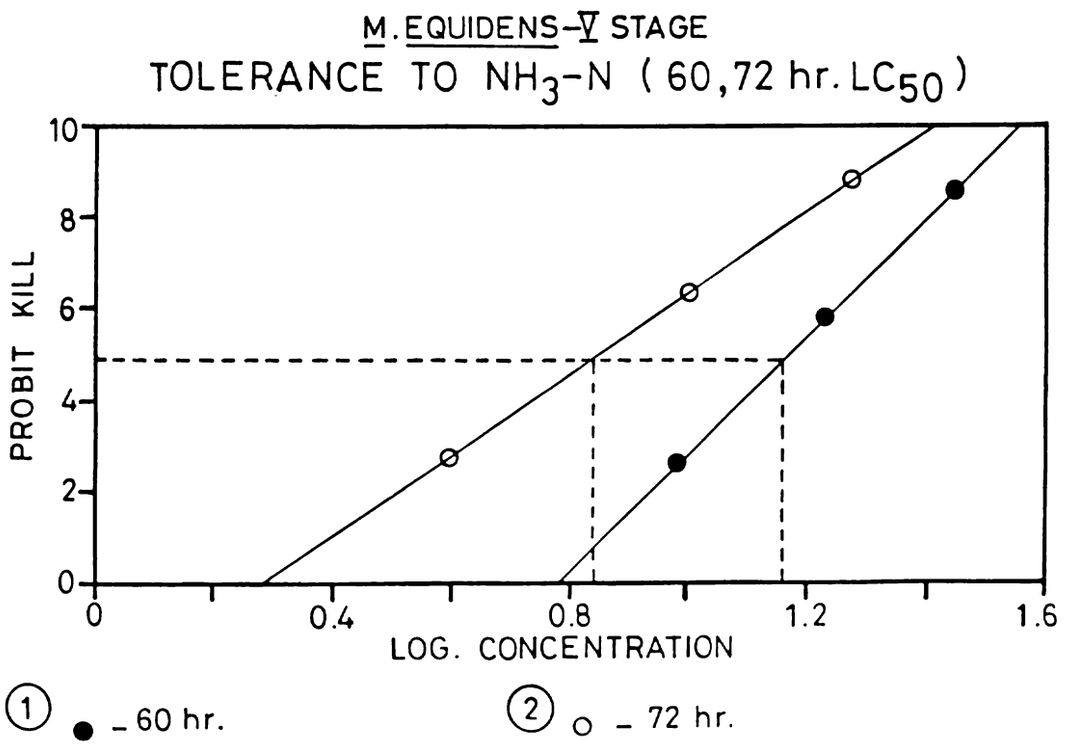

M.EQUIDENS-V STAGE
TOLERANCE TO NH3-N (60,72 hr.LC50)
PROBIT KILL
LOG. CONCENTRATION
1 ● - 60 hr.
2 o - 72 hr.

FIG: 32

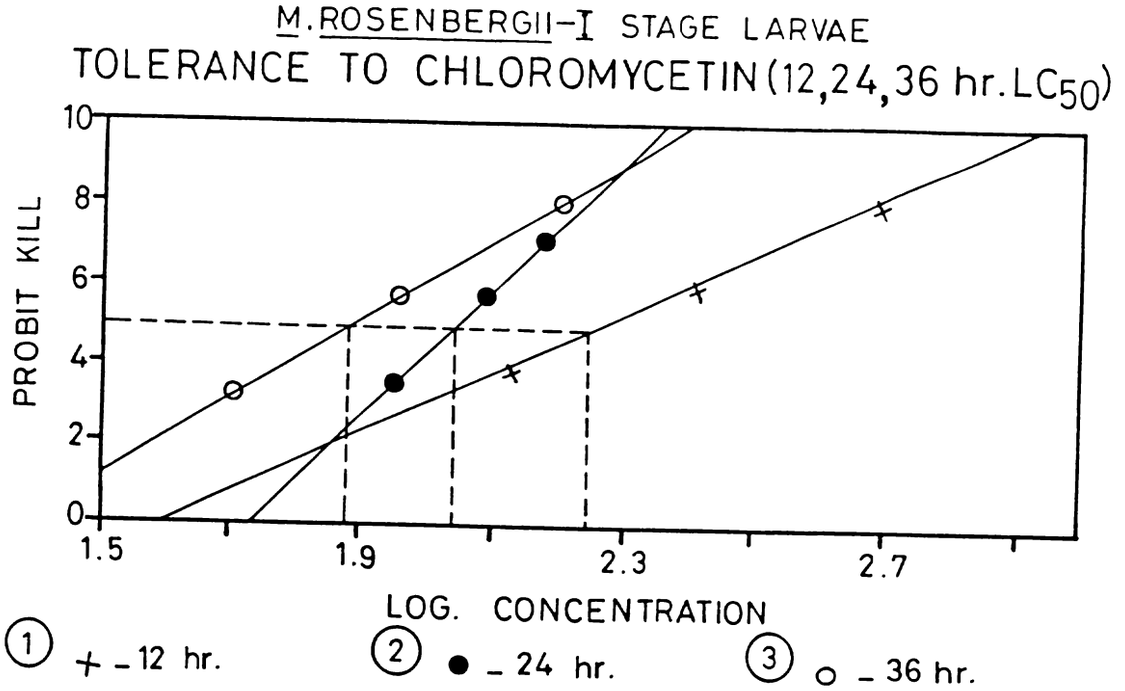

M.ROSENBERGII-I STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (12,24,36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.5
1.9
2.3
2.7
LOG. CONCENTRATION
1 + - 12 hr.
2 ● - 24 hr.
3 o - 36 hr.

FIG: 33

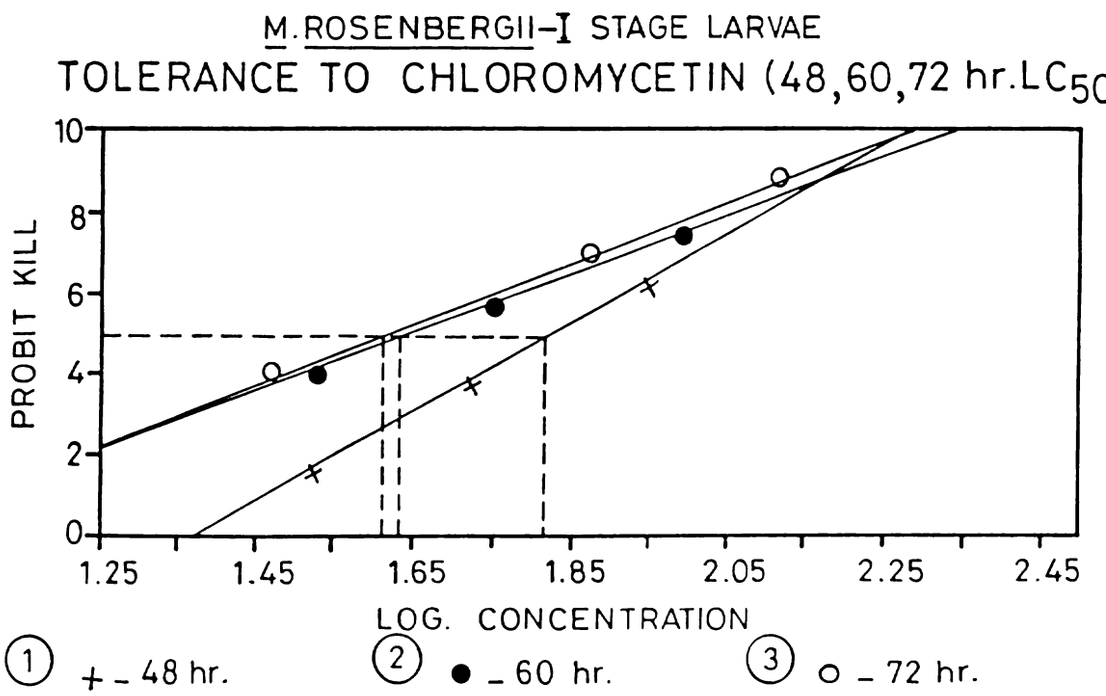

M.ROSENBERGII-I STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.25
1.45
1.65
1.85
2.05
2.25
2.45
LOG. CONCENTRATION
1 + - 48 hr.
2 ● - 60 hr.
3 o - 72 hr.

FIG: 34

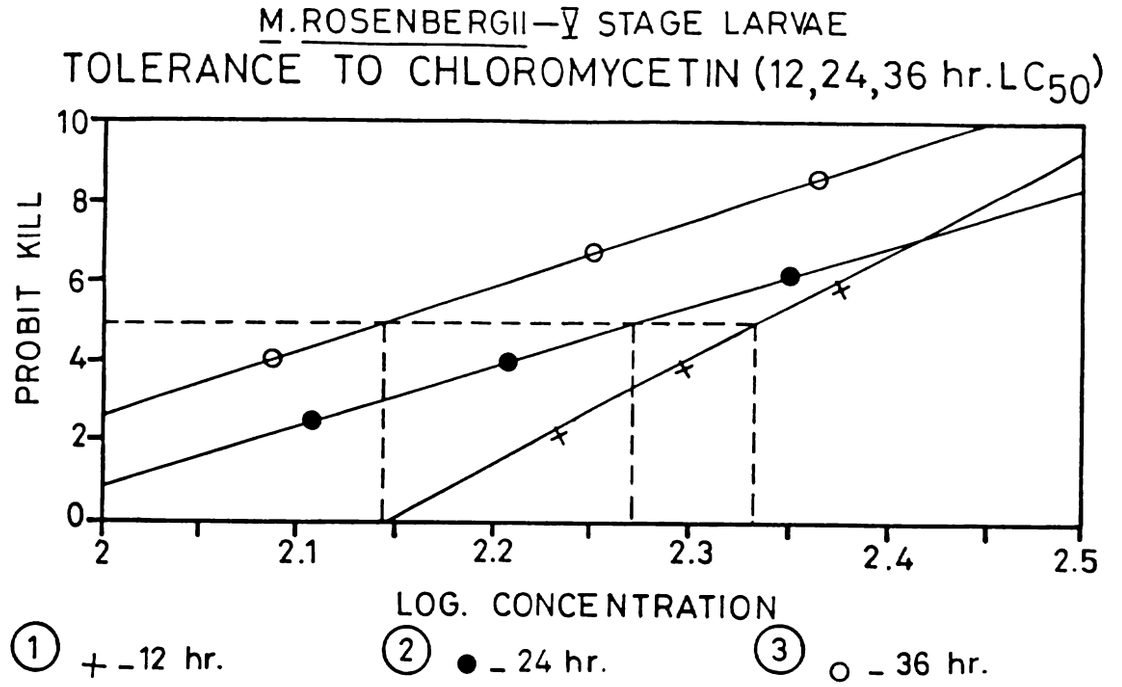

M.ROSENBERGII—V STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (12,24,36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
2
2.1
2.2
2.3
2.4
2.5
LOG. CONCENTRATION
1 + - 12 hr.
2 ● - 24 hr.
3 o - 36 hr.

