In vitro embryo culture and effect of salinity on the embryonic development of the cultivable freshwater prawn *Macrobrachium malcolmsonii* (H. Milne Edwards)

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Five culture media prepared by using varying quantities of NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ and $NaHCO_3$, were used to test their feasibility for the embryo culture of *Macrobrachium malcolmsonii*. A reduction in incubation period (11 days) was obtained in the medium V, compared with other media (I to IV). The hatching percentage was higher in the medium V (69.7%) than in other media. Hence, medium V is suitable for *in vitro* embryo culture of *Macrobrachium malcolmsonii*. In *in vitro* culture, eggs hatched on the 12th day had a survival of 66.7% in 0.5 ppt salinity, whereas in 2, 4, 6, 8 and 10 ppt salinities mortality occurred in III, II and I stages of embryonic development. It was also noted that more than 35 eggs in a cluster lead to mortality.

UNDER captive conditions *Macrobrachium malcolmsonii* often shed their eggs when disturbed, as the gravid females are sensitive. Similar behaviour was also reported in *M. nobilii*[1]. Soundarapandian *et al.*[2] also observed a similar phenomenon in cross-bred females of *M. malcolmsonii*. In view of its culture importance along the South East Coast of India[3], developing techniques for an *in vitro* embryo culture and enhanced larval production is important. The information on *in vitro* embryo culture and the effect of salinity on embryonic development during *in vitro* culture is meager in prawns. Hence, the present investigation was directed towards this aspect in *M. malcolmsonii*.

Mature males (length 155 mm and weight 33 g) and equal number of mature females (length 150 mm and weight 31.4 g) of *M. malcolmsonii* were collected from the freshwater tanks of Manampadi (Lat. 11°29′N and Long. 79°46′E), Tamil Nadu. They were acclimatized to laboratory conditions (salinity 0.5 ppt, temperature 28 ± 2°C, DO 5 ppm and photophase 12/12 h L/D) and maintained in a 180 × 60 cm fibreglass tank. One-third of the water was changed daily and the prawns were fed with clam meat. As soon as the females experienced pre-mating moult, mating was allowed by introducing a newly pre-mating moulted female with a mature male in 1 : 1 ratio in a 50 l fiberglass tank. After spawning, clusters of 12 h embryo were removed from the brooding female. The clusters were teased and separated into individual embryo or into small pieces (a maximum of 30 embryos per piece). *In vitro* culture was carried out at room temperature (28 ± 2°C) and an average of about 200 embryos were placed in each 100 ml glass bowl containing 50 ml culture medium. Five replicates were maintained for each medium. Culture media were prepared by using the same chemicals described by Damrongphol *et al.*[4] for *in vitro* embryo culture of *M. rosenbergii*. But different quantities were used in the present study (Table 1). After finding the suitable medium, a test was conducted to know the effect of salinity on embryonic development during the *in vitro* embryo culture. Different salinities (0.5, 2, 4, 6, 8 and 10 ppt) were prepared by adding required amounts of sodium chloride (NaCl, AR grade) in the medium. Salinities were measured by using Century Water Analyser Kit Model CK 711.

Developmental stages of the *in vitro* cultured embryos were compared with those of the embryos carried by brooding female in the laboratory. Dead embryos in the culture were aspirated out and fresh media were replaced daily. Chi-square test (χ^2), coefficient of variation (CV) and regression analysis were done by using ABSTAT 3.01 statistical package.

Various media were tested for their feasibility in embryo culture of *M. malcolmsonii* and the results are shown in Table 2. A reduction in the incubation period (11 days) was obtained in medium V compared with the control (14 days). Statistically significant variation was observed in the incubation period among different media tested ($\chi^2 = 27.569$; $P < 0.001$). The hatching percentages in media IV and V were 23.5% and 69.8%

Table 1. Chemicals used to prepare various media for *in vitro* embryo culture of *M. malcolmsonii*

Chemicals	Medium (mM)				
	I	II	III	IV	V
NaCl	423.0	242.0	52.0	15.0	0.419
KCl	10.0	20.0	8.0	8.0	0.027
$CaCl_2$	10.0	8.0	5.0	2.0	0.010
$MgCl_2$	23.0	12.0	7.0	2.3	0.023
$MgSO_4$	25.5	20.0	5.6	2.9	0.025
$NaHCO_3$	2.1	2.1	2.1	2.1	0.012

