EGG DEVELOPMENT AND CLASSIFICATION IN THE MOLE CRAB, *Hippa pacifica* (Dana, 1852) (ANUMORA: Hippidae)

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Abstract

The stages of egg development in the mole crab, Hippa pacifica (Dana 1852), were described and classified in this study. Twenty gravid female mole crabs that inhabited the intertidal zone in the sandy beach of Magoong, Linamon, Lanao del Norte were collected. The animal exhibited adaptation such as protective coloration for survival. Eggs were found at the ventral portion of the animal where they are protected by telson and pleopods. Examination of most of the spongy egg masses showed that the eggs are isolecithal and in the same stage of development suggesting for a synchrony between the developing eggs in a single crab. Cleavage pattern as observed is holoblastic. Eggs from different mole crabs are from various developmental stages suggesting that breeding activity in this species might be a continuous process and might occur all year round. The eggs were classified into eight stages starting from the stage where yellow yolk granules can be seen and the egg masses appear bright orange in color to the stage where the egg masses turn pale grey in color and the embryo is almost completely developed. Egg developmental stages were seen to be the same with that of the mole crab Emerita asiatica. However, differences were observed when it was compared with the blue king crab Paralithodes platypus. Further studies should be conducted to determine the reproductive biology of this species of mole crab.

Keywords: *mole crab, Hippa pacifica, egg development, classification*

1.0 Introduction

In sexually reproducing organisms, the developing embryo undergoes complex developmental processes that begin as soon as the egg has been fertilized. The pattern of egg development is neither difficult nor complex to understand as this can be seen and observed in some crustaceans. The Indo -pacific mole crab, *Hippa pacifica* Dana is a rather uncommon species that is found along the shoreline of the Philippines. The mole crab is small in size, growing up to 35 mm long and 25 mm wide with gray or sand -colored and does not have claws or spines. Like other crustaceans, they periodically molt and the empty exoskeleton may be found on the shore. Males and females may look very similar at first glance, but females are larger with a carapace length of 14-35 mm, and the males with 10-22 mm. If a female is carrying eggs, they will be found under the telson and will be a bright-orange mass and if it is not carrying eggs, the pleopods to which the eggs are attach will be visible on the underside of the crab when the telson is lifted. Reproduction in crabs is a complicated process. Reproduction and spawning generally occur in conjunction with molting. During the reproductive season (February-October), females can produce one clutch per month of 50-45,000

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eggs, which take approximately 30 days to develop (Shimek, 2008). Many factors are affecting the rate of embryonic development of most crabs. According to Webb et al., (2007), the rate of embryonic development increase with increasing temperature. Likewise, the patterns of embryonic cleavage are determined by two major parameters: the amount and distribution of yolk protein within the cytoplasm, and factors in the egg cytoplasm that influence the angle of the mitotic spindle and the timing of its formation (Gilbert, 2005). Thus, egg type in terms of the amount and distribution of yolk exhibits a certain type of cleavage.

Many pioneering studies had been conducted to investigate the ecology and behavior of the mole crabs, but less study was made to document and understand the egg development of this species. The embryology of brachyuran crabs has been examined haphazardly throughout the world by a few researchers, but no standard exists for defining the developmental stages (Stevens, 2006). Subramoniam (1979) studied some aspects of reproductive ecology of *Emerita asiatica* Milne Edwards and Stevens (2006) studied the embryo development and morphometry in the blue king crab *Paralithodes platypus* using image and cluster analysis, but no studied were done to investigate the embryology of the mole crab, *Hippa pacifica* Dana. The aims of this study were to document and determine the egg type of this mole crab, its cleavage pattern, and to classify its embryonic developmental stages in reference to the existing studies on other crabs (*E. asiatica* & *P. platypus*). The results of this study will provide a framework and visual key for the morphological identification and classification of embryonic stages for further population or reproductive studies on mole crabs.