FIG: 35

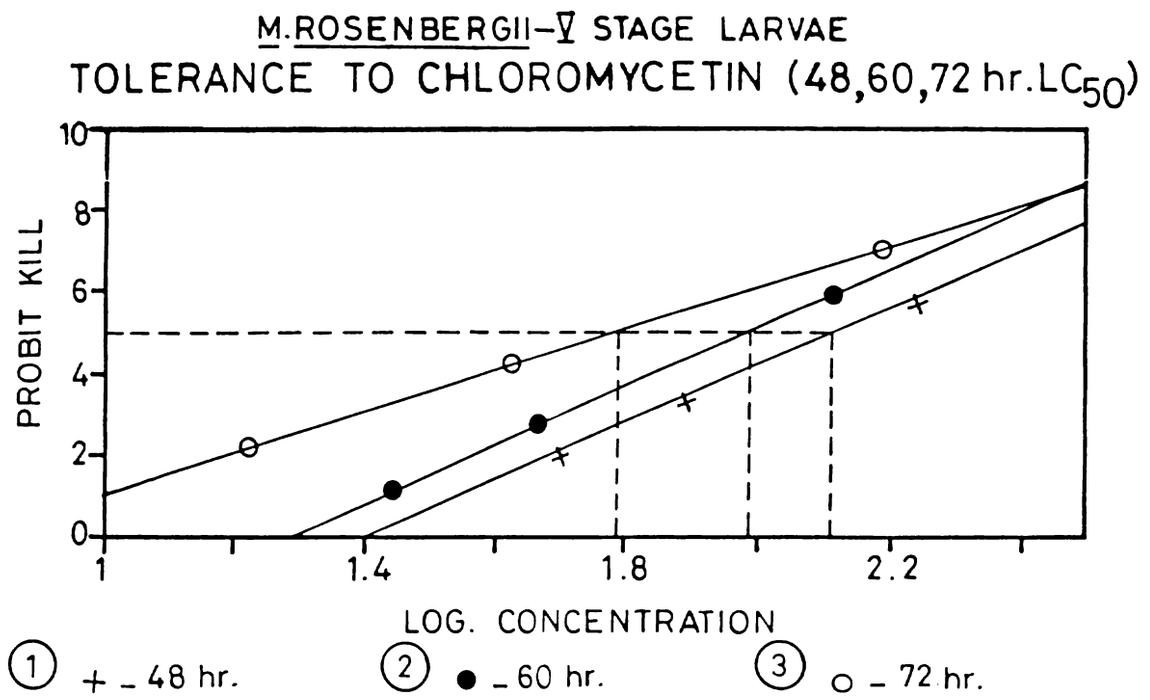

M.ROSENBERGII—V STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.4
1.8
2.2
LOG. CONCENTRATION
1 + - 48 hr.
2 ● - 60 hr.
3 o - 72 hr.

FIG: 36

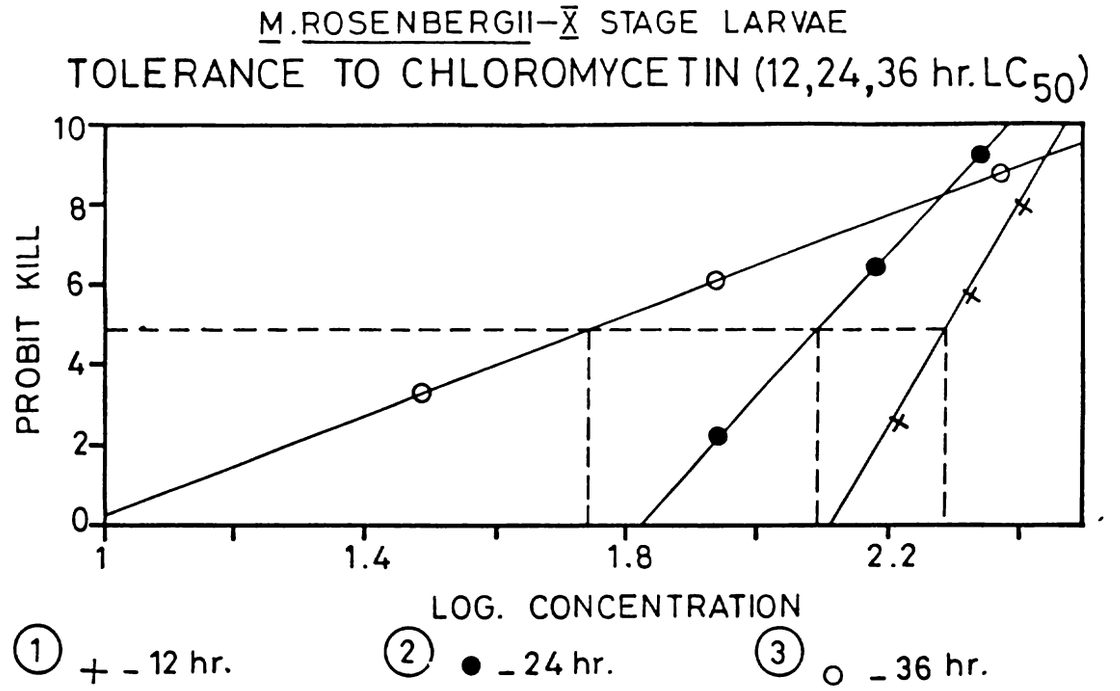

M.ROSENBERGII–X STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (12,24,36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.4
1.8
2.2
LOG. CONCENTRATION
① + – 12 hr.
② ● – 24 hr.
③ o – 36 hr.

FIG: 37

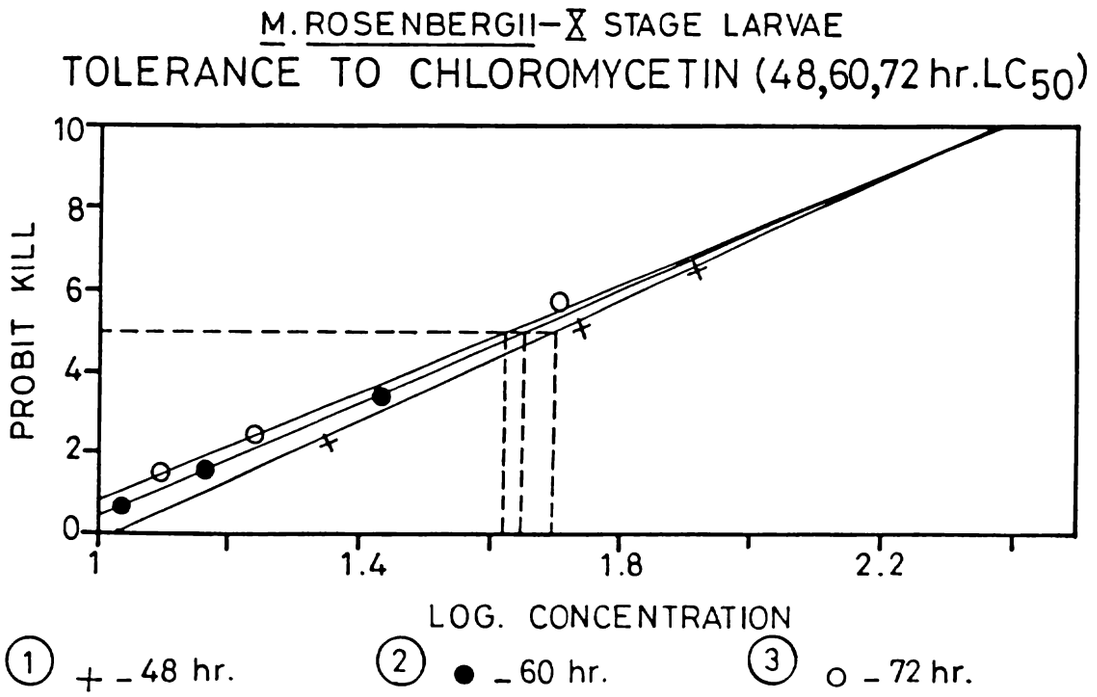

M.ROSENBERGII–X STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.4
1.8
2.2
LOG. CONCENTRATION
① + – 48 hr.
② ● – 60 hr.
③ o – 72 hr.

FIG: 38

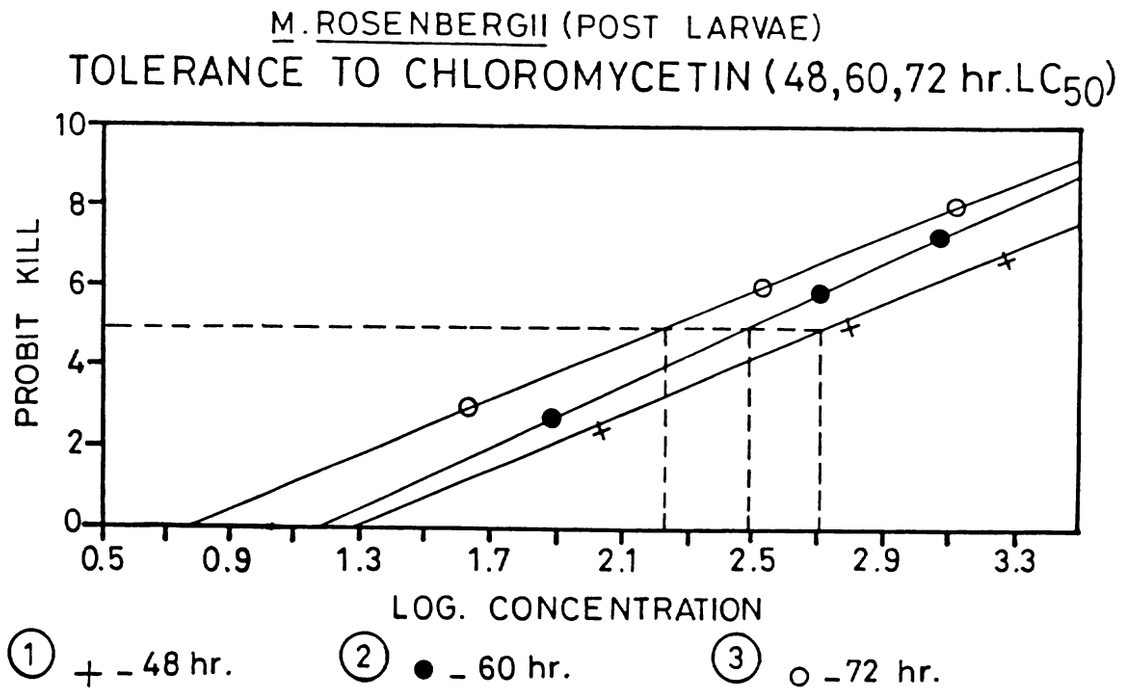

M. ROSENBERGII (POST LARVAE)
TOLERANCE TO CHLOROMYCETIN (48,60,72 hr. LC_{50})
PROBIT KILL
10
8
6
4
2
0
0.5
0.9
1.3
1.7
2.1
2.5
2.9
3.3
LOG. CONCENTRATION
① + - 48 hr.
② ● - 60 hr.
③ o - 72 hr.

