

Embryonic Development of *Macrobrachium idae* (Heller, 1862)

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Abstract

The egg laying usually takes place between 6 and 20 hrs after mating. The laying of one whole batch of eggs is usually completed within 20 mins. The eggs are extruded through the female genital pores into the brood chamber, first on one side then the other. The chamber between the 4th pair of pleopods is filled first, then those between the 3rd, 2nd and 1st pairs, successively. The eggs are held in bundles like grapes by an extremely thin elastic membrane which is believed to be secreted by the ovigerous setae. During the development in prawns the colour of the eggs changed through greenish opaque, light green, brownish-yellow and dull whitish in colour. The embryonic stages are very much useful in recognizing the stages of development of the species. The process of embryonic development includes nuclear division, cleavage (blastomeres), segmentation, formation of optic vesicle, eye pigment development and larva formation. At third minute after mating the sperm fused with the egg membrane and subsequently the male pronucleus entered the egg's cytoplasm. The first and second nuclear divisions were completed without any corresponding division of the cell. Third division begun at 8 h and eight nuclei were formed after 9 hrs. Subsequent divisions of sixteen and thirty two nuclei stage took place at about 1 to 1.30 hrs interval and segmentation was completed at 22-24 hrs. Embryonic development follows the normal blastula and gastrula stages, ending with the closing of the blastopore. When larva hatches out from the eggs, they undergo a series of development stages before metamorphosing to juvenile. During this period, they exhibit marked variations in morphological features. These variations are unique for each and every species according to the developmental stages.

Keywords: Yolk substances; Development; Brown to grey eggs; Embryo; Early larvae

Introduction

The prawn, *M. idae* is a freshwater prawn, it is facultative euryhaline and abundant mostly during monsoon and post-monsoon seasons. It is commonly available in estuarine, backwater and mangrove niches. They migrate into brackish water area for spawning and also sometime for hatching the larval forms and the spent mother return to freshwater area. In general some of the *Macrobrachium* species are grow faster and bigger and some may slow and smaller in sizes. The differential growth between sexes is of great concern in *Macrobrachium* species culture where the males grow much faster than females. The differential growth in males not only create problem in culture but also in mating behavior. Crustacean embryonic and larval systems offer a unique and valuable tool for understanding both the developmental processes and physiological regulatory mechanisms. Palaemonid females carry centrolecithal eggs in an external brood pouch during the development time [1]. This peculiarity of the palaemonids allows a systematic tracking of the embryonic development. Muller [2] described the embryogenesis in *M. Carcinus* and *M. acanthurus*. Recently studied the embryonic development of *M. idella idella* but nobody performed such study in the prawn *M. idae* [3]. So the present study was designed to know the information on the embryonic development of edible unexploited prawn, *M. idae*.

Materials and Methods

The animals were collected from Ponnanthittu (Lat.11°28'41"N; Long. 79°45'30"E) waters which is located 2 km south to Parangipettai and connected with Vellar estuary. The prawns were caught by the fisherman with the help of trap, line and hand- net and scoop net. The specimens collected from the fisherman were ranged from 30 to 110 mm in length. Totally 150 specimens were collected and transported to the laboratory in live condition by keeping them in bucket containing freshwater and aeration. After reaching the laboratory they were washed carefully with distilled water to remove the dust and algal particles.

They were kept in the fiber glass tanks (45×30×37 cm). Optimum temperature (27–28°C) and dissolved oxygen (5 ppm) was maintained in the brood tank. The berried females were fed with commercial feed once in a day. Every day the excess feed, excreta and shed out eggs were siphoned out. The development of the eggs was closely observed every day. Daily colour changes of the eggs during incubation period could be noted. Eggs were sampled aseptically by gently removing a bunch of eggs from the brood pouch using a sterilized forceps in random locations and separated with the help of a needle and forceps without damaging the eggs. After each sampling, brooders were given 1-min prophylactic fungus dip treatment in malachite green (5 mg L⁻¹) before being returned to incubation tanks. All the developing embryos were examined with a light microscope to ensure that only viable embryos were sampled and the colour change corresponding to the development and length of the incubation period was noted. The time courses of embryonic development, as indicated by the appearance of specific morphological features were recorded from spawning time onwards. This includes from fertilization to hatching of first zoea. The gradual changes in the embryonic development and increase in the size of the eggs were recorded to understand the different developmental stages.