2.0 Methodology

A total of twenty gravid female mole crabs, were collected from the intertidal zones of Magoong Beach, Linamon, Lanao del Norte in the month of October. Animals were washed in sea water to remove adhering sand particles. The developing eggs were then carefully stripped from the pleopodal hairs of the gravid female mole crab and placed in a petri dish with sea water. Digital images of the mole crab were taken using a digital camera and that of the live embryo were taken using MicronCam by Nietzie Bebing, connected to a stereoscopic zoom dissecting microscope. Appearance of the animal such as protective coloration was noted. Each gravid female mole crab that carries all the eggs in clusters/sponge were evaluated if almost all stages that are found at any time in different individuals of the population is at the same stage of embryonic development or had the egg development leading to the release of zoea larvae. Egg type and cleavage pattern were also determined. Stages of embryonic development were defined using two different methods. One of the traditional method using developmental changes that are visually observable in the stained or unstained embryos. However, no standard criteria exist by which to define stage endpoints (Stevens, 2006), so stage definitions tend to be subjective. As a general guide, reference was made to descriptions of embryonic development in mole crab, *Emerita asiatica* Milne Edwards by Boolootian et al., (1959) and Eickstaedt (1969) as cited by Subramoniam (1979) and blue king crab, *Paralithodes platypus* by Stevens (2006). Embryonic stages of development of *H. pacifica* were then compared with these two crabs.

Table 1. Classification of egg development in *Emerita asiatica*

Stage	Description
I	Yellow yolk granules seen; egg mass bright orange in color
II	Cleavage has taken place and blastomeres are seen; egg mass bright orange in color
III	A yolk-free white streak makes its appearance in the animal pole; ectoderm cells are present in this transparent part
IV	One quarter of the yolk cleared; the white band circles the yolk material which is now in the center; at the animal pole a periodic twitching is recognized; red pigment spots are seen at the edge of the yolk; colour of the egg mass is dull orange
V	One third of the yolk is utilized; two eye spots have made their appearance; red spots prominent and seen at the animal pole; colour of the egg mass very dull orange
VI	Egg mass brownish orange in colour; eyes are well developed; yolk is found in the vegetal pole; two thirds of the yolk cleared; red pigments are spread all over the white space
VII	Egg mass greyish orange in colour; yellow yolk is found as two clusters in the center; appendages of the embryo are developed; heart beat seen; eye spots very well developed
VIII	Egg mass pale grey in colour; colourless yolk in the form of oil globules seen just below the two eyes as two pockets; heart beat prominent; embryo almost completely developed
IX	Embryo fully formed; egg mass white in colour; no yolk globules seen; about to be released
X	Released zoea larvae

*classification of the stages of egg development leading to hatching of zoea larvae was made using the criteria given by Boolootian *et al.* (1959) and Eickstaedt (1969) as cited by Subramoniam (1979).

3.0 Results and Discussion

The mole crab *Hippa pacifica* Dana have all the adaptations that help them survive in the sandy beach ecosystem. For instance, it closely matches the color of the substrate in which it burrows (Wenner, 1972). This provides protective coloration to avoid their predators. Their main predators are fish, water birds and shore birds. Fish provide the greatest threat, and this may explain why mole crabs are mostly in the upper intertidal zone. Figure 1 shows a mole crab showing its greyish-black color carapace that commonly matches the sandy beach of Magoong, Lanao del Norte where they were collected. The eggs are found under the telson and bright orange in color (fig. 2). According to Costello et al. (1971), females carry their eggs for a period of four to five months. In contrast to the brachyuran crabs *Eurypanopeus canalensis* Abele & Kim, *Panopeus chilensis* H. Milne Edwards & Lucas, and *Perisesarma bidens* De Haan that the eggs are macrolecithal-centrolecithal, the egg type of the mole crab is isolecithal (fig. 3). This type of egg is characterized by sparse and evenly