FIG:39

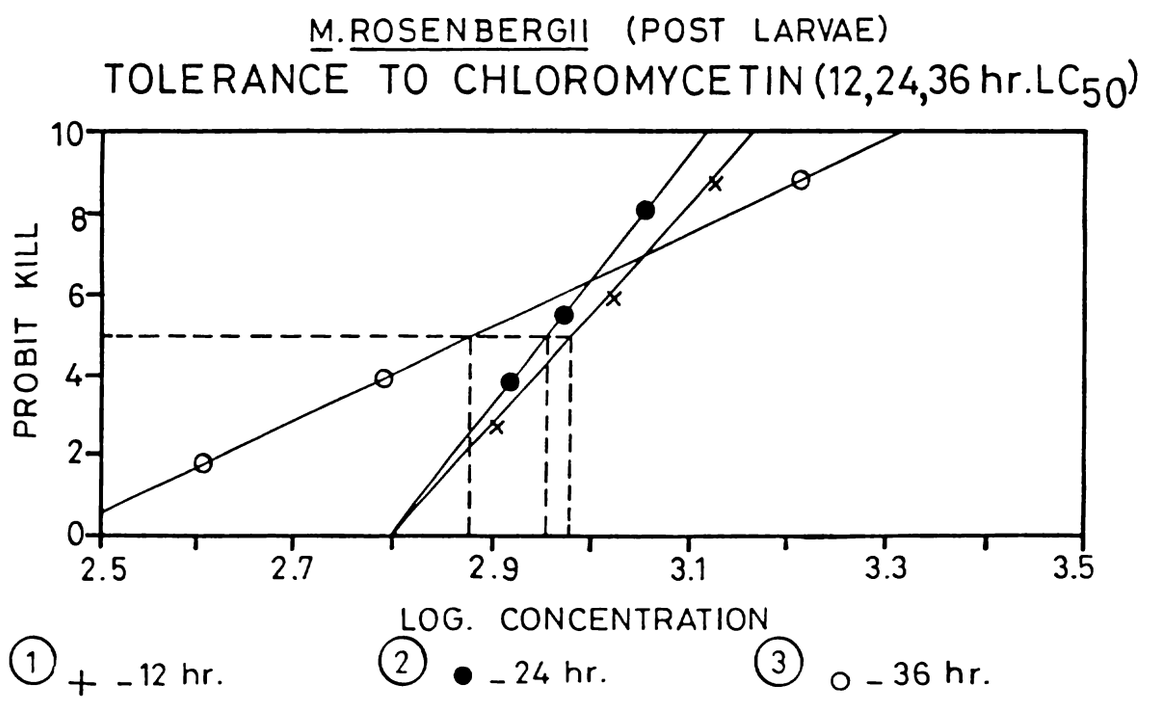

M. ROSENBERGII (POST LARVAE)
TOLERANCE TO CHLOROMYCETIN (12,24,36 hr. LC_{50})
PROBIT KILL
10
8
6
4
2
0
2.5
2.7
2.9
3.1
3.3
3.5
LOG. CONCENTRATION
① + - 12 hr.
② ● - 24 hr.
③ o - 36 hr.

FIG: 40

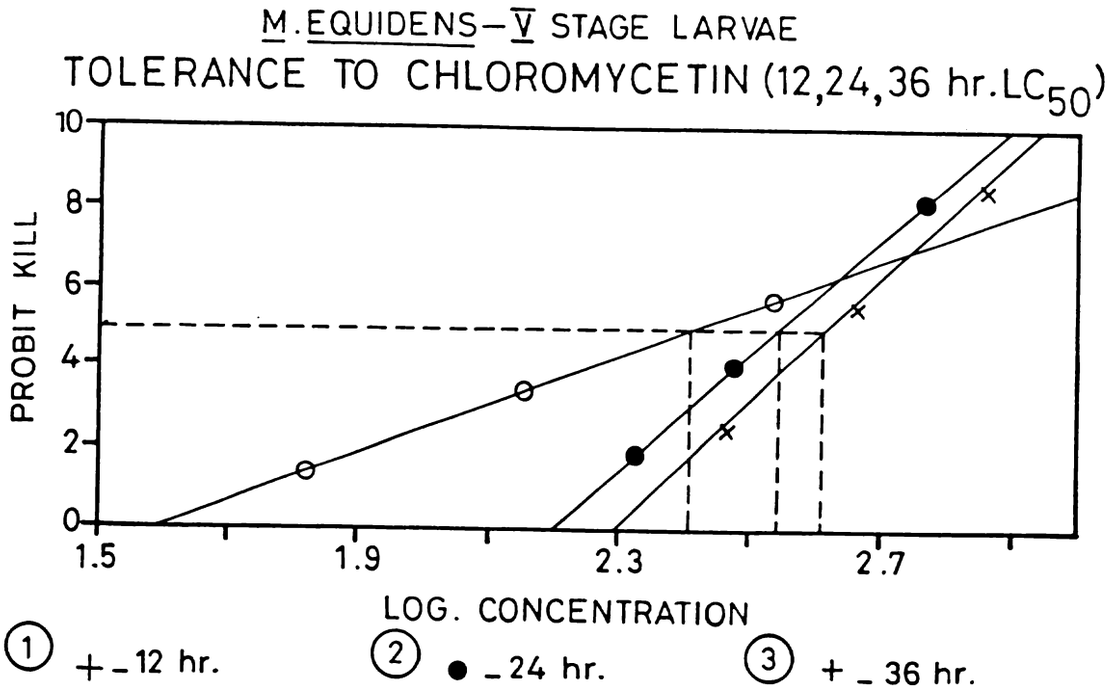

M.EQUIDENS–V STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (12,24,36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.5
1.9
2.3
2.7
LOG. CONCENTRATION
① + – 12 hr.
② ● – 24 hr.
③ + – 36 hr.

FIG: 41

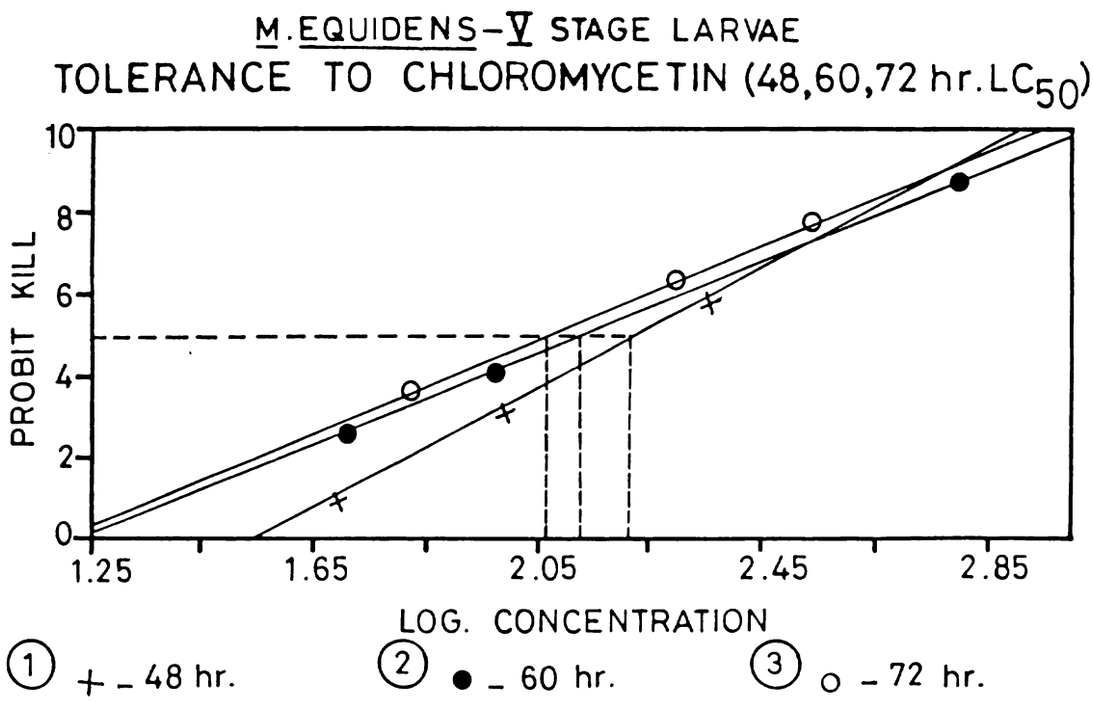

M.EQUIDENS–V STAGE LARVAE
TOLERANCE TO CHLOROMYCETIN (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.25
1.65
2.05
2.45
2.85
LOG. CONCENTRATION
① + – 48 hr.
② ● – 60 hr.
③ o – 72 hr.

FIG: 42

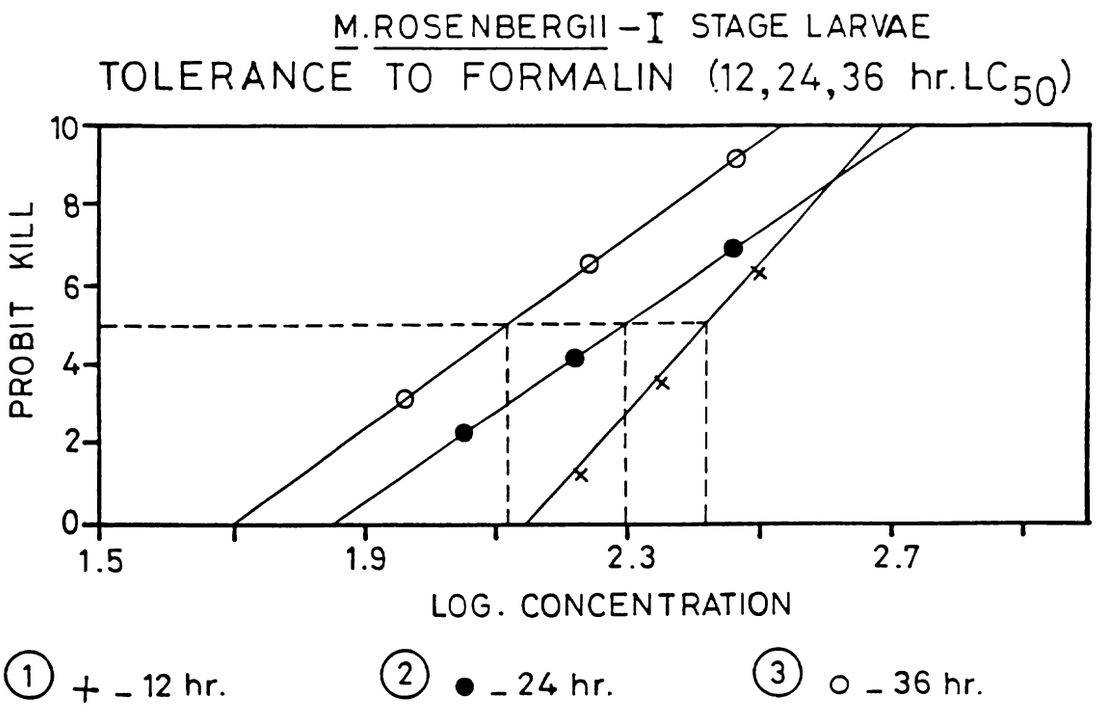

M.ROSENBERGII – I STAGE LARVAE
TOLERANCE TO FORMALIN (12,24,36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.5
1.9
2.3
2.7
LOG. CONCENTRATION
① + – 12 hr.
② ● – 24 hr.
③ o – 36 hr.

FIG: 43

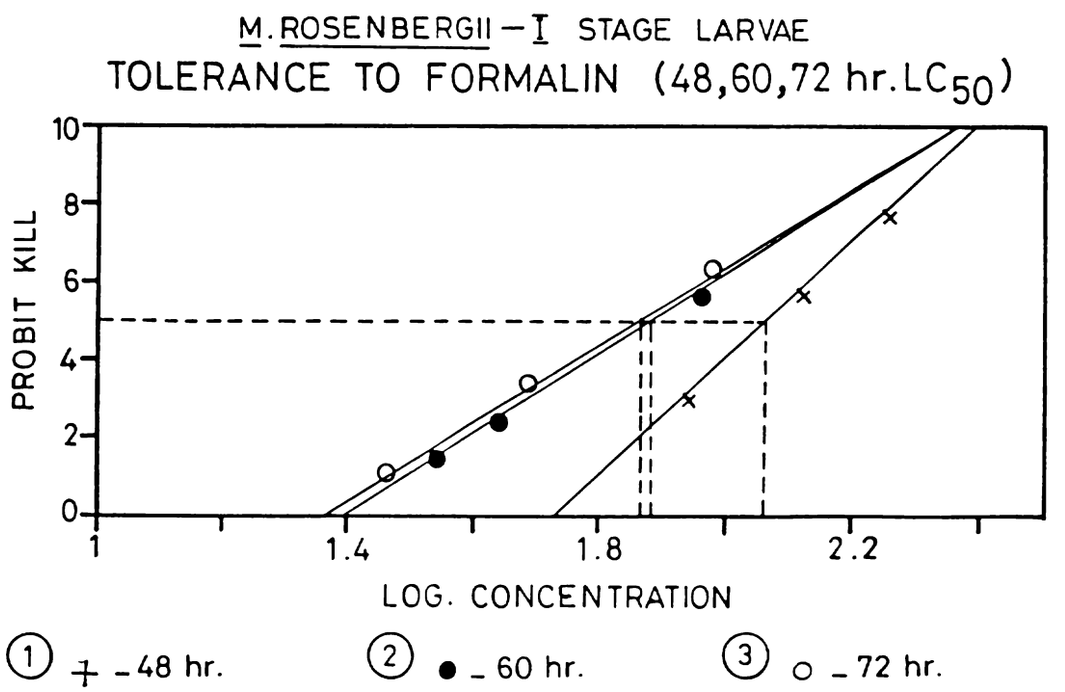

M.ROSENBERGII – I STAGE LARVAE
TOLERANCE TO FORMALIN (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.4
1.8
2.2
LOG. CONCENTRATION
① + – 48 hr.
② ● – 60 hr.
③ o – 72 hr.