Hatching under Laboratory Condition

Once the first stage zoea inside the egg was fully developed, the

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larva was ready to come out of the eggshell to start active life. The process of hatching was studied through hand lens and compound microscope with the developing embryo removed from the brood sac. This slow process was accompanied by continuous vibration at the mouth of the larva and stretching of its rolled body, forcing the egg shell to elongate gradually. Vibration at the mouth became more and more vigorous followed by further stretching of the body. About an hour later the thoracic appendages started to vibrate vigorously but intermittently for about a few minutes with increasing length of pereopods vibration. This became very vigorous and continuous. The body continued to stretch the rostrum and telson, which was held like a mask covering and protecting the eyes and head, which started pushing outward. Suddenly the eggshell break and the telson thrashed out followed by the head and with a forceful flex and stretch of the body the hatched zoea larvae started swimming actively in the water column.

Results

Once there was a dominant male encounter the pre-mating female, the male placed its spermatophore on the thorax of a mature female, near the opening of the gonopores. As eggs were extruded from the oviduct, they passed across the spermatophore and were fertilized externally. Eggs were deposited, or oviposited, on setae of the pleopods of the female. The newly oviposited eggs were containing all the necessary material for synthetic processes associated with embryogenesis and morphogenesis and all the compounds required for oxidative metabolism and energy production. The eggs contain nutritive reserves in the form of proteinaceous yolk and lipid vesicles scattered throughout the cytoplasm. The fertilized eggs were opaque, greenish, round or oval in shape. The diameter of the egg was approximately 0.44-0.45 mm. As the development progresses the greenish colour changed into light green, brownish-yellow and finally to dull whitish in colour about to hatch. At this stage, the developing larvae were observed under microscope. During this period there was considerable increase in the size of the egg in long axis. Fecundity was ranged between 4,842 and 22,372 (60 - 90 mm total length of females). The incubation periods were varied from 13-14 days. The process of embryonic development includes nuclear division, cleavage (blastomeres), segmentation, formation of optic vesicle, eye pigment development and larva formation.

Newly spawned egg and nuclear division

The attachment of sperm to the egg took place within the first minute of spawning. By the second minute of post-spawning, the egg became spherical and clear membrane was observed very beginning to envelop the egg. A third minute after mating the sperm fused with the egg membrane and subsequently the male pronucleus entered the egg's cytoplasm. After about 2 hours the stellate island of protoplasm containing the nucleus was discernable at the center of the egg and became clearly visible at about 1h. Counting from the time of fertilization, the first nuclear division started at about 4 h and was completed within one hour.

Second nuclear division and segmentation

The second nuclear division was started after 6 hrs and completed at 7.30-8 hrs. Third division begun at 8 hrs and eight nuclei were formed after 9 hrs. Subsequent divisions of sixteen and thirty two nuclei stage took place at about 1 to 1.30 h interval and segmentation was completed at 22-24 hrs. In *M. idae* the cleavage was superficial (meroblastic, i.e., a large mass of centrally located yolk confines cleavage to the cytoplasmic rim of the egg). The first and second nuclear divisions were completed without any corresponding division of the cell. Four cleavage furrows

appeared when the third nuclear division was almost completed. They started from four subequidistant points on the surface and extend rapidly almost at right angles to each other to form four quadrants, or blastomeres, whose apexes become joined by a median furrow. The fourth nuclear division was holoblastic. Advanced segmentation stages are shown distinct hexagonal markings on the surface upto this stage. The colour of the embryo was opaque, greenish, round or oval in shape. The diameter of the egg was approximately 0.47 mm.

Embryonic development follows the normal blastula and gastrula stages, ending with the closing of the blastopore. Increase in cell numbers was observed in the first 48 hrs and at 94-96 hrs a clear region at one pole of the embryonic mass was easily discernable. The clear region extended lengthwise forming the trunk of the growing embryo after 120-122 hrs. The diameter of the egg was approximately found to be 0.57 mm translucent, light green in colour and oval in shape. As the clear region developed on the yolk mass lessened.

After 156 hrs, small dark compound eyes appeared on the yolk mass and then at 170 hrs, heartbeat was discernible. The colour was translucent, brownish-yellow in colour. The diameter of the egg was measured as 0.58 mm. In 216 hrs, the clear region which developed into trunk and caudal portion occupied about 2/3 of the embryo mass and the eye spots were enlarged and oval shaped. At 264 hrs the appendages were formed beneath the clear trunk region, the eyes were enlarged and surrounded by striation and the translucent globules at the dorso-caudal portion of the yolk mass clearly exhibited rhythmic contraction. The diameter of the egg was approximately 0.62 mm and transparent, dull whitish in colour. In 302 hrs the eyes were dark rounded and striation was observed, the translucent globules became enlarged and occupied most of the dorsal area of the yolk mass. The diameter of the egg was 0.64 mm and transparent dull whitish in colour. The newly hatched 1st zoeas were released at 330 hrs.