distributed yolk. One parameter that determines the pattern of embryonic cleavage particular to a species is the amount and distribution of yolk protein within the cytoplasm (Gilbert, 2005). Because of the fact that the large amount of yolk retards cleavage, isolecithal egg undergoes holoblastic or complete cleavage as observed in the mole crab (fig.4). Cleavage pattern of *P. bidens* De Haan was reported by Sarker et al., (2009) as complete and holoblastic. This is in contrary to Gilbert (2005) who was according to him; zygote containing large accumulations of yolk (macrolecithal) undergoes meroblastic cleavage, wherein only a portion of the cytoplasm is cleaved. The stages of egg development in reference to sand crab *Emerita asiatica,* are summarized in Table 1. It has been found that all the eggs in a "sponge" were in the same stage of development suggesting that there is a synchrony between the developing eggs in a single crab. According to Subramoniam (1979) in the study made in the mole crab *E. asiatica*, almost all stages are found at any time in different individuals of the population suggesting that the egg development leading to the release of zoea larvae maybe a continuous process in accordance with the continued breeding activity in population.

Embryonic stages of development of *E. asiatica* and *H. pacifica* are almost the same because as observed on this study, embryonic stages of development of the *H. pacifica* fits to the criteria made by Boolootian *et al.* (1959) and Eickstaedt (1969) for *E. asiatica*. This suggest that during early embryonic stages, *H. pacifica* Dana embryos developed at almost the same rate as described for *E. asiatica* by Subramoniam (1979), although the rate of embryonic development will increase with increasing temperature (Webb et al., 2007). Crustacean growth and development rates increase with temperature, as has been shown for larval stages of both red

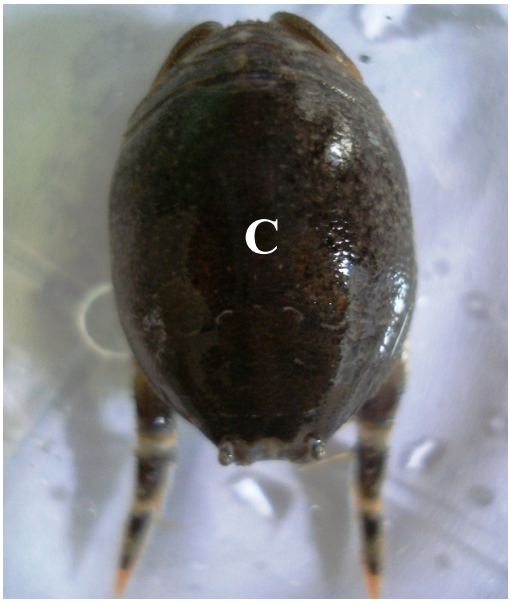

Figure 1. Dorsal view of the female H. pacifica Dana *showing the greyish-black color of the carapace. C, carapace.*

Figure 2. Ventral view of the female H. pacifica Dana *showing bright-orange mass of egg under the telson. T, telson.*

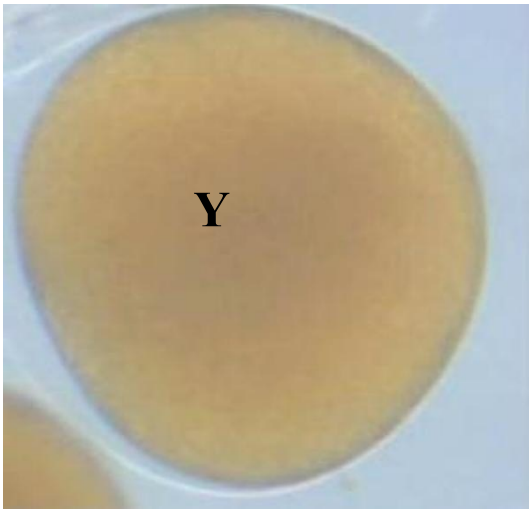

Figure 3. Unfertilized egg of the mole crab showing the evenly-distributed yolk. This is isolecithal type of egg. Y, yolk.