FIG:44

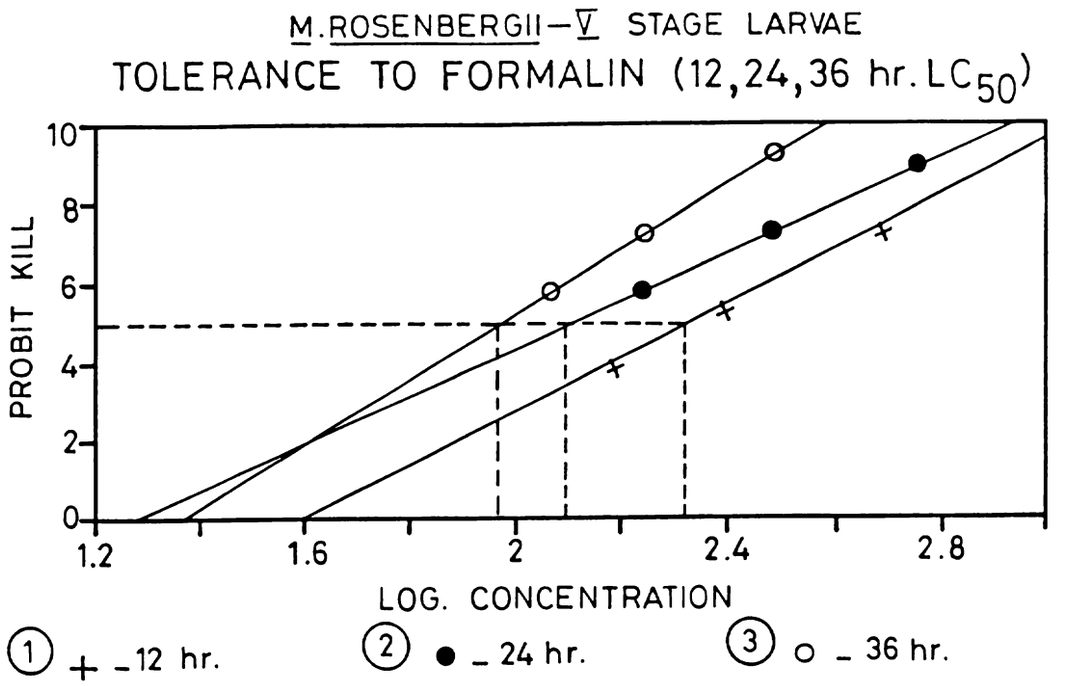

M.ROSENBERGII—V STAGE LARVAE
TOLERANCE TO FORMALIN (12,24,36 hr. LC50)
PROBIT KILL
10
8
6
4
2
0
1.2
1.6
2
2.4
2.8
LOG. CONCENTRATION
1 + - 12 hr.
2 ● - 24 hr.
3 o - 36 hr.

FIG: 45

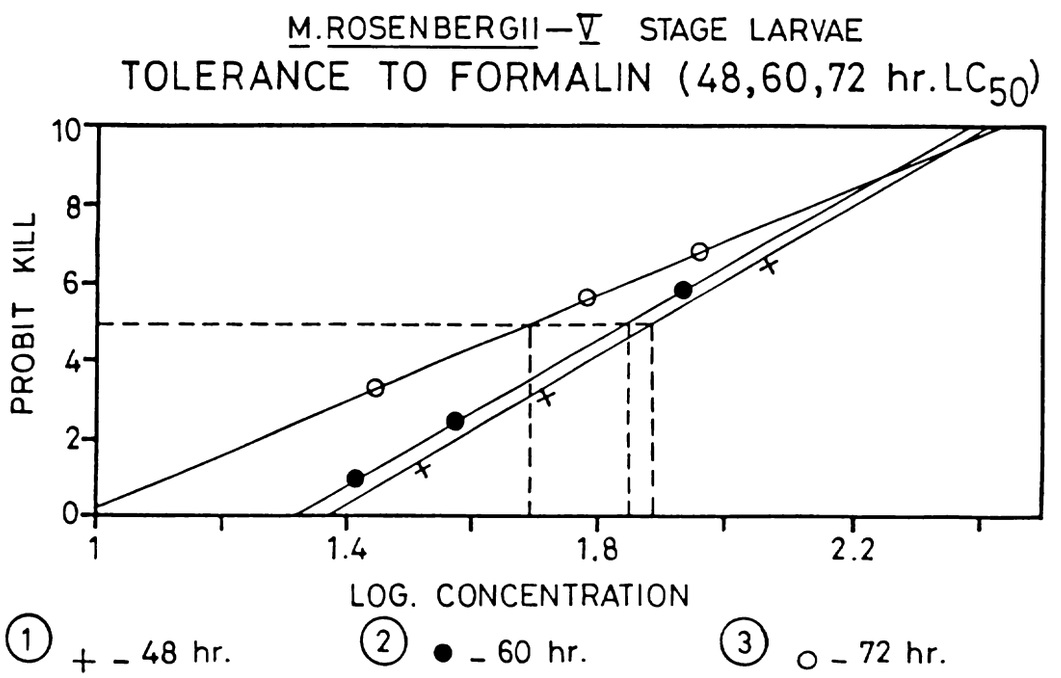

M.ROSENBERGII—V STAGE LARVAE
TOLERANCE TO FORMALIN (48,60,72 hr. LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.4
1.8
2.2
LOG. CONCENTRATION
1 + - 48 hr.
2 ● - 60 hr.
3 o - 72 hr.

FIG: 46

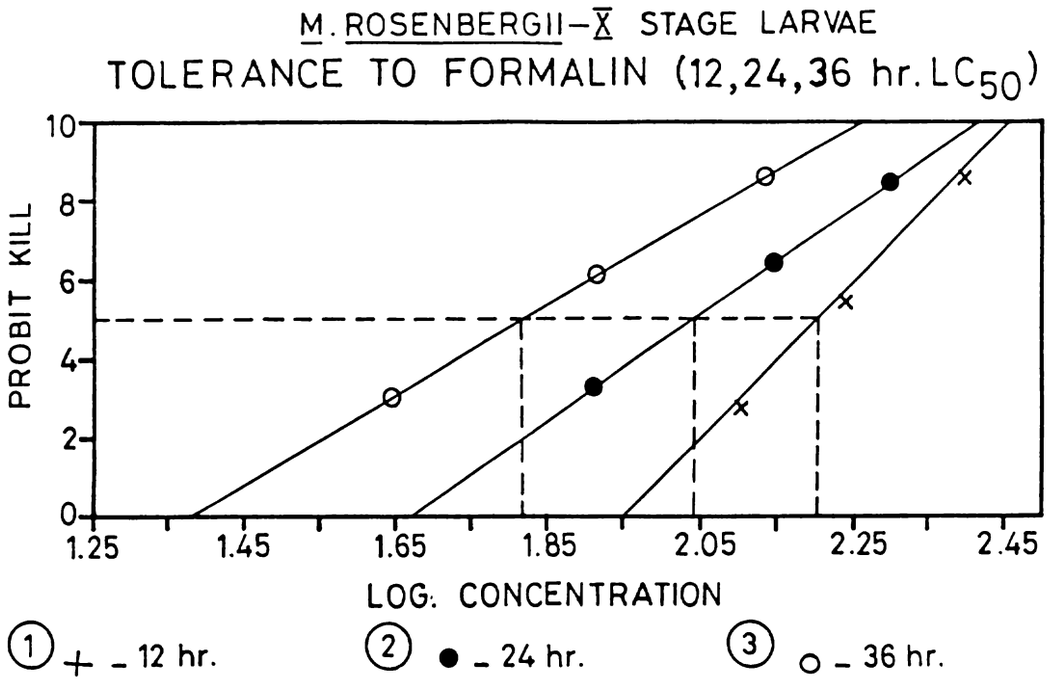

M. ROSENBERGII–X STAGE LARVAE
TOLERANCE TO FORMALIN (12,24,36 hr. LC50)
PROBIT KILL
10
8
6
4
2
0
1.25
1.45
1.65
1.85
2.05
2.25
2.45
LOG. CONCENTRATION
1 + – 12 hr.
2 ● – 24 hr.
3 o – 36 hr.

FIG: 47

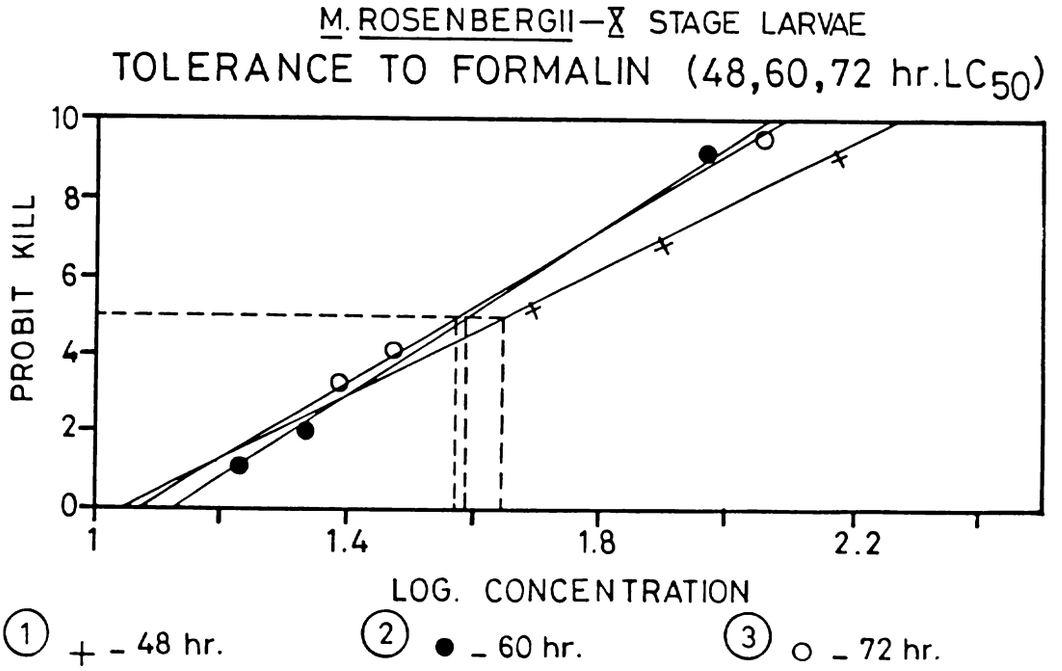

M. ROSENBERGII–X STAGE LARVAE
TOLERANCE TO FORMALIN (48,60,72 hr. LC50)
PROBIT KILL
10
8
6
4
2
0
1
1.4
1.8
2.2
LOG. CONCENTRATION
1 + – 48 hr.
2 ● – 60 hr.
3 o – 72 hr.