Discussion

In *Macrobrachium* spp the females incubate their embryos on pleopods (swimmerettes) of the abdomen until hatching it is common in certain decapod crustaceans. During this period, the embryo's investment coats (egg coats, egg envelopes) protect it from physical and chemical stresses and maintain the internal milieu. The outer investment coat, due to its immediate exposure to the aquatic environment, is of primary importance in this role. The outer coat has also been associated with the attachment of eggs to the maternal pleopods, selective permeability [4] and osmotic hatching [5], and it may serve as a substratum for aquatic microorganisms [6]. Dinakaran [3] also observed egg coat used for egg attachment to the pleopods in *M. idella idella*. In the present study also *M. idae* newly extruded eggs contain egg coats which helped the eggs to attach each other and to the pleopods. This helps to protect the eggs from physical and chemical damages. During oviposition, the female stood upright and the eggs moved into a chamber formed by the pleopods and the lateral epirnera (pleura) on the underside of her abdomen. Eggs were attached to each other and to pleopodal setae by a connecting or adhesive material that formed the outer investment coat. This occurred for both fertilized and unfertilized eggs. At sites of attachment, the adhesive material took the shape of a flattened strand or a twisted stalk [7].

Extruding eggs, fertilized or unfertilized, were connected to the pleopodal setae and to each other by the adhesive material, which simultaneously formed the outer investment coat of the eggs. This mechanism is unlike that suggested for *Homarus* [4] in that cement glands or ducts were not observed in *P. macrodactylus*, and secretion

of adhesive material occurred before, rather than during, oviposition. It also differs from that proposed for *Carcinus* [8] in that fertilization was not necessary for attachment and the outer layer was formed by material secreted from the female pleopods, not from individual eggs. Attachment in *Palaemonetes*, described by Burkenroad [9] and Jefferies [10] was probably the same as that described here for *P. macrodactylus* and *M. idella idella* but the adhesive material escaped detection because of its close conformity with the external surfaces of the pleopods [3]. Similar mechanism was observed in the present study.

According to Parson and Tucker [11] fecundity can vary seasonally, annually and between areas. The number of eggs produced by crustaceans varies widely [12]. In several crustaceans there is a linear relationship between the number of eggs per brood and the size of the females. This has also been observed in *M. lamarrei* [13], the freshwater crayfishes *Astacus leptodactylus* [14] and *P. (Austrocambarus) ilamasi* [15]. According to Manush et al. [16] fecundity of *M. rosenbergii* varies from 40,000 to 60,000 eggs (body weight 100 g). Hamasaki et al. [17] emphasized that oocyte number increased with increasing female's body size and predicted estimates ranged between 0.8 and 4.5 million for the carapace width of 130-240mm in *P. trituberculatus*. The prawn *M. idae* carries approximately about 40-160 developing eggs [18]. According to Dinakaran [3] fecundity varied from 6,158 to 29,272 (60 and 92 mm total length) in *M. idella idella*. In the present study fecundity was varied between 4,842 to 22,372 (60 and 90 mm total length).

In *M. idae* the colour of the eggs changed through greenish opaque, light green, brownish-yellow and dull whitish in colour. At the time of development, the colour of the egg changed through brown to grey as the yolk is used up and the outline of the embryo becomes visible. The eyes and pigment spots appear first followed by the outlines of the abdomen and cephalothorax [19]. The colour change was caused by absorption of the yellow yolk and development of dark pigment in the eyes [20-23]. Extruded eggs of *Macrobrachium* spp are of two colours either green, like in *M. acanthurus* [23] and *M. amazonicum* [24] or orange in colour as in *M. heterochius*, *M. ohione* [25], *M. rosenbergii* [26] and *M. Carcinus* [27]. In *Macrobrachium* spp eggs with embryos turn either grey or dark brown prior to exclusion [28]. Whereas in *M. gangeticum* the colour of eggs is green yellow and become grey corresponding to embryonic development [29]. In *M. lamarrei* and *M. lamarrei lamarrei* the eggs were green in colour [29,30]. According to Aubson and Patlan and Rodriguez [31-33] classified the eggs into four different developmental stages. However Ajith Kumar [34] classified eggs into 4 stages based on the colour of the eggs in *M. idella idella*. The colour of the eggs changed opaque, light green, brownish-yellow and dull whitish in colour in *M. idella idella* [3] and the present study too. Many workers have divided the crustacean egg stages based on the appearance of distinctive morphological features such as eye, heart beat and appendages formation. However, such morphological characters only begin to appear mid-way during embryonic development. Cellular differentiation starts soon after gastrulation and requires enormous energy expenditure. Subramoniam [35] emphasized the importance of a detailed classification of early development of decapod crustaceans to understand the changes in the metabolic pathways involving interconversion of already stored substrates within the closed system of egg development.