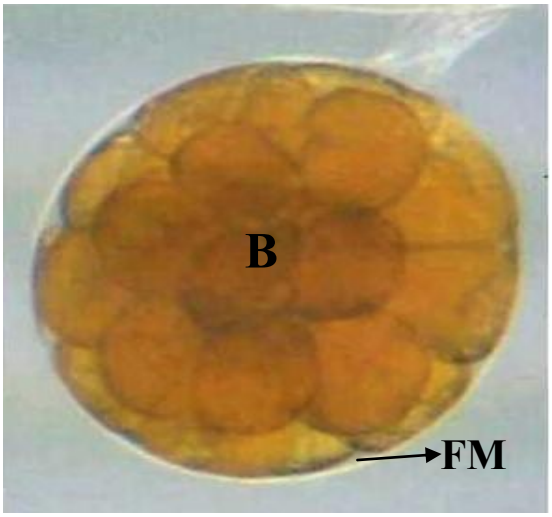

Figure 4. Fertilized egg of the mole crab showing holoblastic type of cleavage pattern. B, blastomere; FM, fertilization membrane.

(Nakanishi, 1981) and blue king crab (Stevens, 2006), southern king crab (Anger et al. 2004), and snow crab (Webb at el. 2007; Kogane et al. 2005). Crustaceans from warmer water environments typically have shorter embryonic development on the order of days to weeks. With short developmental periods, observations that will be made at daily intervals are often different enough to be characterized as individual stages. Because of the great disparity in development time, there is no standardized scheme for characterizing developmental stages of crabs or any other decapods crustaceans (Stevens, 2006).

Most authors recognized 5 or fewer embryonic stages and as many as 15 embryonic stages before hatching (Sarker et al., 2009). The rarity of the stage which represents the release of zoea larvae may be because this process is rapid and probably takes place at night as in the case in the freshwater prawn. This might explain that no zoea larva was observed during sampling made for this study. The timing of larval release in wild populations of mole crabs as in the snow crabs may be associated with specific environmental cues, but more studies may be conducted to confirm these claims. Stevens (2006) first used both visual and cluster analysis of morphometric measurements of crab embryology. In the present study, the general terminologies of fundamental embryology were followed in describing the developmental stages of the mole crab *H. pacifica* Dana. This has been compared and contrasted with that of the blue king crab as described by Stevens (2006) (see below). Undivided eggs were identified as stage 1, which is similar to the findings of Stevens (2006) in blue king crab, Sarker et al. (2009) in mangrove crab and Guerrero et al. (2006) in decapods crustaceans, *Eurypanopeus canalensis* Abele & Kim and *Panopeus chilensis* Milne Edwards & Lucas. In the present study,

2-16 cell embryos were not observed and were identified as a single stage (Fig.5b). Undivided (just-spawned or- fertilized) eggs characteristically differ from cleaved eggs and were distinguished as stage 1, which is more appropriate in the embryology of brachyuran crabs (Sarker, 2009).

According to Subramoniam (1979), a feature of interest in the egg development of *E. asiatica* is that the frequency of the early egg developmental stages is more than that in the later stages, suggesting that the early stages may take more time for development than the later stages. In this study however, all stages of egg development in *E. asiatica* were also observed but the frequency of early egg developmental stages could not be deduced because sampling for this study was made only once. The number of described stages ranges from 10-15, depending on the length of development and utility of each stage in describing changes observable by eye. According to Stevens (2006), the use of embryo morphometrics is a more quantitative method, and may be a useful approach for comparing development between different populations, environmental conditions or species. In the present study, it was observed that each developmental stage is almost similar to the criteria for classification and egg development given by Boolootian *et al.* (1959) and Eickstaedt (1969) for the mole crab, *E. asiatica* Milne Edwards. However, when the developmental stages of the mole crab embryos were compared to that of the blue king crab, *Paralithodes platypus,* nauplius, prenauplius and metanaupius stages were not observed. This could be due to the different methods and environments where the embryos were grown.

NORMAL DEVELOPMENT:

A. The Unfertilized Ovum: The eggs are orange to scarlet in color. They are isolecithal and spherical and are evenly filled with yolk components (fig. 3). They are attached to the egg-mass by thin stalks, and remain on the ventral surface of the female until the zoeae hatch; presumably, the eggs do not develop further if they are detached from the egg-mass. The thin distinct periplasm is homogenous throughout the egg. Neither the micropyle nor signs of egg polarity were observed (fig. 3).

