



Alterations in Size, Weight and Morphology of the Eggs of Blue Swimmer Crab, *Portunus pelagicus* Linnaeus, 1758 (Decapoda, Brachyura, Portunidae) during Incubation

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Abstract

Portunus pelagicus Linnaeus, 1758 is an important candidate species for culture in India and trials are for developing its hatchery technology. The present work investigates the changes occurring in diameter, volume, wet weight and morphology of *P. pelagicus* eggs during incubation which would improve the knowledge base on embryology of species. Mated females from wild were maintained under similar physico-chemical conditions. Once spawned, the egg samples were collected from each animal daily and their diameter, volume, and wet weight were assessed. Based on the changes occurring in egg morphology at 24 hour intervals, the embryo development was classified into eight 'periods'. The egg size and wet weight varied significantly ($P < 0.01$) among crabs and egg size was unrelated with the carapace width of female. The average diameter, volume and wet weight of the egg were 0.324 mm, 0.016 mm³ and 0.048 mg, respectively. Diameter, volume and wet weight of the eggs increased to 13.58, 46.52 and 49.05%, respectively and the major share of increment occurred in last two days.

Keywords: Embryo development, egg diameter, egg volume.

Introduction

Crustaceans show a great diversity in embryonic development, especially due to a significant variation in egg size (Hines, 1982). In brachyuran crabs, eggs are attached to the pleopods beneath the abdomen of the females from spawning to hatching (Anderson, 1982). The incubation period depends on species (Nagao *et al.*, 1999) and temperature (Garcia-Guerrero *et al.*, 2003). The reproductive output per brood for brachyuran crabs is strongly correlated with body size and weight within a species (Hines, 1982) and the size of eggs (Rabalais, 1991). Embryonic development is a continuous process, but staging of embryos is based on static morphology of the embryo when certain physiological and morphological features are apparent through the course of development (Moriyasu and Lanteigne, 1998). Embryonic development in crabs has been staged by many authors based on different criteria and most of the studies were based on general egg morphology (e.g. Gimenez and Anger, 2001) and a few based on egg histology (e.g. Jun-Zeng *et al.*, 2001). During development, the eggs undergo a lot of changes in their size, shape, weight, contents and morphology. For example, in the eggs of the brachyuran crab

Xantho bidentatus, the organic material and dry weight decreased, whereas the water and ash content increased constantly during incubation (Babu, 1987).

The blue swimming crab *Portunus pelagicus* is a candidate species for culture because of its fast growth, attractive appearance and taste. *P. pelagicus* occurs in shallow, tropical and temperate coastal and estuarine waters throughout the Indo-West Pacific from Africa to India, southeast Asia and Australia (Smith and Sumpton, 1989; Chande and Mgaya, 2003). In India, the species is available all along the coast, prominently in the south-east and the south-west regions and breeds round the year (Pillai and Nair, 1973). Throughout its distribution, it is important in many commercial and recreational fisheries (Sukumaran and Neelakantan, 1997; Sumpton *et al.*, 2003). The fecundity of *P. pelagicus* ranges between 900,000 and 1,600,000 (Meagher, 1971). The larval development comprises of 4 zoeal stages and a megalopa stage (Shinkarenko, 1979). Since commercial hatcheries are not available for the species, the farmers are forced to depend on the wild for juvenile crabs for culture practices. Difficulties in obtaining juveniles from wild, their non-uniform size and concerns of stock depletion due to over exploitation have encouraged research activities to

frame out hatchery technology for the species. Production of seeds on a large scale is also important in formulating conservation management strategies. Sea ranching of crab juveniles is a good option to enhance the natural stock. The study on alterations in the diameter, volume, wet weight and morphology of eggs during incubation will help in understanding the process of egg and embryo development and this knowledge is of immense use while maintaining the broodstock in hatcheries. Investigations on the embryo development of *P. pelagicus* from Indian waters are scanty and it is in this backdrop the present study to trace out the changes taking place in the diameter, volume, wet weight and morphology of eggs of *P. pelagicus* during incubation was attempted.