FIG: 48

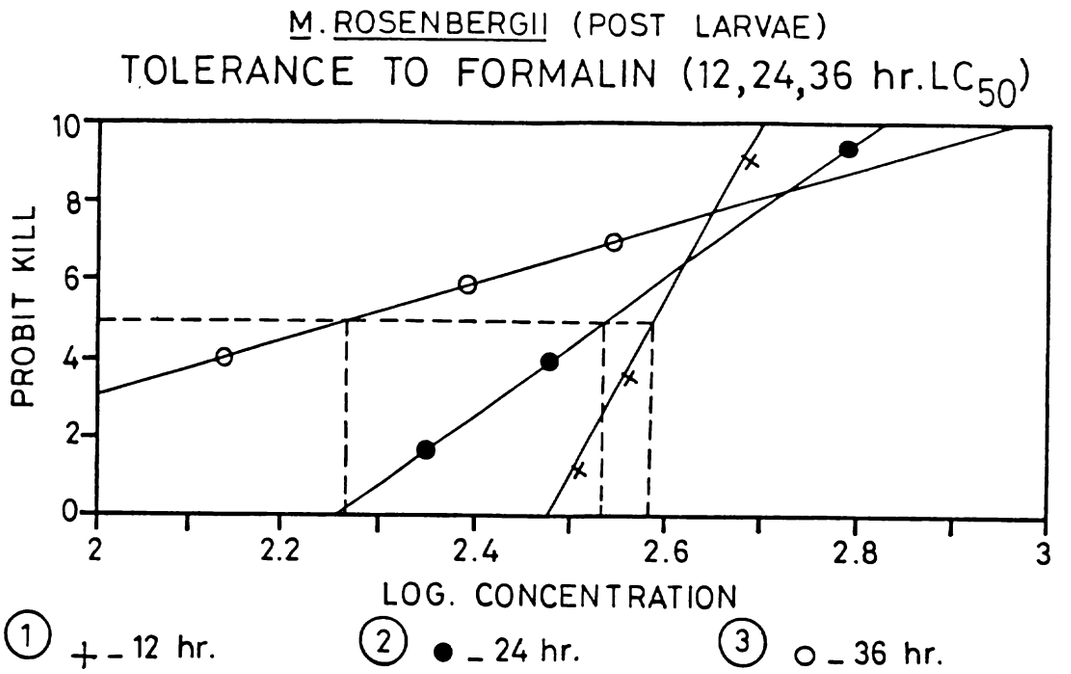

M. ROSENBERGII (POST LARVAE)
TOLERANCE TO FORMALIN (12,24,36 hr. LC50)
PROBIT KILL
10
8
6
4
2
0
2
2.2
2.4
2.6
2.8
3
LOG. CONCENTRATION
1 + - 12 hr.
2 ● - 24 hr.
3 o - 36 hr.

FIG: 49

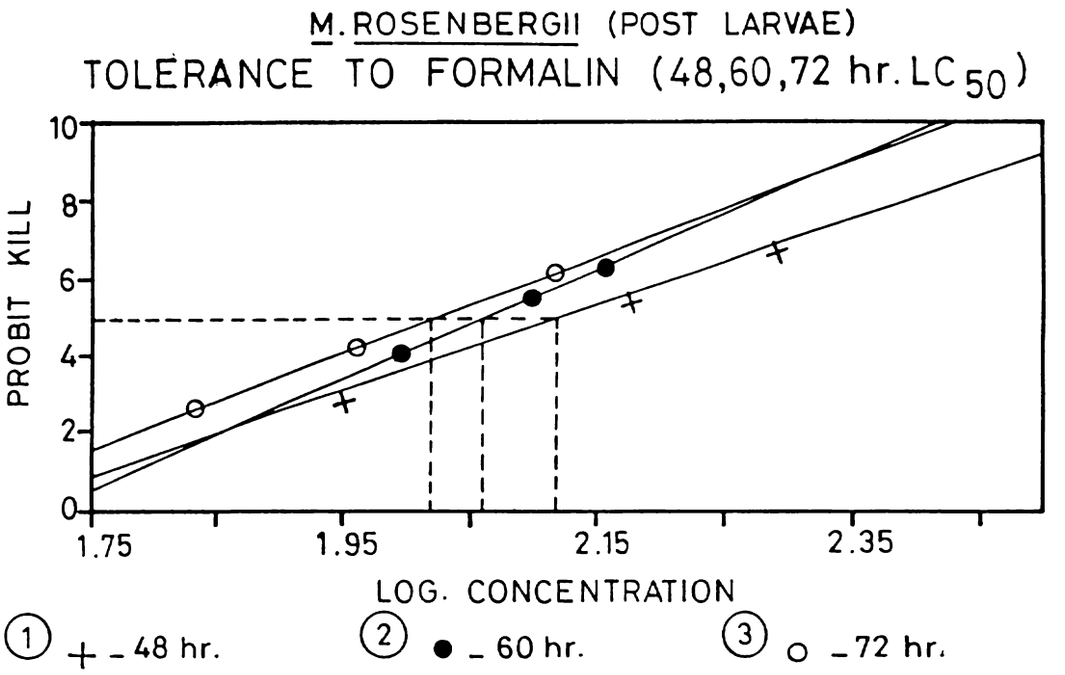

M. ROSENBERGII (POST LARVAE)
TOLERANCE TO FORMALIN (48,60,72 hr. LC50)
PROBIT KILL
10
8
6
4
2
0
1.75
1.95
2.15
2.35
LOG. CONCENTRATION
1 + - 48 hr.
2 ● - 60 hr.
3 o - 72 hr.

FIG: 50

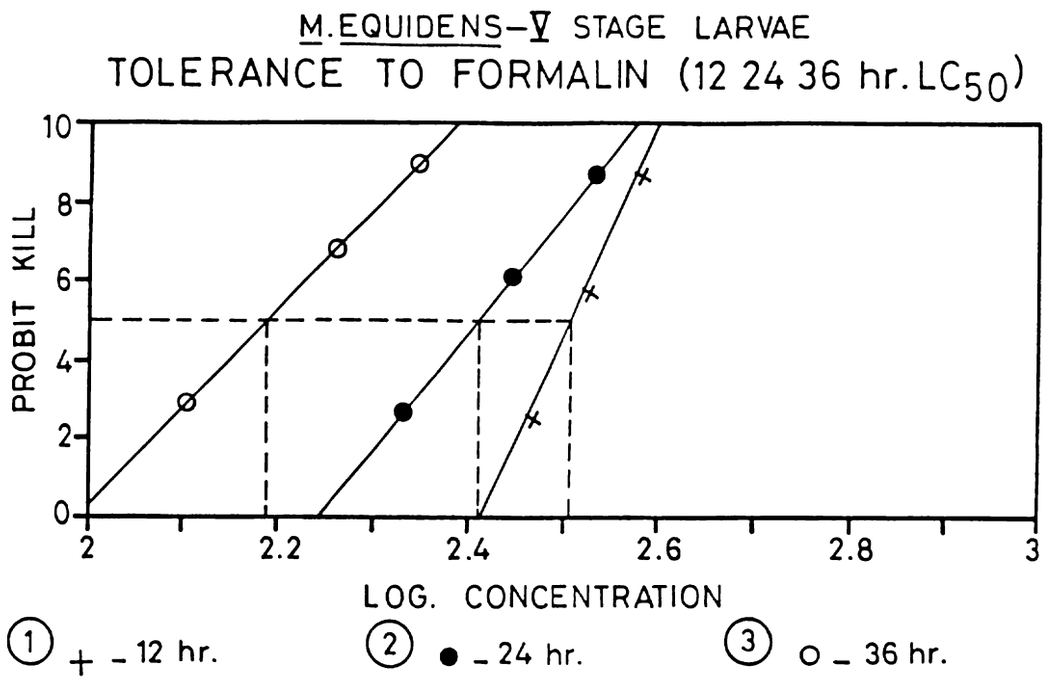

M.EQUIDENS–V STAGE LARVAE
TOLERANCE TO FORMALIN (12 24 36 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
2
2.2
2.4
2.6
2.8
3
LOG. CONCENTRATION
1 + – 12 hr.
2 ● – 24 hr.
3 o – 36 hr.

FIG: 51

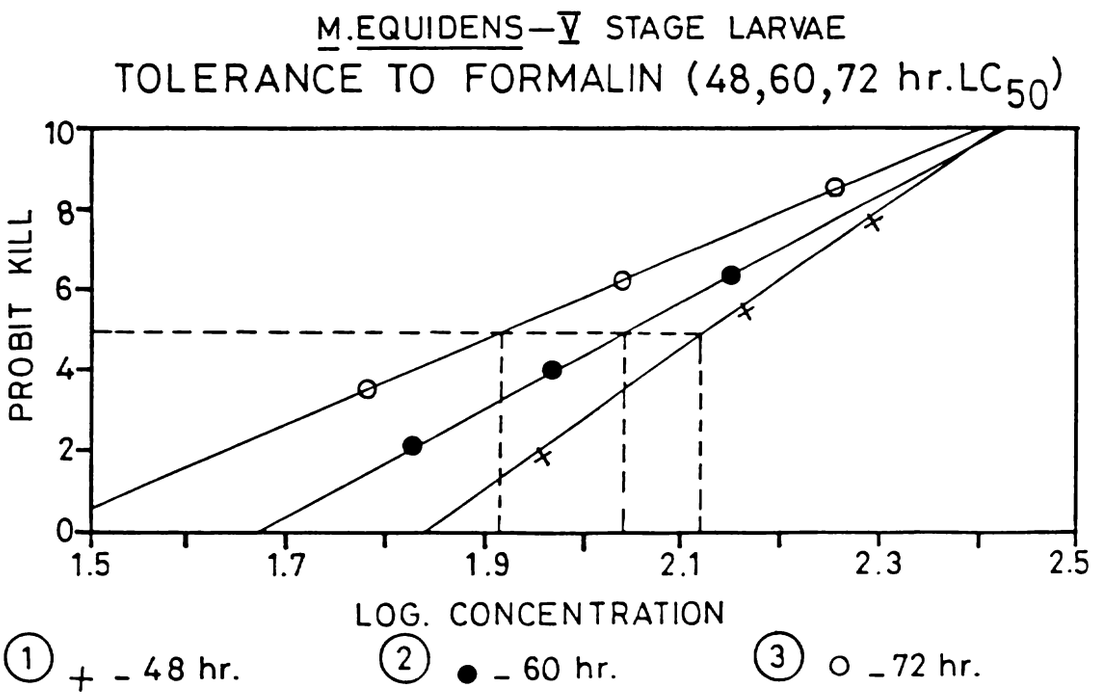

M.EQUIDENS–V STAGE LARVAE
TOLERANCE TO FORMALIN (48,60,72 hr.LC50)
PROBIT KILL
10
8
6
4
2
0
1.5
1.7
1.9
2.1
2.3
2.5
LOG. CONCENTRATION
1 + – 48 hr.
2 ● – 60 hr.
3 o – 72 hr.