Holoblastic or total cleavage usually occurs in eggs containing a small amount of yolk (oligolecithal eggs), in which the establishment of morphological characteristics occurs relatively fast, resulting in the development of the typical free nauplius larvae with three pairs of appendages [36,37]. This pattern is observed in most branchiopods and

maxillopods, and in penaeids of the Malacostraca [38]. The quantity and distribution of yolk in the eggs of different crustacean species is closely related to cleavage and embryonic development patterns [39,40]. Crustaceans with yolky eggs (centrolecithal eggs) present meroblastic or partial cleavage. The large amount of yolk triggers a delayed embryonic development that results in further structuring of the embryonized - nauplius (also called egg-nauplius), with the formation of paired appendages, growth of the caudal papilla and organization of appendages in the post-naupliar region [41,42]. This pattern is observed in most malacostracans in which the hatching form is the zoea [39]. In *M. idae* the embryonic development followed the general pattern of embryogenesis described for other species that have centrolecithal eggs, as *M. Carcinus* [2], *M. idella idella* [3], *P. varians* [43], *P. pugio* [44] and *M. acanthurus* [45]. The formation of the zoea structures follows the organization of the basic body plan observed in the development of oligolecith species eggs [38,46] where first larval phases correspond to the embryonized post-zoea in the meroblastic pattern [47].

The cleavage process observed in stage II indicates that development follows a holoblastic pattern, since cleavage furrows can be seen in the surface of the whole egg, individualizing the blastomeres. However, these cleavage furrows are shallow and they do not reach the central yolk mass the subsequent organization of the germinal disc seen in stage III, followed by the organization of the embryonized zoea and post-zoea, are typical of the meroblastic developmental pattern [2,48]. The recognition of both holoblastic and meroblastic developmental traits during the cleavage stage is common in most decapods species, due to particular quantity and distribution of the yolk in the eggs [39]. The development of *M. idae* shows that the initial morphogenesis is quite intense until organization of the embryonized zoea. The zoea could be visualized due to large size of the egg, superficial position of the embryo and colour contrast between embryonic cells and the yolk mass.

In the present study, the egg size of the *M. idae* increased mainly in long axis during the embryonic development. These changes in egg size were also reported to most of the malacostracan species, as the brachyuran *Eriocheir japonicus* [49] and the prawns, *M. offersi* [50] and *M. idella idella* [3]. According to Odinetz-Collart and Rabelo [51] and Narciso and Morats [52], in crustaceans the egg diameter tends to increase until hatching. Churchill GJ [53] explained that the egg diameter was not related in any way to female size and also the egg diameters increased at a relatively steady pace throughout ontogeny. Under constant environmental conditions, the variability in egg size and biomass has been attributed to variation in female size or age [54-56] and genetic factors [57-59]. The growth of the egg size is associated, among other factors, to increase the water content in eggs, as the embryo develops [49]. The eggs of aquatic invertebrates range widely in size. Even within a single taxonomic group such as decapods or amphipods, egg size can vary enormously between species, and also within species. For example echinoderm eggs vary in diameter from 50 to 1500 μm [60]. In general, of course, species with smaller eggs have higher fecundities than those with larger eggs, and the selective advantages of the different egg size have been discussed extensively in the literature of marine invertebrate reproduction [61-63]. The number of eggs containing embryos during development depends on the size of the mother prawn, as is known for *M. lamarrei* [13], *M. ohione* [25], *M. amazonicum* [64] and *M. idae* [65]. Associated with these variations in egg size are differences in the time taken for the embryo to develop and hatch. This can vary from a few days in some tropical species to at least 18 months in some Polar isopods [66-68].