Materials and Methods

Animal Collection and Maintenance

Twenty eight mated females of *P. pelagicus*, understood by the presence of spermatophores in the spermatheca (Robertson and Kruger, 1994) were collected from Palk Bay off Mandapam (9°17'N, 79°9'E), along the south-east coast of India from May to August 2007. The animals were brought live to the wet laboratory, transferred to 1 ton black FRP (Fiberglass Reinforced Plastic) tanks with a sandy substratum of four inches thickness and continuous aeration and acclimatized for two days. The most active among them were tagged and stocked separately in black rectangular 500 L FRP tanks with sandy substratum (4 inches thick) and continuous aeration. The range and the average of carapace width of the animals selected were 140-160 mm and 151.5±5.42 mm (± standard deviation, SD) and that of the body weight were 180-230 g and 205.32±12.81 g. The sandy substratum provided refuge, aided the proper development of clutch and also minimised egg loss during incubation (Davis *et al.*, 2004). Feeding was done once daily with raw cuttlefish (*Sepia pharaonis*) and clam (*Meritrix meritrix*) meat (1:1), *ad libitum* and the feed waste was removed early morning every day after stopping the aeration. The temperature, pH, salinity and photoperiod regimen maintained were 29.0±0.1°C (± standard error, SE), 8.1±0.1 (± SE), 35 and 12 hL:12 hD (hL/D = hours light/dark), respectively. The water temperature was maintained using aquarium heaters with thermostats (Azoo, USA) and the pH was adjusted by adding 1N sodium carbonate or 1N hydrochloric acid, as required. Tanks were covered with black sheets after the photophase. Water exchange (70%) was carried out daily and the animals were checked regularly for spawning.

Assessment of Egg Dimensions

In order to study the variations in the egg

diameter, twenty eggs were randomly taken from the clutch of each animal daily and measured across their longest and shortest axis with an ocular micrometer. The eggs of *P. pelagicus* are almost spherical when laid and throughout the development (earlier observations). Furthermore, to minimize any possible error, two axes were measured for each egg and its average was taken as the diameter. The average of 20 such diameters gave the mean egg diameter. The egg was taken as a perfect sphere and the volume was calculated using the equation $1/6\pi d^3$, where 'd' is the mean diameter of the egg. The volume also was calculated separately for each egg every day. In order to study the variations in egg wet weight, five egg subsamples (of weighable size, which varied) each were collected from the clutch of twenty females daily, blotted dry on a paper towel and weighed separately to the nearest 1 mg on an electronic balance. The number of eggs in each subsample was counted. Using the weight and number of eggs in each subsample, the weight of a single egg in that subsample was worked out. The average of the single egg weight from five subsamples gave the mean wet weight of the egg of that female. The average of 20 such wet weights gave the mean egg wet weight. All the parameters were calculated separately for each individual all through the incubation period. The diameters, volumes and wet weights of the eggs were separately subjected to one-way analysis of variance (ANOVA) daily to find out whether there is any significant difference ($P<0.01$) in each of these parameters among the crabs.

Study on Egg Morphology

In order to study the morphological changes in the egg, 10 eggs each from five females, taken consistently from the middle region of the clutch (to minimize the possible variations in developmental stage within the clutch) every day were fixed in aqueous Bouin's solution for 24 hours, washed repeatedly in seawater and preserved in 70% alcohol to improve the descriptions (Turra and Leite, 2007). The eggs were examined under a light microscope in different magnifications and photographed. The developmental stages were classified based on the changes occurring in the quantity of the yolk, the space occupied by the embryo and the appearance and subsequent growth of the eyes, appendages, pigments and heart (Felder *et al.*, 1985). Since embryo development is a continuous process, the term 'periods' were used instead of 'stages' to describe a phase of 24 hours (García-Guerrero and Hendrickx, 2004).

Results

Routine statistical analysis using one-way ANOVA of the parameters showed that the differences in the values of the parameters among

crabs were significant ($P < 0.01$). All the females spawned within 8-10 days of stocking. Since the egg deposition occurred in the sandy substrata, exact time of spawning could not be recorded. However, the spawning seemed to have occurred during the night or early morning hours. The eggs were spherical; light yellow and laden with yolk when deposited. The egg/clutch, which was light yellow at the time of spawning transformed to yellow-orange after two days, then to light grey and finally dark grey prior to hatching. The complete embryonic development took place in eight days ('incubation period' at $29 \pm 0.1^\circ\text{C}$ (\pm SE)) and hatching occurred during the early morning hours in all the females studied.

Egg Diameter, Volume and Wet Weight

The eggs of *P. pelagicus* in the present study had a mean diameter of 0.324 ± 0.014 mm (\pm SD), average volume of 0.016 ± 0.002 mm³ (\pm SD) and a mean wet weight of 0.048 ± 0.007 mg (\pm SD). The mean egg diameter increased gradually as embryogenesis progressed from day 1 to day 6, and increased rapidly from day 6 till hatching (Figure 1).