FIG: 52

M. ROSENBERGII

EFFECT OF WATER MEDIUM USED FOR MORE THAN ONCE ON THE REARING OF LARVAE

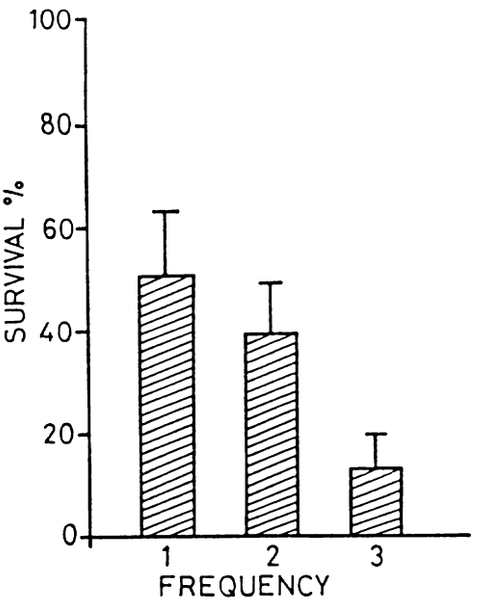


FIG: 53

M. EQUIDENS

EFFECT OF WATER MEDIUM USED FOR MORE THAN ONCE ON THE REARING OF LARVAE

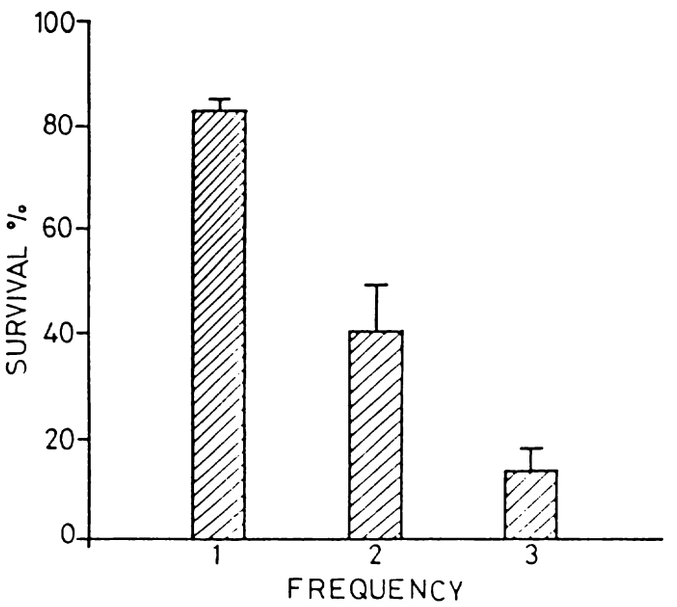


FIG: 54

NUMBER OF STAGES OF LARVAE OBSERVED UNDER VARIOUS STAGES OF EVERY FIFTH DAY

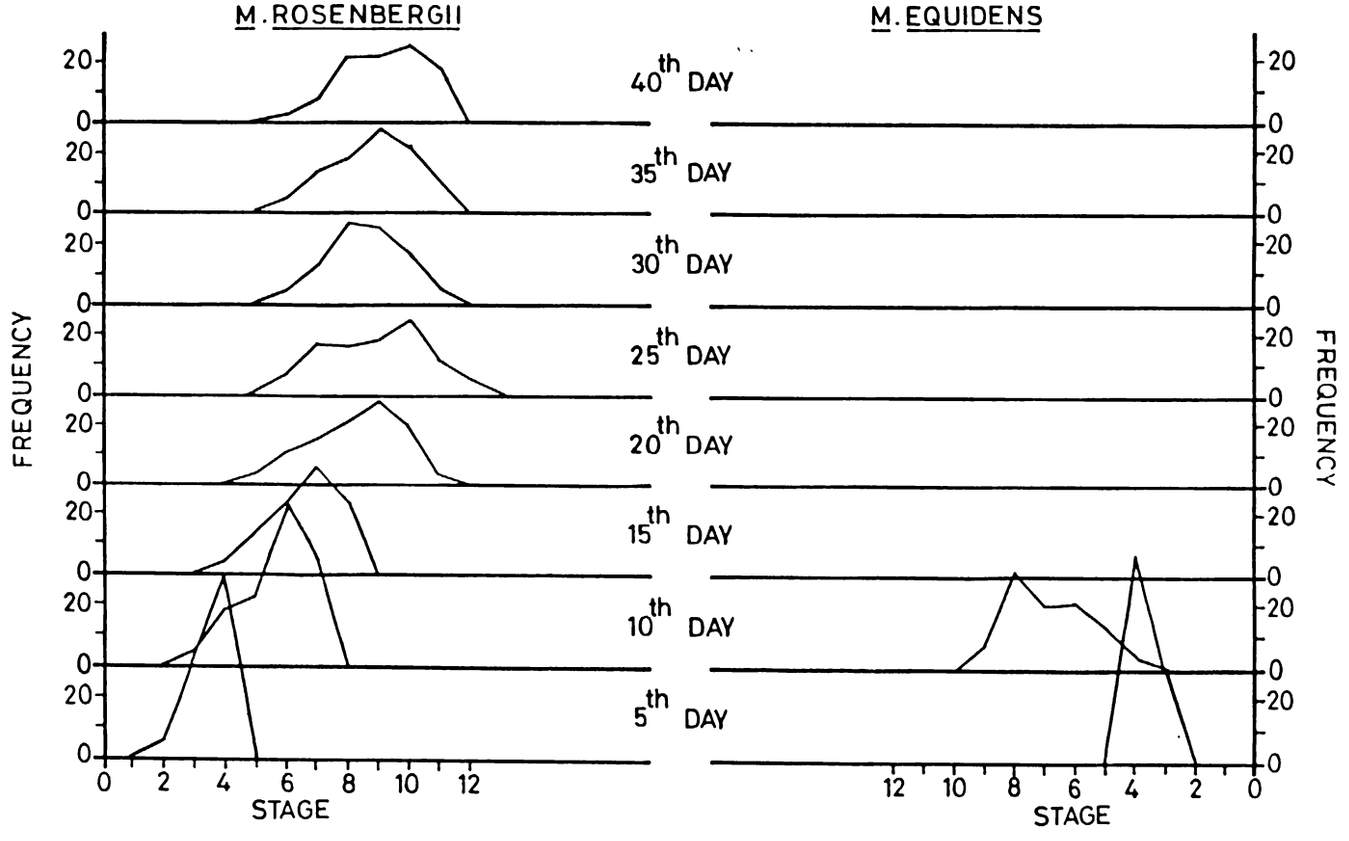


FIG: 55

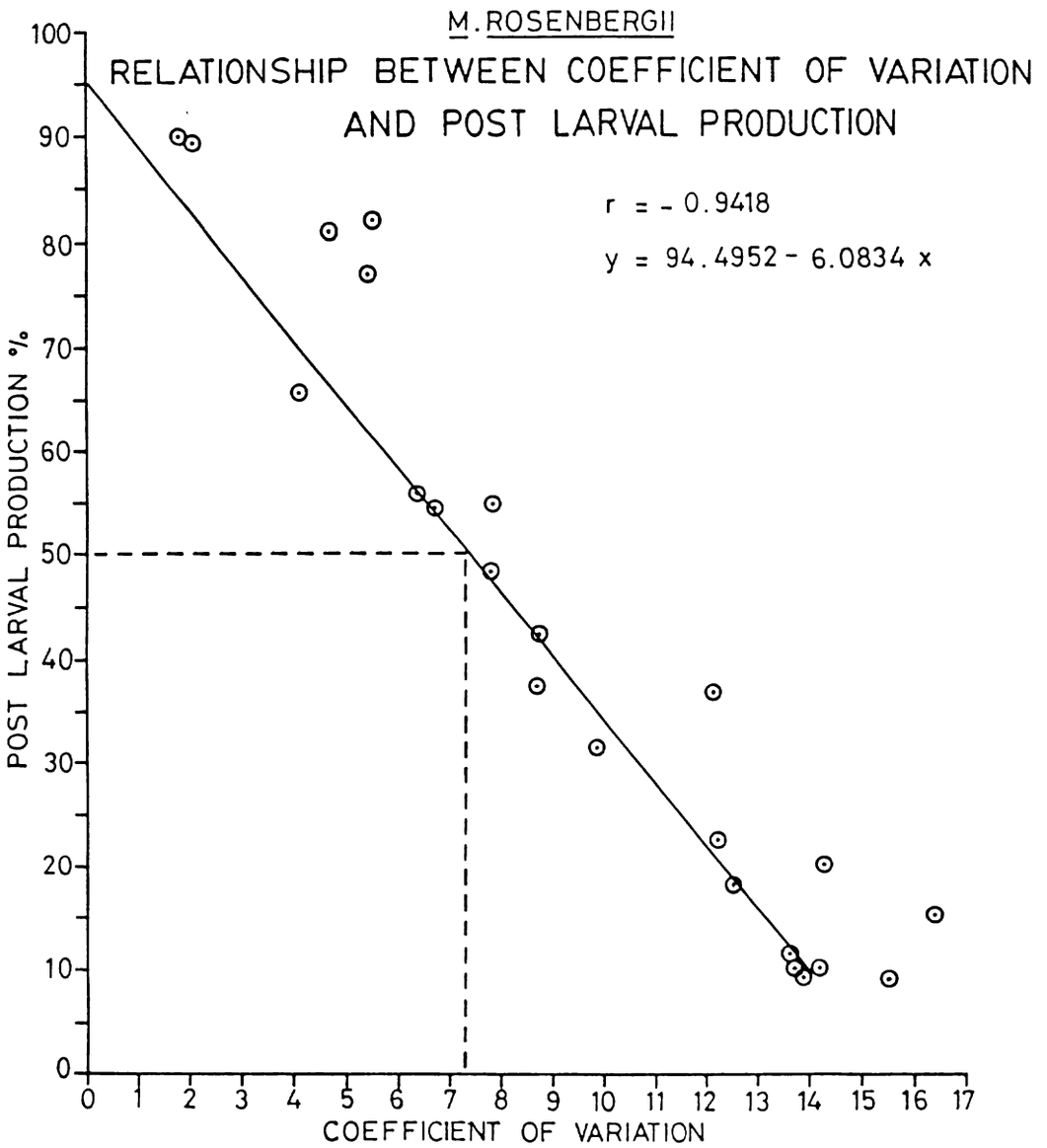

M. ROSENBERGII
RELATIONSHIP BETWEEN COEFFICIENT OF VARIATION
AND POST LARVAL PRODUCTION
r = - 0.9418
y = 94.4952 - 6.0834 x
POST LARVAL PRODUCTION %
COEFFICIENT OF VARIATION
0
10
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