The incubation of developing embryos by most female decapod crustacean may be one of the reasons for the success of this group. This insures greater survival against predators and other adverse environmental conditions [28]. These different incubation times are related to the endogenous factors of development and can also be influenced by exogenous factors like water temperature, as described by Celada et al. [66]. In *P. sanguinolentus* embryonic development was last for 8-11 days [69]. In *M. rosenbergii* embryonic development was last for about 18.5 days [70]. The incubation time is 12-15 days for *M. malcolmsonii* and comparatively less duration of 12-13 days for *M. gangeticum*. Whereas in giant freshwater prawn *M. rosenbergii* is longer period for incubation and embryonic development was reported at 18-25 days [30,71]. In *M. idella idella* embryonic developments were last for about 13-14 days [3]. However, in the present study the water temperature was controlled, suggesting that the endogenous factors, like egg size and the amount of yolk were decided to the variation of the development time. The embryonic developments of *M. idae* are last for about 13-15 days as in *M. idella idella* [3]. The species belonging to the genus *Macrobrachium* are known to migrate from fresh water to brackish water for breeding purposes [72]. Populations of *M. idae* inhabiting the rice fields along the west coast of south India are known to migrate into the backwaters during the breeding season. During the embryonic development the eggs of *M. idae* increase their salt (ash) content from 4 to about 7% (dry body weight) by absorbing salts from the surrounding water [18]. The gravid females of *M. rosenbergii* that were gradually exposed to salinities of 8 ppt during the last part of incubation had a higher number of larvae released [26]. In the present study the *M. idae* brooder were maintained at 4-5 ppt during incubation period.

The species of *M. olfersi*, *P. pandaliformis* and *P. argentinus* have similar sized eggs and have presented similar development times. On the other hand, the longest length of development was observed in *M. potiuna*, whose voluminous eggs allowed for a more prolonged embryogenesis, which results in a development of more complex structures [73]. According to Jalihal et al. [74], the species of *Macrobrachium*, which have larger eggs, tend to show a smaller fecundity, fewer larval stages, and a reduction of the larval period. These features have also been observed in other palaemonids, such as *M. nattereri* [75], *M. iheringi* [76], *M. borellii* [77] and *M. jelskii* [78].

A similarity of the length of the embryonic periods shows that a specific amount of time to the organization of embryonic features is necessary. The prezoa and zoea periods are faster due to the organization of less complex embryonic structures. The post-zoea period is longer because the structures have to be finalized and to acquire functionality before hatching. In *M. potiuna* the postnaupliar stage is even longer, because this species hatches as a more complex larvae [73]. In the present study also prezoa and zoeal periods are faster than the post-zoeal period.