The total increase in the diameter (day 1 to day 8) was 13.58% of which almost 74% took place in the last two days. The lowest and the highest increments in egg diameter occurred between periods III-IV and VII-VIII, respectively. The initial and final mean egg volumes were calculated as 0.015 ± 0.002 mm³ (\pm SD) and 0.021 ± 0.004 mm³ (\pm SD), respectively. The total increment in the egg volume was estimated to be 46.52% and of that around 75% took place in the last two days (Figure 1). The total increase in wet weight of egg was 49.05% with the last two days contributing nearly 78%. The lowest and the highest increments in the egg wet weight occurred between periods II-III and VII-VIII, respectively.

Egg Morphology

The development of egg was classified into 24 hour 'periods'. The variations in the morphology of

the eggs of *P. pelagicus* were continuous and the embryo developed in a gradual manner (Figure 2).

Period I (Day 1): Macrolecithal spherical yellow eggs; entire volume filled uniformly with yolk mass; no other spots could be seen; surface appearance not very smooth; cleavage not commenced; clutch was light yellow.

Period II (Day 2): Yellow eggs; small bud like invaginations could be seen mainly on one side which denoted the formation of a cluster of presumptive primordial cells; fragmented oil globule like appearance on the egg surface was more pronounced; clutch continued to be light yellow.

Period III (Day 3): Yolk content along the periphery at one side has been reduced by a sizeable amount; certain other peripheral regions also were transparent; clutch was dark yellow.

Period IV (Day 4): Yolk content reduced by around 35%; more portions of egg were transparent; invaginations moved further inwards; clutch continued to be dark yellow.

Period V (Day 5): Not much change in the egg volume transparency; light yellowish-orange eye spots appeared (not easily distinguishable); clutch was yellow-orange.

Period VI (Day 6): Egg volume transparency was more than 50%; developing embryo was fairly visible; middle of the yellow-orange eye streak began to darken (eyes could be spotted easily); head and the main body parts could be differentiated; pigmentation faintly visible; clutch was light grey.

Period VII (Day 7): Heart became functional; ocular lobes large, brownish-black, oval-shaped and have developed a dark core; heavy pigmentation due to the high deposition of chromatophores throughout the head region of the embryo; embryo occupied the entire space inside the egg; slight pigmentation on abdominal somites; body parts began to acquire shape; clutch continued to be light grey.

Period VIII (Day 8): Eye spots very large and nearly black; appendages could be seen; heavy pigmentation on head and rest of the body; yolk

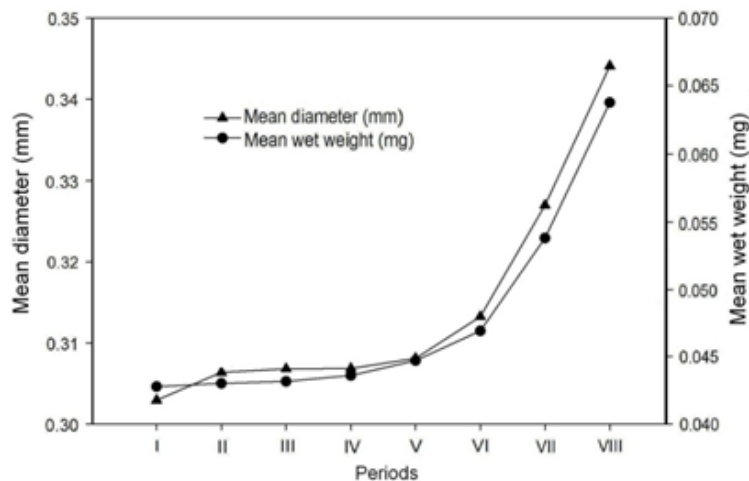


Figure 1. Variation in the mean diameter and wet weight of eggs of *Portunus pelagicus* during incubation.