FIG: 56

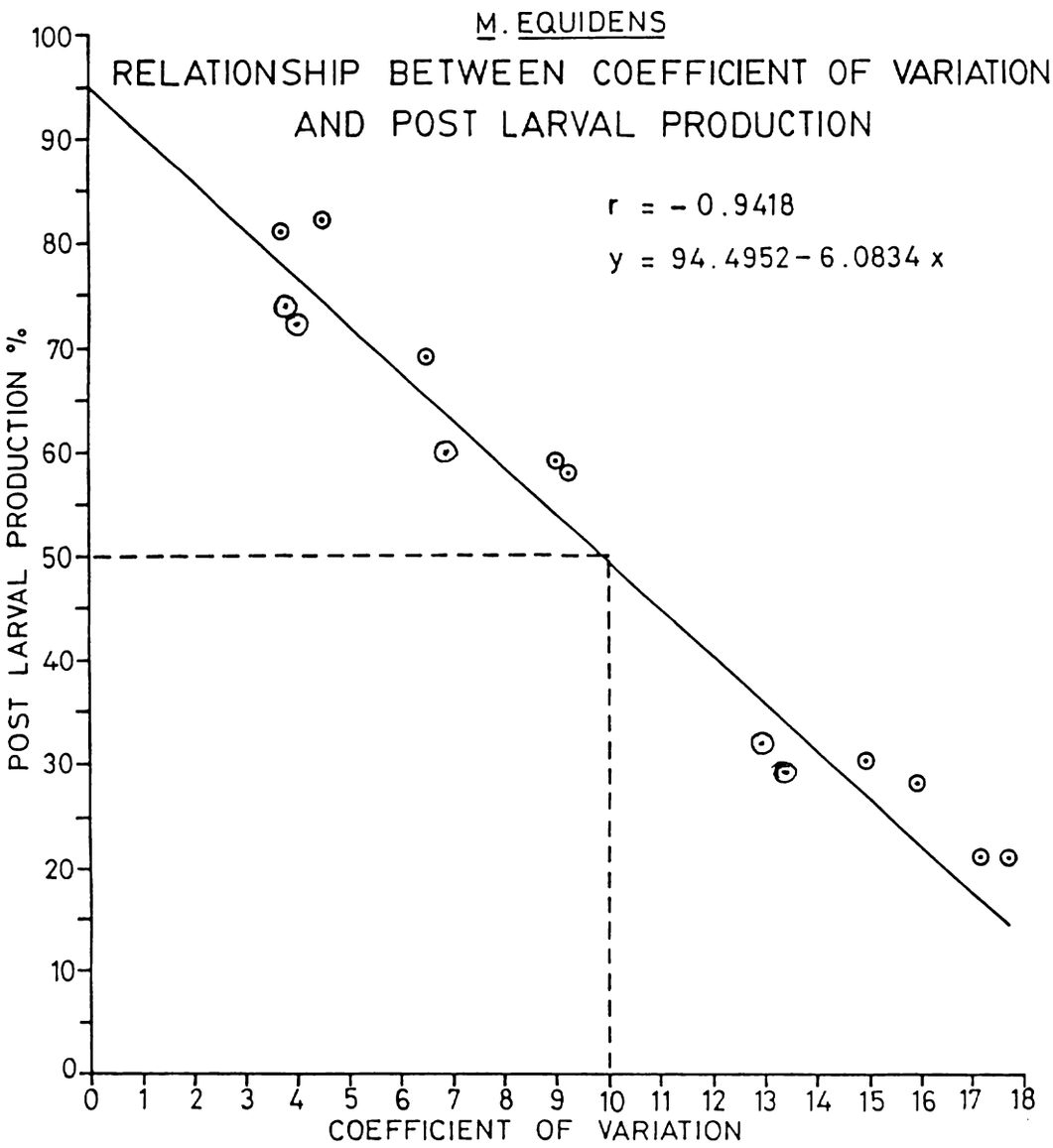

M. EQUIDENS
RELATIONSHIP BETWEEN COEFFICIENT OF VARIATION
AND POST LARVAL PRODUCTION
r = - 0.9418
y = 94.4952 - 6.0834 x
POST LARVAL PRODUCTION %
COEFFICIENT OF VARIATION
0
10
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

FIG:57

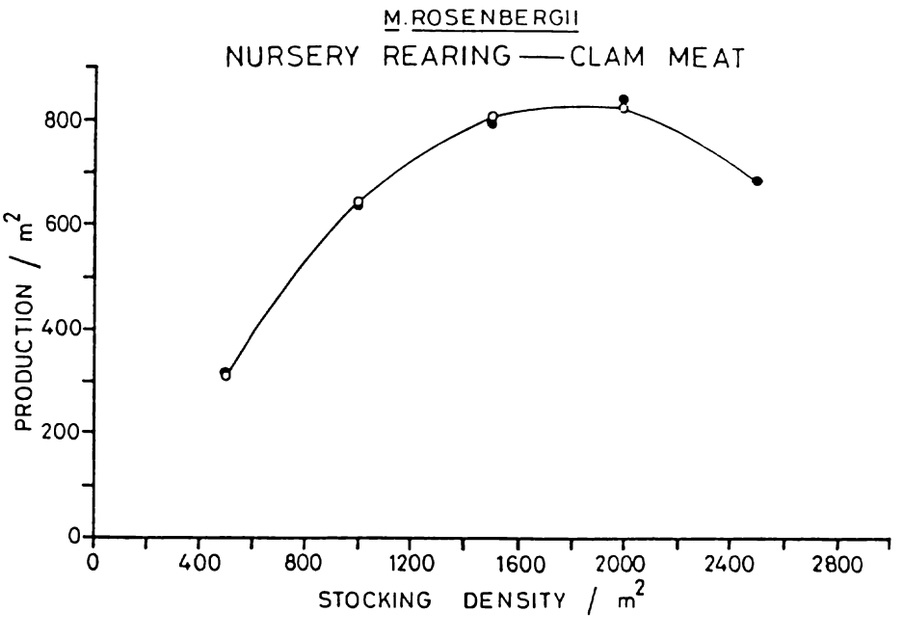

M.ROSENBERGII
NURSERY REARING — CLAM MEAT
PRODUCTION / m2
0
200
400
600
800
0
400
800
1200
1600
2000
2400
2800
STOCKING DENSITY / m2

FIG: 58

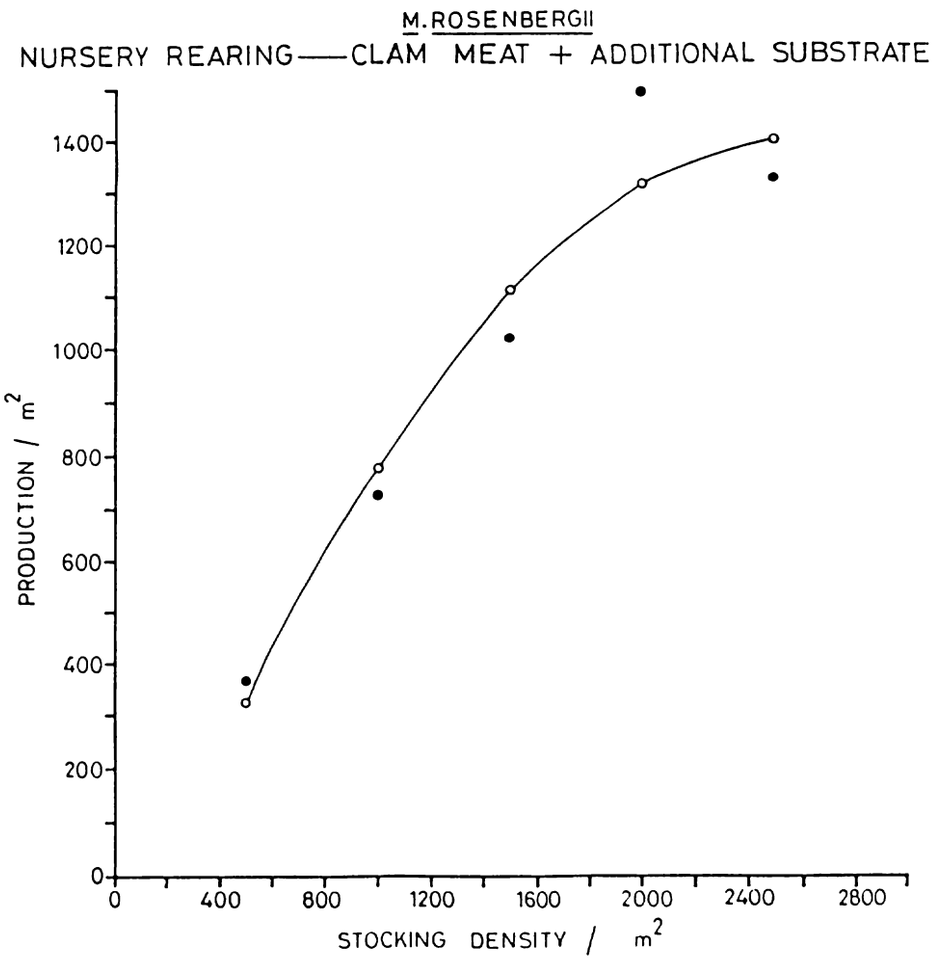

M.ROSENBERGII
NURSERY REARING — CLAM MEAT + ADDITIONAL SUBSTRATE
PRODUCTION / m2
0
200
400
600
800
1000
1200
1400
0
400
800
1200
1600
2000
2400
2800
STOCKING DENSITY / m2

FIG:59

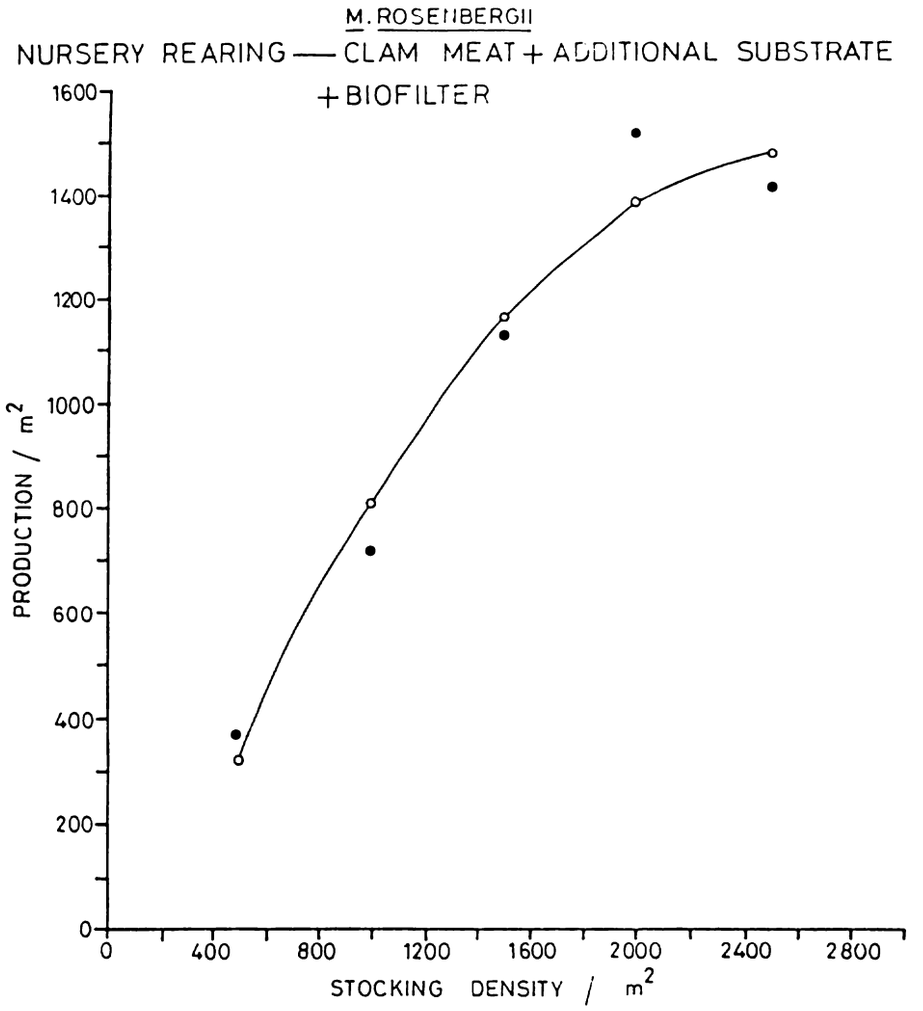

M. ROSENBERGII
NURSERY REARING — CLAM MEAT + ADDITIONAL SUBSTRATE
+ BIOFILTER
PRODUCTION / m2
STOCKING DENSITY / m2
0
200
400
600
800
1000
1200
1400
1600
0
400
800
1200
1600
2000
2400
2800

FIG: 60

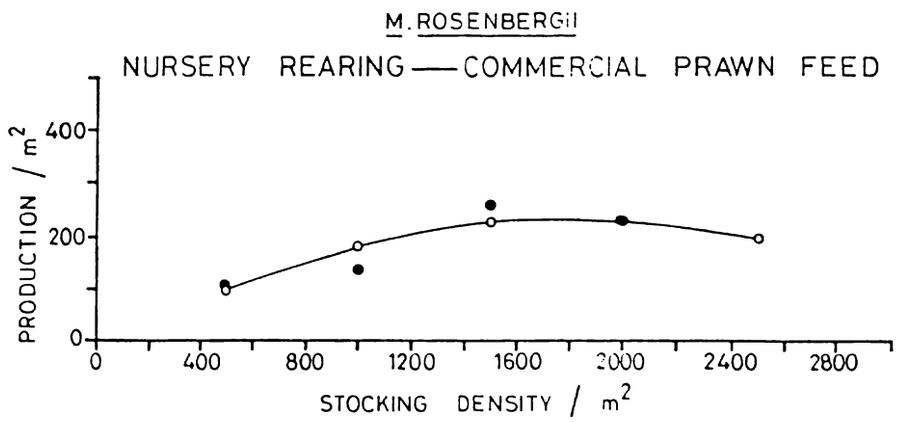

M. ROSENBERGII
NURSERY REARING — COMMERCIAL PRAWN FEED
PRODUCTION / m2
STOCKING DENSITY / m2
0
200
400
0
400
800
1200
1600
2000
2400
2800