References

1. Ammer D, Muller YMR, Nazair EM (2001) Biologia reproductiva de *Macrobrachium olfersii* (Wiegman) (Crustacea, Decapoda, Palaemonidae) coletados na Ilha de Santa Catarina, Brasil. Rev Bras Zool 18: 529-537.
2. Muller YMR (1984) Die Embryonalentwicklung von *Macrobrachium Carcinus* (L.) (Malacostraca, Decapoda, Natantia). Zool Jahrb Anatol Jena 112: 51-78.
3. Dinakaran GK (2010) Mating behaviour, embryonic development, biochemical composition and mass seed production of edible prawn *Macrobrachium idella idella* (Hilgendorf, 1898). Ph. D Thesis, Annamalai University.
4. Yonge CM (1937) The nature and significance of the membranes surrounding the developing eggs of *Homarus vulgaris* and other Decapoda. Proc Zoo Soc London A107: 499-517.
5. Davis CC (1965) A study of the hatching process in aquatic invertebrates. XIV. An examination of hatching in *Palaemonetes vulgaris* (Say). Crusta 8: 233-238.
6. Johnson PW, Sieburth JMCN, Sastry A, Arnold CR, Doty MS (1971) Leucothrix mucor infestation of benthic crustacea, fish eggs and tropical algae. Limnol Oceanogr 16: 962-969.
7. Williamson DI (1982) Larval morphology and diversity. In: L.G. Abele (Ed.). Embryology, morphology and genetics, Academic Press, New York, USA.
8. Cheung TS (1966) The development of egg-membranes and egg attachment in the shore crab, *Carcinus maenas*, and some related decapods. J Mar Biol Assoc UK 46: 373-400.
9. Burkenroad MD (1947) Reproductive activities of decapod Crustacea. Am Nat 81: 392-398.
10. Jefferies DJ (1964) The moulting behavior of *Palaemonetes varians* (Leach) (Decapoda: Palaemonidae). Hydro 24: 457-488.
11. Parson DG, Tucker GE (1986) Fecundity of northern shrimp *Pandalus borealis* (Crustacea, Decapoda) in areas of North West Atlantic. Fish Bull US 84: 549-558.
12. Sastry AN (1983) Ecological aspects of reproduction. T. H. Waterman. Biol Crusta. 8. Environ adap Acad press, New York.
13. Shakuntala K (1977) The relation between body size and number of eggs in the freshwater prawn *Macrobrachium lamarrei*. Crusta 33: 17-22.
14. Koksai G (1988) *Astacus leptodactylus* in Europe. In: Freshwater Crayfish: Biology, management and exploitation (eds. Holdich, D.M. and A. Kuris). Crusta 117-141.
15. Rodriguez-Serna M, Carmona-Osaide C, Olvera-Novoa MA, Arredondo-Figueroa JL (2000) Fecundity, egg development and growth of juvenile crayfish *Procambarus (Austrocambarus) llamasii*, Villalobos, 1955. Under Laboratory conditions. Aquacult Res 31: 173-179.
16. Manush SM, Pal AK, Das T, Mukherjee SC (2006) The influence of temperatures ranging from 25 to 36°C on developmental rates, morphometrics and survival of freshwater prawn (*Macrobrachium rosenbergii*) embryos. Aquacult 256: 529-536.
17. Hamasaki K, Fukuanga K, Kitada S (2006) Batch fecundity of the swimming crab *Portunus trituberculatus* (Brachyura: Portunidae). Aquacult 253: 359-365.
18. Shankuntala Katar, Pandian TJ (1972) On the hatching mechanism of a freshwater prawn *Macrobrachium idae*. Hydrobiol 40: 1-17.
19. Warner GF (1977) The Biology of Crabs. Elek Science, London, UK.
20. Lin S, Li S, Wang GZ (1994) Studies on the biochemical composition during ovarian development of mud crab, *Scylla serrata*. J Xiamen Univ 33: 116-120.
21. Veera Ravi A (1994) Biochemical changes during embryonic and larval development of the edible Portunid crab *Charybdis lucifera* (Fabricius). Ph.D. Thesis, Annamalai University, India.
22. Parimalam K (2001) Embryonic and larval development of the hermit crab, *Clibanarius longitarsus* (De Haan) (Crustacea: Decapoda: Anomura). M.Phil Thesis, Annamalai University, India.
23. Choudhury PC (1971) Laboratory rearing of larvae of the palaemoni: shrimp *Macrobrachium acanthurus* Wiegmann, 1836. Crusta 21: 113-125.
24. Guest WC (1979) Laboratory life history of the palaemonid shrimp *Macrobrachium amazonicum* (Heller) (Decapoda, Palaemonidae). Crusta 37: 141-151.
25. Truesdale FM, Mermilliod WJ (1979) The river shrimp *Macrobrachium ohione* (Smith) (Decapoda, Palaemonidae): Its abundance, reproduction and growth in the Atchafalaya river basin of Louisiana, U.S.A. Crusta 36: 61-73.
26. Ling SW (1969) Methods of rearing and culturing *Macrobrachium rosenbergii* (De Man). Ibid 3: 607-619.
27. Lewis JB, Ward J, McIver A (1966) The breeding cycle, growth and food of the fresh water shrimp *Macrobrachium Carcinus* (LINNAEUS). Crusta 10: 48-52.
28. Ching CA, Velez MJ Jr (1985) Mating incubation and embryo number in the freshwater prawn *Macrobrachium heterochirus* (Wiegmann, 1836) (Decapoda, Palaemonidae) under laboratory conditions. Crusta 49: 43-48.