Table 2. Effect of various media on incubation period and hatching percentage of *M. malcolmsonii* during *in vitro* embryo culture

Medium	Incubation period (days)	Developmental stages	Hatching percentage
I	0.88 ± 0.44	I	–
II	3.18 ± 0.295	I	–
III	4.32 ± 0.740	II	–
IV	13.18 ± 0.536	IV	23.5 ± 0.265
V	11.08 ± 0.414	IV	69.76 ± 0.716
Control*	14.0 ± 0.682	IV	95.2 ± 0.837

*Embryos carried by the laboratory maintained brooding female.

respectively. However, a higher percentage (95.2%) was obtained in the control. No survival was noticed in media I, II and III after 0.88 (I stage), 3.18 (I stage) and 4.32 (II stage) days respectively. When CV was tested among the replicates for the incubation period in different media and in control, medium V showed less variation of 3.74% compared to 50.0%, 9.3% 17.1%, 4.1% and 4.9% in media I, II, III, IV and in control respectively. Similarly, CV was 0.9% in control than 1.1% and 1.0% respectively in media IV and V for hatching percentage.

In 0.5 ppt salinity, the eggs hatched out on the 11th day with a survival of 66.7%, whereas in 2, 4, 6, 8 and 10 ppt salinities, total mortality occurred before hatching (Table 3). The number of days in each developmental stage increased linearly as the salinity increased ($r^2 = 0.9370$). A similar trend was also observed between increased salinity and embryonic mortality rate in each stage ($r^2 = 0.9722$).

The following changes were observed in the egg during *in vitro* embryo culture in 0.5 ppt salinity (Figure 1). Fifteen hours after spawning, the cultured embryos developed to 32 cell stage. The 3-day-old embryo showed a clear region at one pole. The clear region extended lengthwise forming the trunk of the growing embryo on the 5th day. Two eye spots were visible on 6-day-old embryo and they developed to oval-shaped eyes on the 8th day. During 7 to 9 days of culture period, the yolk mass lessened significantly followed by the formation of appendages beneath the clear trunk region and on the 9th day, the eyes were large and surrounded by striations and the translucent globules at the dorso-caudal portion of the yolk mass clearly exhibited rhythmic contraction.

Fully developed embryos were seen on the 11th day of culture followed by hatching on the 12th day. The activities and size of the zoea hatched out from cultured embryos are similar to the zoea obtained from the brooding mother. It is interesting to note that mortality occurred when more than 35 eggs in a cluster were cultured. When healthy 7th day embryos (stage III) reared in 0.5 ppt salinity were transferred to 6 ppt salinity, 50% mortality occurred within 24 h of transfer and 50% eggs successfully hatched to zoea but they were found to be weak and less active compared to the normal ones (obtained from the brooding mother) and they died after 2 h of hatching.

According to Fisher[5], optimal physiological conditions such as nutrient, temperature and pH are essential for embryonic growth, and a high level of nutrient, in contrast, retards the growth of embryo. The unsuitability of the media I to IV in the present study may be owing to the high level of their nutrient content which retards the growth of embryo due to the increased microbial contamination resulting in embryonic mortality. This increased bacterial contamination in turn, reduced the oxygen supply reaching the embryos[4]. Since better growth and higher percentage of hatching were observed in medium V, it seems that medium V provides the nutrient requirement of embryo at an optimum level. Damrongphol *et al.*[4] observed best growth of *M. rosenbergii* embryo in the medium containing 423 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 23 mM $MgCl_2$, 25.5 mM $MgSO_4$, and 2.1 mM $NaHCO_3$ which is similar to medium I in the present study. From the results obtained in the present study, it is inferred that *M. malcolmsonii* requires comparatively lower quantities of nutrients for embryonic development compared to *M. rosenbergii*, showing its adaptability to survive in low nutrient content medium.