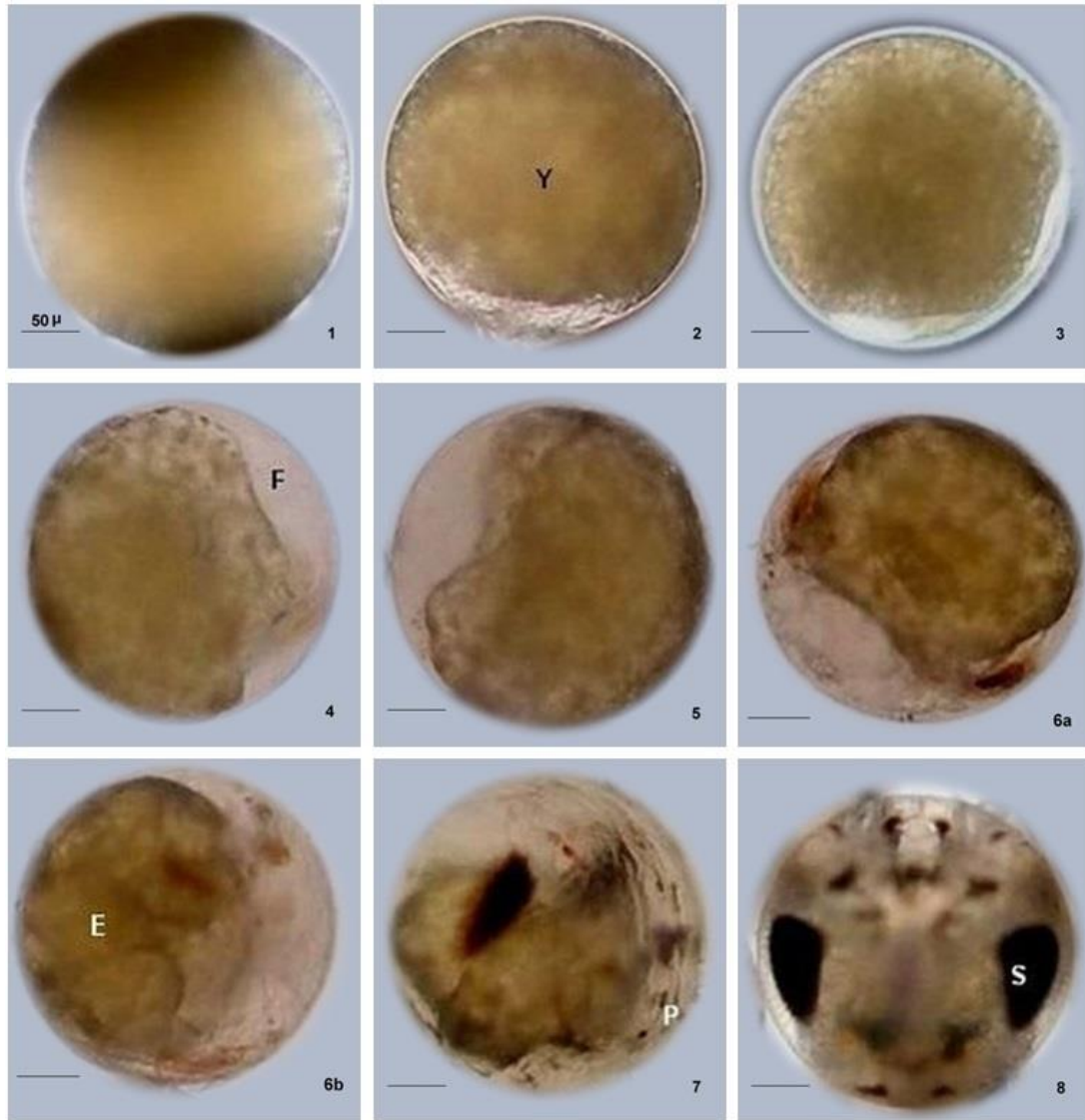


Figure 2. Morphological changes in the egg of *Portunus pelagicus* during incubation. Days 1, 2, 3, 4, 5, 6a* and 6b*, 7 and 8. Yolk (Y), developing embryo (E), free space (F), eye spot (S) and pigments (P).

*from different angles. Scale bar = 50 µm.

nearly used up apart from a small patch in the cephalic region; heart beating vigorously; transparent embryo; clutch was dark grey.

Zoea 1 (Day 9): Free swimming zoea 1 larvae.

Discussion

Egg developmental studies are of great significance in the comprehensive understanding of the reproductive biology of any species. Knowledge on the reproductive biology of a species is one of the most important aspects in evaluating the harvesting strategies of exploited populations (Addison and Bennet, 1992). Several studies have indicated that variable egg quality is one of the most critical factors underlying the inconsistent success of controlled seed production (Davis, 2003).