29. Kanaujia DR (2003) Indian River prawn *Macrobrachium malcolmsonii* and minor species of commercial importance. In: Souvenir, International Symposium of Freshwater Prawns 2003, College of Fisheries, Kerala Agricultural University, Kochi, India.
30. Uno Y, Sao KC (1969) Larval development of *Macrobrachium rosenbergii* (de Man) reared in the laboratory. J Tokyo Univ Fish 55: 179-190.
31. Aubson B, Jr, Patlan D (1974) Color changes in the ovaries of Penaeid shrimp as determinant of their maturity. Mar Fish Rev 36: 23-26.
32. Rodriguez A (1977) Contribucion al conocimiento de la biologia y pesca dellangostino *Penaeus kerathurus*. Forskal, 1775 del Golfo de Cadiz (region Sudatlantica Espanola). Investigacion Pesq 41: 603-635.
33. Rodriguez A (1985) Biologia del langostino *Penaeus kerathurus*. Forskal., 1775 del Golfo de Cadiz. I Reproduction. Investigacion pesq 49: 581-595.
34. Ajith Kumar M (1990) Studies on the Proximate Composition of the Prawn *Macrobrachium idella* (Hilgendorf). M. Phil. Thesis, Annamalai University.
35. Subramoniam T (1991) Yolk utilization and esterase activity in the mole crab *Emerita asiatica* Milne Edwards. Crustacean Issues 7: 19-30.
36. Ducker, DM, Dobkin S (1975) A contribution to knowledge of the larval development of *Macrobrachium olfersii* (Wiegman, 1836) (Decapoda, *Palaemonidae*). Crusta Leiden 29: 1-30.
37. Hertzler PL (2002) Development of the mesendoderm in the Dendobranchiata shrimp *Sicyonia ingentis*. Arthropod Struc Develop Oxford 31: 33-49.
38. Hertzler PL, Clark WH Jr (1992) Cleavage and gastrulation in the shrimp *Sicyonia ingentis*: invagination is accompanied by oriented cell division. Development 116: 127-140.
39. Anderson DT (1982) Embryology, morphology and genetics. Academic Press. New York, USA.
40. Fioroni P (1992) Allgemeine und vergleichende Embryologie der Tiere. Springer.
41. Helluy SM, Beltz BS (1991) Embryonic development of the American lobster (*Homarus americanus*): quantitative staging and characterization of an embryonic molt cycle. Biol Bull Woods Hole 180: 355-371.
42. Scholtz G (2000) Evolution of the nauplius stage in malacostracan crustaceans. J Zool Sys Evol Res Berlin 38: 175-187.
43. Weygoldt P (1961) Beitrag zur Kenntnis der ontogenie der Dekapoden: embryologische untersuchungen an *Palaemonetes varians* (Leach). Zool jahrbucher Anat jena 79: 223-270.
44. Glas PS, Countney LA, Rayburn JR, Fisher WS (1997) Embryonic coat of the grass shrimp *Palaemonetes pugio*. Biol Bull Woods Hole 192: 231-242.
45. Bressan CM (1999) Postnaupliar Embryonic Development of *Macrobrachium acanthurus* (Crustacea Decapoda). Brazilian J Morpho Sci Sao Paulo 16: 155-160.
46. Nazari EM, Ammar D, Petersen RL, Muller YMR (1998) Desenvolvimento embrionario do camarao rosa *Penaeus paulensis*. Perez Farfante, 1967. (Crustacea, Decapoda). Anais Aquac Brasil 98: 2641-648.
47. Talbott P, Helluy S (1995) Reproduction and embryonic development. J.R. Factor. Biology of the lobster *Homarus americanus*. Academic Press. New York, USA.
48. Weygoldt P (1979) Significance of later embryonic stages and head development in Arthropod Phylogeny. New York, USA.
49. Kobayashi S, Matsuura S (1995) Egg Development and Variation of Egg Sizes in the Japanese Mitten Crab *Ericheir japonica* (de Hann). Benthos Res 48: 29-39.
50. Mossoe EC, Bueno SLS (2002) Reproductive Biology of *Macrobrachium olfersi* (Decapoda, Palaemonidae) in Sao sebastiao, Brazil J Crust Biol Woods Hole 22: 367-376.
51. Odinetz-Collart O, Rabelo H (1996) Variation in Egg Size of the Fresh-Water Prawn *Macrobrachium amzonicum* (Decapoda: Palaemonidae). J Crust Biol Woods Hole 16: 684-688.
52. Narciso L, Morats S (2001) Fatty Acid Profile of *Palaemon serratus* (*Palaemonidae*) Eggs And Larvae During Embryonic And Larval Development Using Different Live Diets. J Crust Biol Woods Hole 21: 566-574.
53. Churchill GJ (2003) An Investigation into the Captive Spawning, Egg Characteristics and Egg Quality of the Mud Crab (*Sylla serrata*) in South Africa. M. Sc Thesis., Rhodes University, South Africa.
54. Mashiko K (1983) Differences in the egg and clutch sizes of the prawn *Macrobrachium nipponense* (De Haan) between brackish and fresh waters of a river. Zool Mag Tokyo 92: 1-9.