Most of the adults of *Macrobrachium* sp. are known to migrate to the brackishwater for breeding purposes[6]. Ling[7] found that the presence of a small quantity of sea water (4 to 6 ppt) provides a better medium for hatching of *M. rosenbergii* eggs. Katre and Pandian[8] confirmed that the eggs of *M. idae* are able to 'pick up' salts from brackishwater more readily than from freshwater. Damrongphol *et al.*[4] observed that 15% artificial sea water improved the embryonic development of *M. rosenbergii* in *in vitro* culture. Interestingly, in the present study cultured embryos of *M. malcolmsonii*

Table 3. Embryonic development (days) of different stages of *M. malcolmsonii* at various salinities in *in vitro* culture (mortality in percentage is provided in parentheses)

Developmental stages	Salinity (ppt)					
	0.5	2	4	6	8	10
I	3.0 ± 0.5 –	2.8 ± 0.76 –	4.4 ± 0.40 (16.6)	5.3 ± 0.67 (26.7)	5.4 ± 0.40 (33.3)	6.5 ± 0.45 (50)
II	2.1 ± 0.31 –	2.7 ± 0.44 (16.6)	4.5 ± 0.47 (50)	– (73.3)	– (66.7)	– (50)
III	2.0 ± 0.1 (20)	4.2 ± 0.57 (50)	– (33.4)	–	–	–
IV	4.1 ± 0.47 (13.3)	– (33.4)				

hatched only in 0.5 ppt salinity but not in 2 to 10 ppt salinity. This is contrary to the above observations, but coincides with the speculations of a few workers[9-12] that *M. malcolmsonii* shows no breeding migration towards brackishwater whereas hatching takes place in freshwater and the zoea I move towards the slight saline areas. When the healthy 7th day embryos, reared in the culture medium containing 0.5 ppt salinity, were transferred to 6 ppt salinity, mortality occurred. This may be due to osmotic stress, followed by the shrinkage of cells at higher salinities[13]. Surprisingly, *M. malcolmsonii* brooders reared in 6 ppt salinity have not shown any adverse effects on developing embryos, and hatching occurs normally. This may be due to the maternal secretion which protects the developing embryos from the adverse external conditions. The incubation of developing embryos on the pleopods beneath the abdomen till hatching ensures greater survival against predators and other hostile environmental conditions[14].

Higher mortality occurring in clusters containing more than 35 eggs is attributed to the reduction of oxygen supply reaching the embryos. Therefore it is necessary to separate the large embryo clusters into individual embryo so as to expose a large portion of the embryo surface to the medium for free oxygen exchange. Egg incubation by the mother involves ventilation of the developing eggs by fanning her pleopods, so that water percolates between individual eggs, facilitating gaseous and ionic exchange[15].

Moulting and breeding are not only the major energy demanding processes[16] but also egg carriage and ventilation of the developing eggs[15]. It was also observed, that the relieved females of *M. nobilii* which skipped spawning moult resulted in producing more brood/annum compared to normal females. This shows that removal of eggs from berried females provides ample scope to save energy. So it is likely that energy cost on incubation is channeled for the next brood production. Since, the

