Asian Fisheries Science 1(1988):197-201.
Asian Fisheries Society, Manila, Philippines
https://doi.org/10.33997/j.afs.1987.1.2.009

An Improved Design for *In Vitro* Hatching of *Macrobrachium* Eggs

S. MATHAVAN and S. MURUGADASS

School of Biological Sciences
Madurai Kamaraj University
Madurai 625 021, India

Abstract - An artificial incubator was designed for the mass hatching of freshwater prawn eggs. The design and efficiency of the incubator are described in detail. Three-day-old eggs of *Macrobrachium malcolmsonii, M. nobilii, M. idella* and *M. lamarrei* were incubated in the new incubator and about 84% of incubated eggs successfully hatched. The advantages of the design are discussed.

In general, prawns of the genus *Macrobrachium* are iteroparous and diecdysic in which spawning is always preceded by a moult. *Macrobrachium* species spawn once in about 20 to 25 days. At the commencement of reproductive activity, ovaries become active, grow and may be clearly seen under the carapace as dark green bodies from the posterior third rostral spine to first abdominal segment (Ling 1969; Pandian and Balasundram 1982). Complete ovary development in *Macrobrachium* requires about 20 days, after which they moult and then spawn.

The fertilized eggs adhere to ovigerous setae of the first four pairs of pleopods (Ling 1969; George 1969). Egg incubation is an energy demanding process. The developing eggs are ventilated by the fanning activity of pleopods of the mother to facilitate gaseous and ionic exchange for the eggs during the incubation period of about 12 days. Subsequently the eggs hatch in batches over a period of up to 7 days (Balasundram and Pandian 1982).

Relieving the female prawns from the task of incubation has resulted in the following advantages: (i) avoidance of egg loss during

in situ incubation, reported to be about 33 to 53% in *M. nobilii* (Balasundram and Pandian 1982), 20 to 80% in *Palaemon serratus* and about 30% in *M. rosenbergii* (Wickins and Beard 1974); (ii) prevention of batching effect in hatching; *in situ* hatching extends for a period of 15 to 20 days (Pandian 1970a, 1970b; Ennis 1973) and results in the production of weaker larvae (Katre and Pandian 1972); (iii) increase of egg production by about 50% due to increase in the spawning frequency in the relieved females (Pandian and Balasundram 1982).

Despite these strong reasons for *in vitro* culture of decapod eggs, only a few researchers have attempted to incubate developing eggs of crustaceans artificially. Some have concentrated on the *in vitro* culture of barnacle eggs (e.g., Barnes and Barnes 1953; Crisp 1959; Lewis 1975). Others endeavored to incubate developing eggs of lobster (Templeman 1940) and crabs (Sandoz and Rogers 1944; Costlow and Bookhout 1968) in standing water. However, they failed to develop a technique in which a minimum of 50% of eggs could consistently be hatched. Phillips (1971) was perhaps the first to mass incubate the developing eggs of *Palaemon serratus* maintaining a constant flow of water. However, he did not report consistency in percentage of hatching. Balasundram and Pandian (1981) made a preliminary design of an artificial incubator for *Macrobrachium* eggs. We have vastly improved that incubator and the present paper reports the details and efficiency of the new design.

Healthy individuals of *Macrobrachium nobilii, M. malcolmsonii, M. idella* and *M. lamarrei* were collected from the River Cauvery, vellar estuary, and maintained in laboratory aquaria. *Tubifex tubifex* served as a feed for the prawns. Females spawned within 12 hours after mating. The time at which the female became berried was counted as 0 hours of incubation. Eggs were removed on the third day of incubation as recommended by Balasundram and Pandian (1981), and used for experiments.

The new design of the incubator is shown in Fig. 1. The incubation chamber consists of an inverted 1,000-ml conical flask with an opening at the top through which the eggs can be introduced; the eggs rest on a diaphragm made of bolting silk (mesh size 137 µm) fixed at 1/3 height of the flask. There is an inlet at the bottom of the flask and two outlets, one at the shoulder of the flask for larval collection and the other at the top for flow of excess water into the collection tank. The inner surfaces of the outlets are covered with bolting silk to prevent egress of the eggs.

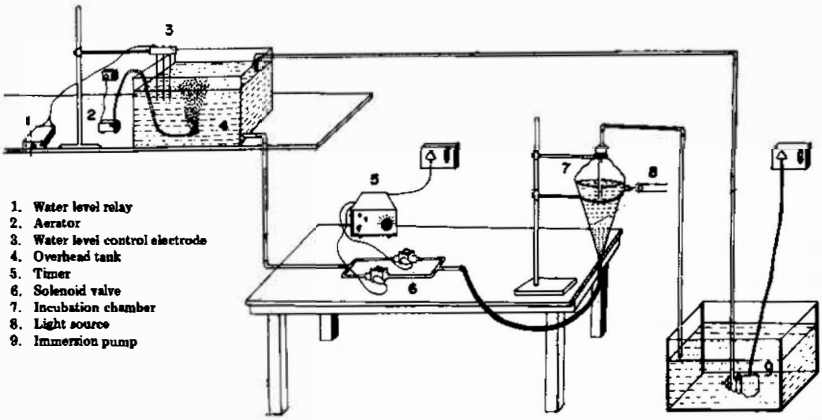

Fig. 1. Prawn incubator design.