55. Qian P, Chia F (1992) Effect of aging on reproduction in marine polychaetes, *Capitella* sp. J Exp Mar Biol Ecol 156: 23-38.
56. Stella V, Lope L, Rodriguez E (1996) Fecundity and brood biomass investment in the estuarine crab, *Chasmognathus granulata* Dana, 1851. Crusta 69: 307-312.
57. Eyster L (1979) Reproduction and developmental variability in the opestobranche *Tenellia pallida*. Mar Biol 51: 133-140.
58. Glazier D (1992) Effects of food, genotype, and maternal size and age on offspring investment in *Daphnia magna*. Ecol 73: 910-926.
59. Gimenez L, Anger K (2001) Relationships among salinity, egg size, embryonic development, and larval biomass in the estuarine crab *Chasmagnathus granulata* Dana, 1851. J Exp Mar Biol Ecol 260: 241-257.
60. Turner RL, Lawrence JM (1979) Volume and composition of echinoderm eggs: implications for the use of egg size in life-history models. In Reproduc Ecol Mar Inverteb. University of south Carolina Press, DC, USA.
61. Vance RR (1973) More on reproductive strategies in marine benthic invertebrates. Am Nat 107: 339-352.
62. Christiansen FB, Fenchel TM (1979) Evolution of marine invertebrate reproductive patterns. Theor Popul Biol 16: 267-282.
63. Perron FE, Carrier RH (1981) Egg size distribution among closely related marine invertebrate species: are they bimodal or unimodal. Am Nat 118: 749-755.
64. Rojas J, Silva (1979) Estudio preliminar de *Macrobrachium amazonicum* (Heller). Conf Nac Biol Chiclayo Peru 6: 163-164.
65. Pandian TJ, Katre S (1972) Effect of hatching time on larval mortality and survival of the prawn *Macrobrachium idae*. Mar Biol 13: 330-337.
66. Celada JD, Carral JM, Gonzalez J (1991) A Study on Identification and Chronology of the Embryonic Stages of the Freshwater Crayfish *Austropotamobius pallipes* (Lereboullet, 1858). Crusta 61: 225-232.
67. Luxmoore RA (1982) The reproductive biology of some sterolid isopods from the Antarctic. Polar Biol 1: 3-11.
68. Wageler JW (1987) On the reproductive biology of Ceratoserolis trilobitoides (Crustacea: Isopoda): Latitudinal variation of fecundity and embryonic development. Polar Biol 7: 11-24.
69. John Samuel N (2008) Embryonic development and larval culture in a commercially important Portunid crab *Portunus sanguinolentus* (Herbst) Ph.D. Thesis, Annamali University: 1-142.
70. Damrongphol P, Eanghuan N, Poolsanguan B (1990) Simple in vitro culture of embryos of the giant freshwater prawn *Macrobrachium rosenbergii*. J Sci Soc Thailand 16: 17-24.
71. Kanaujia DR, Mohanty AN, Mitra G, Prasad S (2005) Breeding and seed production of the Ganga river prawn *Macrobrachium gangeticum* (Bate) under captive condition. Asian Fish Sci 18: 371-388.
72. Panikkar NK (1967) Osmotic behaviour of shrimps and prawns in relation to their biology and culture. FAO.
73. Muller Y, Ammar D, Nazari E (2004) Embryonic development of four species of palaemonid prawns (Crustacea, Decapoda): pre-naupliar, naupliar and post-naupliar periods. Revi Brasil De Zool 21: 27-32.
74. Jalihal DR, Sankolli KN, Shenoy S (1993) Evolution of larval developmental patterns and the process of freshwaterization in the prawn *Macrobrachium Bate*, 1868 (Decapoda, *Palaemonidae*). Crust Leiden 65: 365-376.
75. Magalhaes C (1989) The larval development of Palaemoid shrimps from the amazon region reared in the laboratory. VI. Abbreviated development of *Macrobrachium nattereri*. Heller, 1862 (Crustacea, Decapoda). Amazoniana Manaus 4: 379-392.
76. Bueno SLS, Rodrigues SA (1995) Abbreviated larval development of the freshwater prawn, *Macrobrachium iheringi* (Ortmann, 1897) (Decapoda, *Palaemonidae*), reared in the laboratory. Crust Leiden 68: 665-686.

77. Bond G, Buckup L (1982) O ciclo reproductor de *Macrobrachium borelli*. Nobili, 1896 *Macrobrachium potiuna* Muller., 1880. (Crustacea, Decapoda, *Palaemonidae*) e suas relacoes com a temperature. Rev Brasileira De Biol, Rio De Janeiro 42 : 473-782.
78. Gamba L (1984) Different egg-associated and larval development characteristics of *Macrobrachium jelskii* and *Macrobrachium amazonicum* (Arthropoda: Crustacea) in a Venezuelan continental lagoon. J Invert Repro Develop Rehovot 7: 135-142.