Crustacean eggs vary much in shape from spherical to elliptical, but the brachyuran eggs are

generally spherical (Garcia-Guerrero and Hendrickx, 2004). In some crustaceans, the shape of the eggs may vary as the embryo development progress; however the eggs continued to be spherical all through the developmental period in the present study. The diameter of the mature egg (which are about to hatch) in the present study was 344 µm. Nearby sized eggs were reported in many brachyuran crabs (eg. the mangrove crab, *Perisesarma bidens* (Sarker *et al.*, 2009)). However, brachyuran crab eggs are found to show inter-specific variations in size (Wear, 1974). As observed in the present study, a lack of proportional relationship between carapace width and egg size have been reported in many other crabs (eg. the New Zealand crab, *Ovalipes catharus* (Haddon, 1994)).

The increment in egg diameter and egg volume with the progress of embryogenesis in the present study is comparable to the increase reported in many

crabs like the mangrove crabs *Goniopsis pulchra* and *Aratus pisonii* (Garcia-Guerrero and Hendrickx, 2004). Different reasons have been attributed to the increase in egg size by various authors. The increase may be due to a progressive absorption of water (Pandian, 1967) or by the retention of metabolic water from lipid oxidation during larval development (Lardies and Wehrmann, 1997). Eggs of benthic marine decapods normally increase their initial water content by nearly 33-40% during incubation (Pandian, 1967, 1970; Babu, 1987). Apart from water absorption and/or retention, the increase in egg size may also be due to a forced expansion of the egg shell in response to the embryo growth – ‘plastic response’ (Giménez and Anger, 2001). Though an investigation to find out the cause of increase in diameter and volume was not carried out here, it is envisaged that all the possibilities cited might have contributed in various proportions. The space created by the expansion of the egg due to the growth of the embryo also might have facilitated further intake of water. In the horseshoe crabs, *Limulus polyphemus* and *Tachypleus tridentatus*, 95% of the egg swelling was caused by the influx of water into the perivitelline space and 5% by the embryo growth (Hayakawa *et al.*, 1985). Specific and detailed study is necessary to find out any further bases for the increase in the size of the eggs during incubation.

In the present study, the wet weight of the egg increased with embryo development as in the case of the egg diameter. Influx of water together with the increase in the weight of the embryo might have contributed to the increase in total weight of the egg. Similar increase in wet weight of egg during incubation was reported in the spider crab, *Hyas araneus* (Petersen and Anger, 1997). The substantial increase in the egg size and wet weight during the last two days of incubation shown here is similar to the observations were made in many other crabs (eg. the fiddler crabs, *Uca lactea* (Yamaguchi, 2001) and *U. cordatus* (Pinheiro and Hattori, 2003). This sudden increase in size and weight towards the end of incubation period is due to the enhanced water intake, which is assumed to be a precursor to hatching (Petersen and Anger, 1997). In the process of hatching, the natural swelling of the eggs is followed by osmotic swelling of the inner egg membrane, which ruptures the chorion (Davis, 1965). The rate of water absorption is influenced by temperature (Kobayashi and Matsuura, 1995) and salinity (Gimenez and Anger, 2001). Though, a study on the rate of absorption of water in the egg was not attempted here, since the animals were maintained in similar physico-chemical conditions, the influence of temperature and salinity on the rate of water absorption is presumed to be the same.

In the present study, the incubation period was found to be the same for all the animals studied. The incubation period in brachyuran crabs was found to increase with the decrease in temperature and *vice*

versa (Wear, 1974). However, the temperature conditions were maintained the same and constant through out here, which might have resulted in the uniform incubation period in all the females studied. Alterations in the egg colour during embryogenesis are common in crustaceans (Sigana, 2002; Pinheiro and Hattori, 2003). The colour change is caused by the absorption of yellow yolk and the development of dark pigment in the eyes (Parimalam, 2001). The availability of yolk in the egg is the chief factor that determines the stage at which the young are to be hatched out (Adiyodi and Subramoniam, 1983).

Staging of embryo development based on egg morphology is of great significance in hatchery operations since it will help in the accurate prediction of the date of hatching. So, arrangements for the timely preparation of live feed and rearing system can be made. Richter *et al.* (1995) opined that the egg size determines the larval size, but it seems more likely the other way since the growth of embryo also contributes to the increase in the size of the eggs. As a study on the pattern in variation of the diameter, volume and wet weight of egg during incubation of *P. pelagicus* from Indian waters has not been carried out before, the results obtained and the inferences drawn add on to the information on the embryology of this species in relation to tropical conditions.

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