FIG: 61

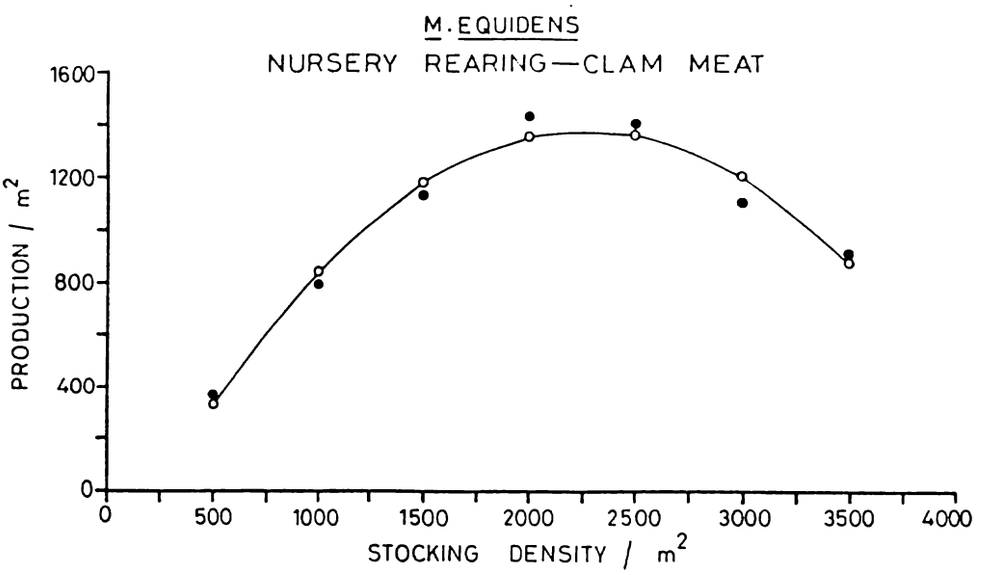

M. EQUIDENS
NURSERY REARING—CLAM MEAT
PRODUCTION / m²
1600
1200
800
400
0
0
500
1000
1500
2000
2500
3000
3500
4000
STOCKING DENSITY / m²

FIG: 62

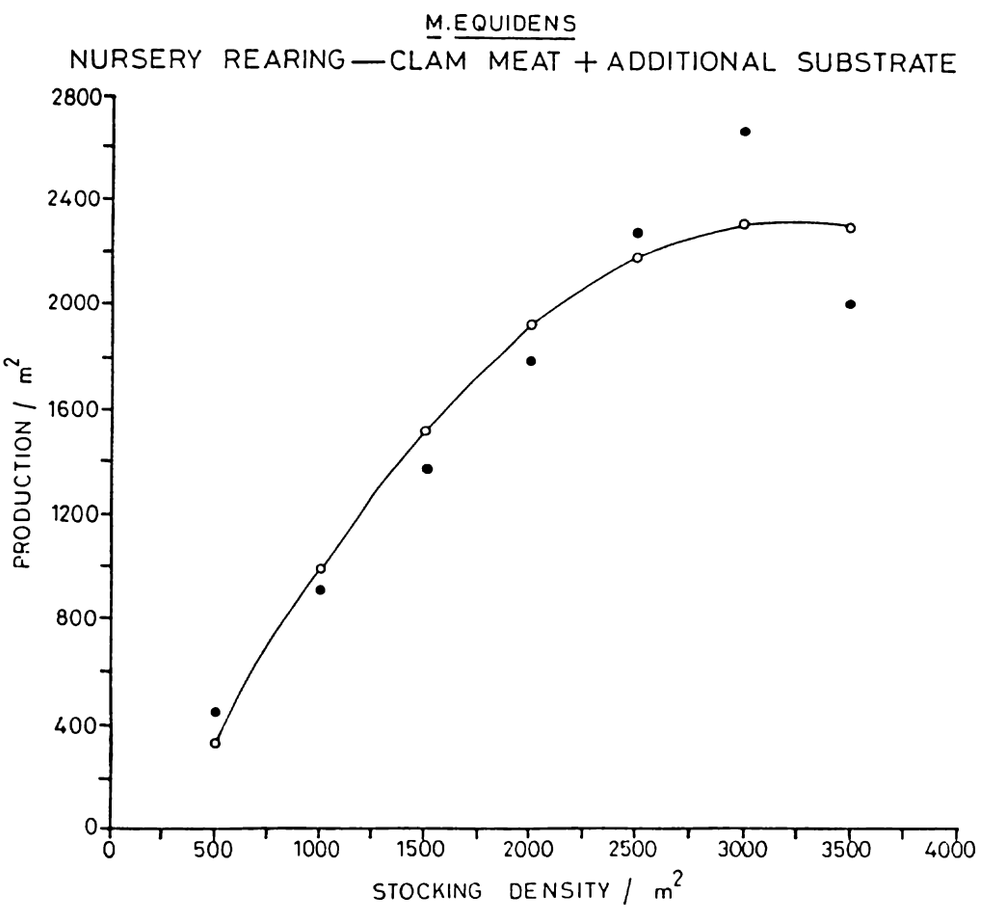

M. EQUIDENS
NURSERY REARING—CLAM MEAT + ADDITIONAL SUBSTRATE
PRODUCTION / m²
2800
2400
2000
1600
1200
800
400
0
0
500
1000
1500
2000
2500
3000
3500
4000
STOCKING DENSITY / m²

FIG: 63

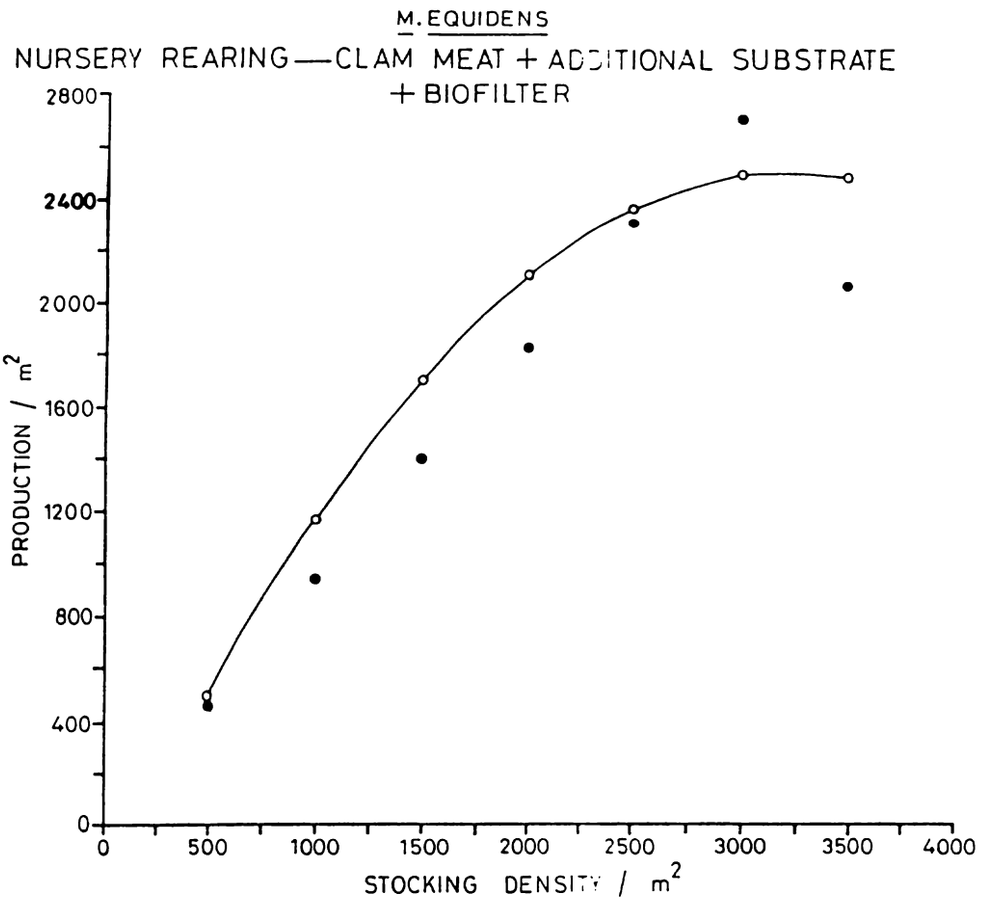

M. EQUIDENS
NURSERY REARING—CLAM MEAT + ADDITIONAL SUBSTRATE
+ BIOFILTER
PRODUCTION / m2
STOCKING DENSITY / m2
0
400
800
1200
1600
2000
2400
2800
500
1000
1500
2000
2500
3000
3500
4000

FIG: 64

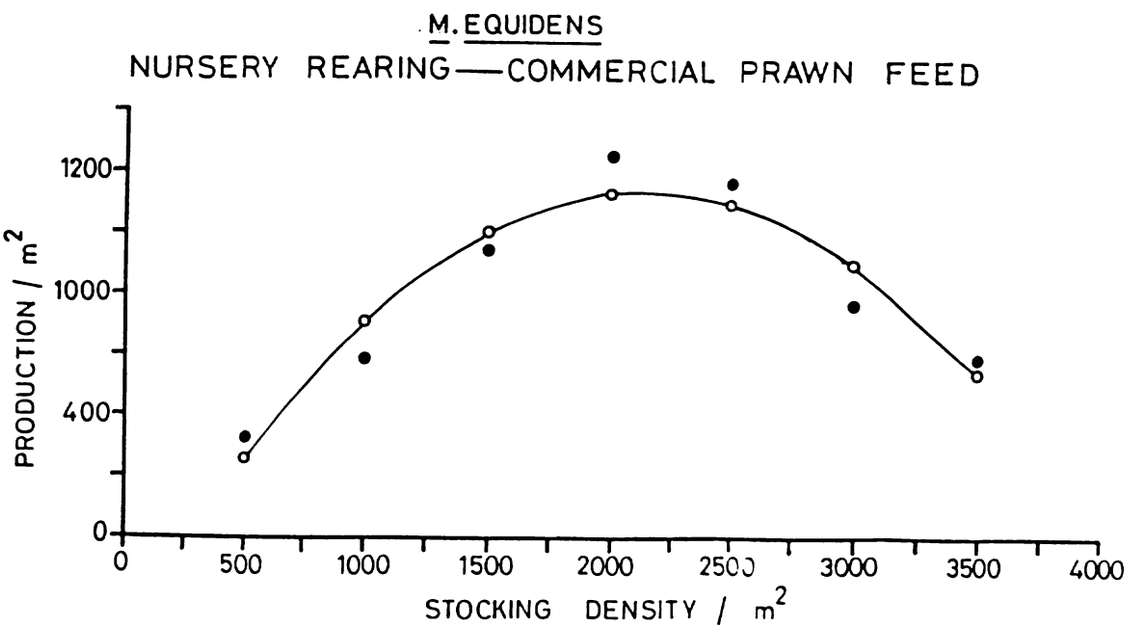

M. EQUIDENS
NURSERY REARING—COMMERCIAL PRAWN FEED
PRODUCTION / m2
STOCKING DENSITY / m2
0
400
1000
1200
500
1000
1500
2000
2500
3000
3500
4000

FIG: 65