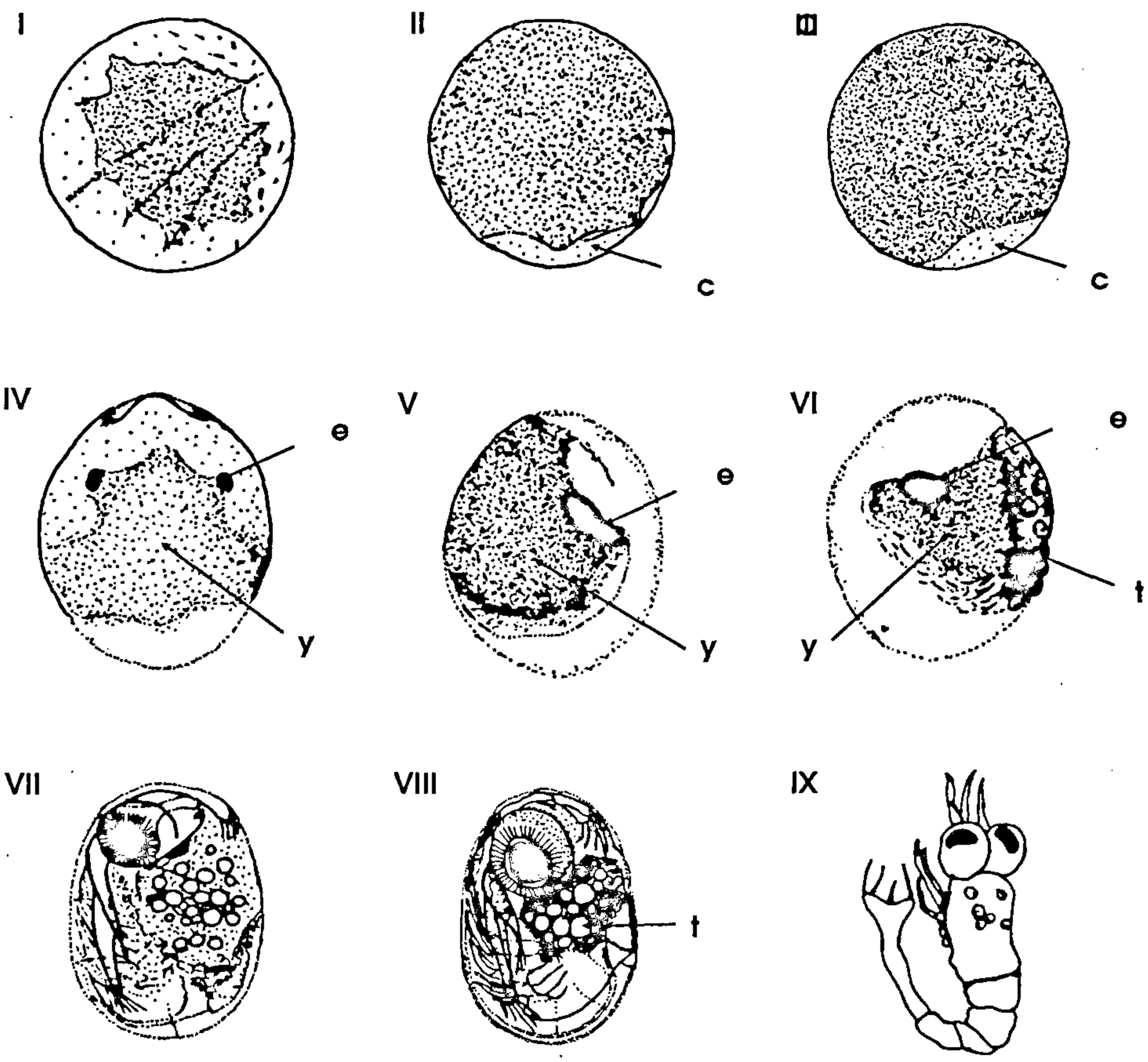

Figure 1. I, 15-h-old embryo of about 32 cells; II, 3-day-old embryo showing a clear region (c) at one pole; III, 5-day-old embryo; IV, 6-day-old embryo developing two eye spots (e) in the yolky mass (y); V, 8-day-old embryo showing an enlarged oval-shaped eyes; VI, 9-day-old embryo with large oval-shaped eyes and translucent globules (t) at the dorso caudal region of the yolky mass (y); VII, 10-day-old embryo; VIII, 11-day-old embryo with prominent dark round eyes and the translucent globules (t) enlarged; IX, Newly hatched larvae on the 12th day.

embryo of *M. malcolmsonii* is highly adaptive to survive well in low nutritive medium, the eggs may be removed soon after fertilization from the mother and cultured in *in vitro* medium, thereby relieving the female to save energy, which can be used for producing the next brood. Thus the brood production per year can be increased.

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MEETINGS/SYMPOSIA/SEMINARS

Workshop on Fractals in Earth Sciences

Date: 22–26 September 1997
Place: Hyderabad

The aim of the Workshop is to expose younger scientists through lectures and demonstration on Fractals and its applications to Earth Sciences.

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First International Conference on Parthenium Management (ICPM)

Date: 6–8 October 1997
Place: Dharward, India

Themes include: Global view of parthenium and its management; Advances in parthenium management; Utility values of parthenium; Future strategies and course of action.

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South Asian Countries' Seminar on Medicinal Plants (SACSOMP-97)

Date: 9–12 November 1997
Place: Patna, India

Highlights of the seminar: Safeguarding and conservation of medicinal plants in the hills and forests which are becoming extinct on account of deforestation; Cultivation of medicinal plants (including aromatic as well as those plants which give us spices) on scientific lines; Environmental pollutions and health hazards are increasing because of setting up of industries which are the source of exhaust gases and fumes; Role of medicinal plants in eradication of diseases like AIDS, Kala-Azar, Cancer, Malaria, Filaria, Leprosy, Renal failure, Cardio-Myopathy, Thalassemia, etc.; The use of biotechnology and genetic engineering for improvement of quality of medicinal plants; Recent discoveries/researches in dentistry, animal diseases, extracting cosmetics and nutrients from plant life, etc.; Lectures on the commercial value of medicinal plants, methods of processing packaging, market potentialities, etc. for the benefit of those engaged in cultivation of medicinal plants.

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