The overhead tank has a holding capacity of 300 l of filtered tap water. The medium is continuously aerated and mixed with 1 ppm of malachite green which is added as a fungicide against the infection of *Aphanomyces saprolegnia* on the developing eggs (Balasundram and Pandian 1981). The collection tank automatically fills the overhead tank when the water level of the latter reaches a critical point. There are two solenoid valves which work alternatively; this provision is made to extend their longevity. The frequency of functioning of the solenoid valve is regulated by an electronic timer. From the solenoid valve a polythene tube is connected to the inlet of the incubation chamber. When the valve opens, water enters the incubation chamber and flushes the eggs upwards from the diaphragm of the chamber. The eggs become suspended in the column of water in the chamber and slowly settle down again as the valve closes. The timer regulates the frequency of this process, which simulates the irrigation technique of the berried prawn during *in vivo* incubation. When hatching commences, the larvae are attracted by the light and are collected via the side outlet.

We incubated up to about 40,000 eggs each of *M malcolmsonii, M. nobilii, M. idella* and *M. lamarrei* (Table 1). The flow rate used was increased from about 60 l/hour on the fourth day of incubation to 80 l/hour on the twelfth day. In later tests the rate was increased, since ventilatory movements of the pleopods of berried female prawns increase from 2,700 times/hour on the first day of incubation to 7,500 times/hour on the final day (Balasundram 1980).

Table 1.Hatching efficiency of *in vitro* incubator for different *Macrobrachium* species.

Species	No. Observations	Water Salinity (ppt)	No. eggs	Hatching rate (%)
M. malcolmsonii	5	0	43,176 ± 917	88 ± 3
M. nobilii	4	0	37,753 ± 855	82 ± 2
M. lamarrei	3	0	22,669 ± 772	87 ± 3
M. idella	4	14	36,813 ± 822	80 ± 2

Acknowledgement

This work was supported by a grant from the International Foundation for Science, Stockholm to Dr. S. Mathavan: Grant A/706.

References

Balasundram, C. 1980. Ecophysiological studies in prawn culture *(Macrobrachium nobilii)*. Madurai Kamaraj Univ., Madurai. 213 p. Ph.D. dissertation.

Balasundram, C. and T.J. Pandian. 1981. *In vitro* culture of *Macrobrachium* eggs. Hydrobiologia 77: 203-208.

Balasundram, C. and T.J. Pandian. 1982. Egg loss during incubation in *Macrobrachium nobilii*. J. Exp. Mar. Biol. Ecol. 59: 289-299.

Barnes, H. and M. Barnes. 1963. *In vitro* development of cirripede eggs. Vidensk. Medd. Dan. Naturhist. Foren. 125: 93-100.

Costlow, J.D. and C.C. Bookhout. 1968. A method for developing the brachyuran eggs *in vitro*. Limnol. Oceanogr. 5: 212-225.

Crisp, D.J. 1959. The rate of development of *Balanus balanoides* (L.) embryos *in vitro*. J. Anim. Ecol.: 119-132.

Ennis, G.P. 1973. Endogenous rhythmicity associated with larval hatching in the lobster *Homarus gammarus*. J. Mar. Biol. Assoc. U.K. 53: 531-538.

George, M.J. 1969. Genus *Macrobrachium* Bate 1968. *In* Prawn fisheries of India. Bull. Cont. Mar. Fish. Inst. Mandapam 14: 178-216.

Katre, S. and T.J. Pandian. 1972. On the hatching mechanism of a freshwater prawn *Macrobrachium idae*. Hydrobiologia 40: 1-17.

Lewis, C.A. 1975. Some observations on factors affecting embryonic and larval growth of *Pollicipes polymerus* (Cirripedia: Lepadomorpha) *in vitro*. Mar. Biol. 32: 127-139.

Ling, S.W. 1969. The general biology and development of *Macrobrachium rosenbergii* (de Man). FAO Fish. Rep. 57, Vol.3. 589-606.

Pandian, T.J. 1970a. Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus*. Mar. Biol. 5: 153-167.

Pandian, T.J. 1970b. Yolk utilization and hatching time in the Canadian lobster *Homarus americanus*. Mar. Biol. 7: 249-254.

Pandian, T.J. and C. Balasundram. 1982. Moulting and spawning cycles in *Macrobrachium nobilii* (Henderson and Mathai). Int. J. Invertebr. Reprod. Dev. 5: 21-30.

Phillips, G. 1971. Incubation of the eggs of the English prawn *Palaemon serratus*. J. Mar. Biol. Assoc. U.K. 51: 43-48.

Sandoz, M. and R. Rogers. 1944. The effect of environmental factors on hatching, moulting and survival of zoea larva of the blue crab *Callinectes sapidus* Rathbun. Ecology 25: 216-228.

Templeman, W. 1940. Embryonic developmental rates and egg laying of Canadian lobsters. J. Fish. Res. Board Can. 5: 71-83.

Wickins, J.F. and T.W. Beard. 1974. Observations on the breeding and growth of the giant freshwater prawn *Macrobrachium rosenbergii* (de Man) in the laboratory. Aquaculture 3: 159-174.