B. Cleavage and Gastrulation: Divisions appear to be total. Cleavage is equal and holoblastic and may be characterized by the early occurrence of a cleavage activity. Cleavage stages are very pronounced (Fig. 5d-f). Due to the large density of blastomeres, the latter stages of cleavage and blastomeres could not clearly be detected. The surface of each blastomere is so granular that the internal feature could not be detected using only a microscope and without applying any special technique for counting the blastomeres (Fig. 5d-f). The phase between blastula and gastrulation is not clearly marked but is a gradual transformtion.

C. Later Stages of Development: The yolk-free portion and evidence of tissue formation is more distinct and clearer in the yolk-free portion (Fig. 5j-l). Crescent-shaped eyes first became visible and further development of heart and heart-beat were observed. All structures of the embryo will become distinct and accordingly, as hatching approaches, the embryo frequently moves its abdomen and appendages in the egg membrane (Sarker, et al., 2008) (Fig. 5u).

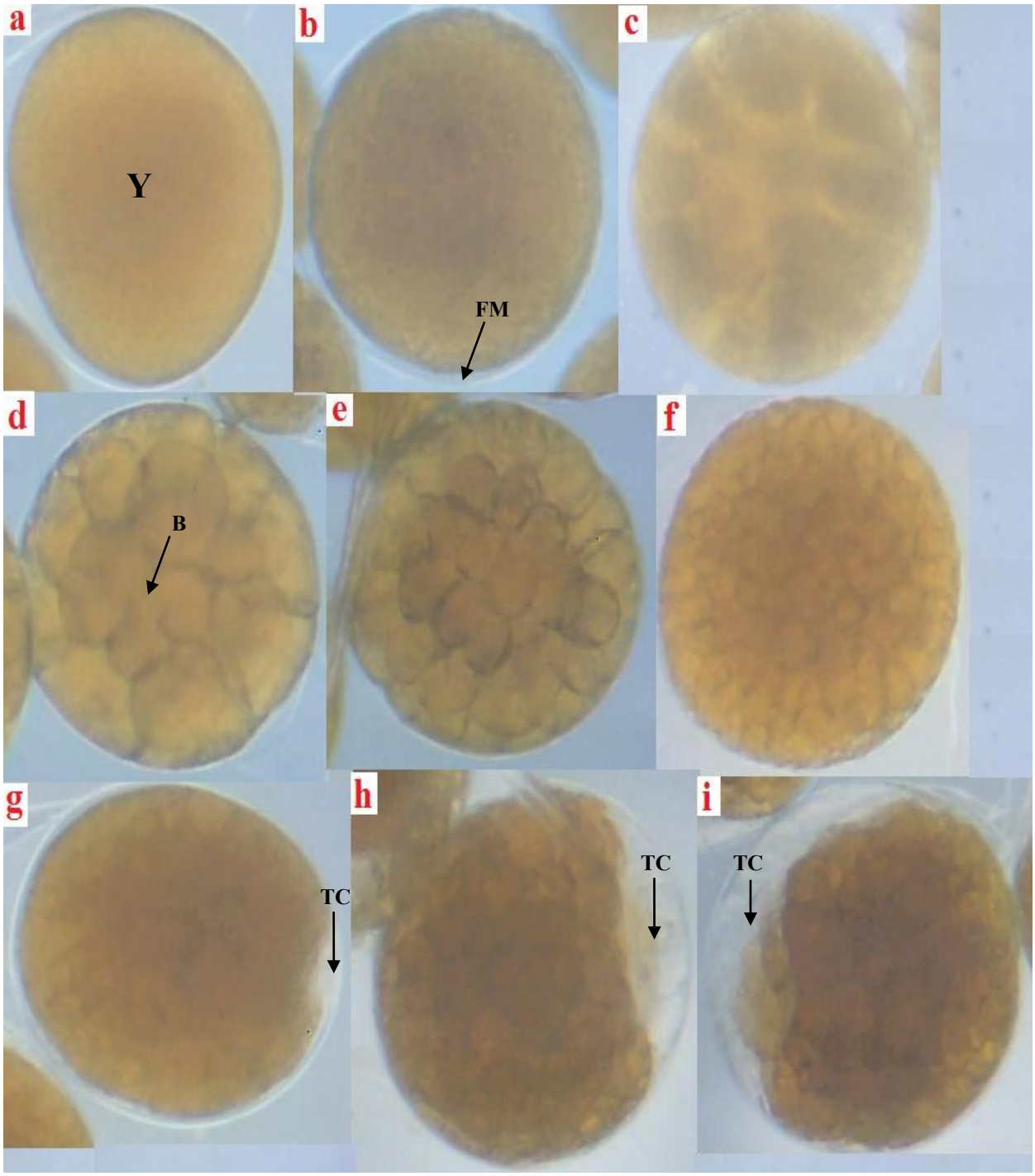

Figure 5. *Stages of development in embryos of Hippa pacifica* Dana. *(a) Unfertilized egg. Yellow yolk granules seen; egg mass bright orange in color. Y, yolk; (b) stage I: Fertilized egg before cleavage. FM, fertilization membrane (c-f) stage II: Cleavage has taken place and blastomeres are seen; egg mass bright orange in color. B, blastomere; (g-i) stage III: A yolk-free white streak makes its appearance in the animal pole; ectoderm cells are present in this transparent part.TC, tissue cap.*

Stages of Embryo Development

(In reference to the development of the blue king crab P. platypus as described by Bradley G. Stevens. Comparison with the development of mole crab H. pacifica Dana *was made).*

Stage 1 Cleavage

During this period, dividing cells were easily distinguishable prior to blastodisc formation, and little change in morphometry occurs. Eggs were initially lavender colored. This observation is different from the *H. pacifica* because the eggs are light orange in color even after fertilization (Fig.5a-b). Cleavage is more or less the same, equal and holoblastic, creating a large density of blastomeres (Fig.5c-f). Because of this, latter stages of cleavage and blastomeres could not clearly be detected. The blastomeres are distinct and granular. The onset of cleavage is more or less the same, which is day 4 after fertilization in blue king crab and not really known in the *H. pacifica*, but accordingly this happens earlier. Early stages of cleavage were not observed but 16, 32, and 64-cell stages are observable in both *H. pacifica* and *P. platypus*

Stage 2 (Blastula-Gastrula)

For both *H. pacifica* and *P. platypus*, individual cells and structures are not visible. The blastodisc (not observed in the *H. pacifica*) became apparent and embryos continued to decrease in size, but developmental changes were not discernible. The phase between blastula and gastrulation is not clearly marked but is a gradual transformation for the two crabs.

Stages 3-5 and 12 were not observed in the H. pacifica

Stage 3 (V-embryo to Nauplius)

During this period embryonic lobes are becoming visible and are increasing in size. Most embryos had distinct lobes that would become the antennules, antennae and mandibles. Optic lobes are diffuse and indistinct, and the abdomen is a diffuse round lobe at the base of the "V". By the end of this stage, the antennules and antennae are elongated, and the abdomen is distinct.

Stage 4 (Prenauplius)

The optic lobes are large and rounded. Rudiments of the antennules and biramous antennae are clearly defined, the latter with a medial epipodite. The mandible is forming medial to the antennae. The abdomen is folded over the embryo for about half of its length.

Stage 5 (Metanauplius)

Optic lobes extend lateral to the rest of the embryo. The tail is about two-thirds the length of the embryo. The telson is forked, but setae are not apparent. Maxilliped rudiments are barely visible lateral to the tail.

Stage 6 (Eye Formation)

The eyes are large, lightly pigmented, and extend almost to the edges of the egg (Fig.5l). The telson has 6 or 7 spines (or setae) and reaches the anterior margin of the optic lobes (not so apparent in *H. Pacifica*). Lateral appendages have setae (not observed in *H. pacifica*). Up to four chromatophores can be seen (Fig.5n).

Stage 7 (Chromatophore Formation)

This is a period of rapid eye growth and formation. The eyes changed from strongly pigmented crescents to being oval-shaped. Six to eight chromatophores are visible on each side. Maxillipeds are elongated with rudimentary setae (not observed in *H. pacifica*). In top view, the embryo takes up one-third of the egg (Fig.5o). In blue king

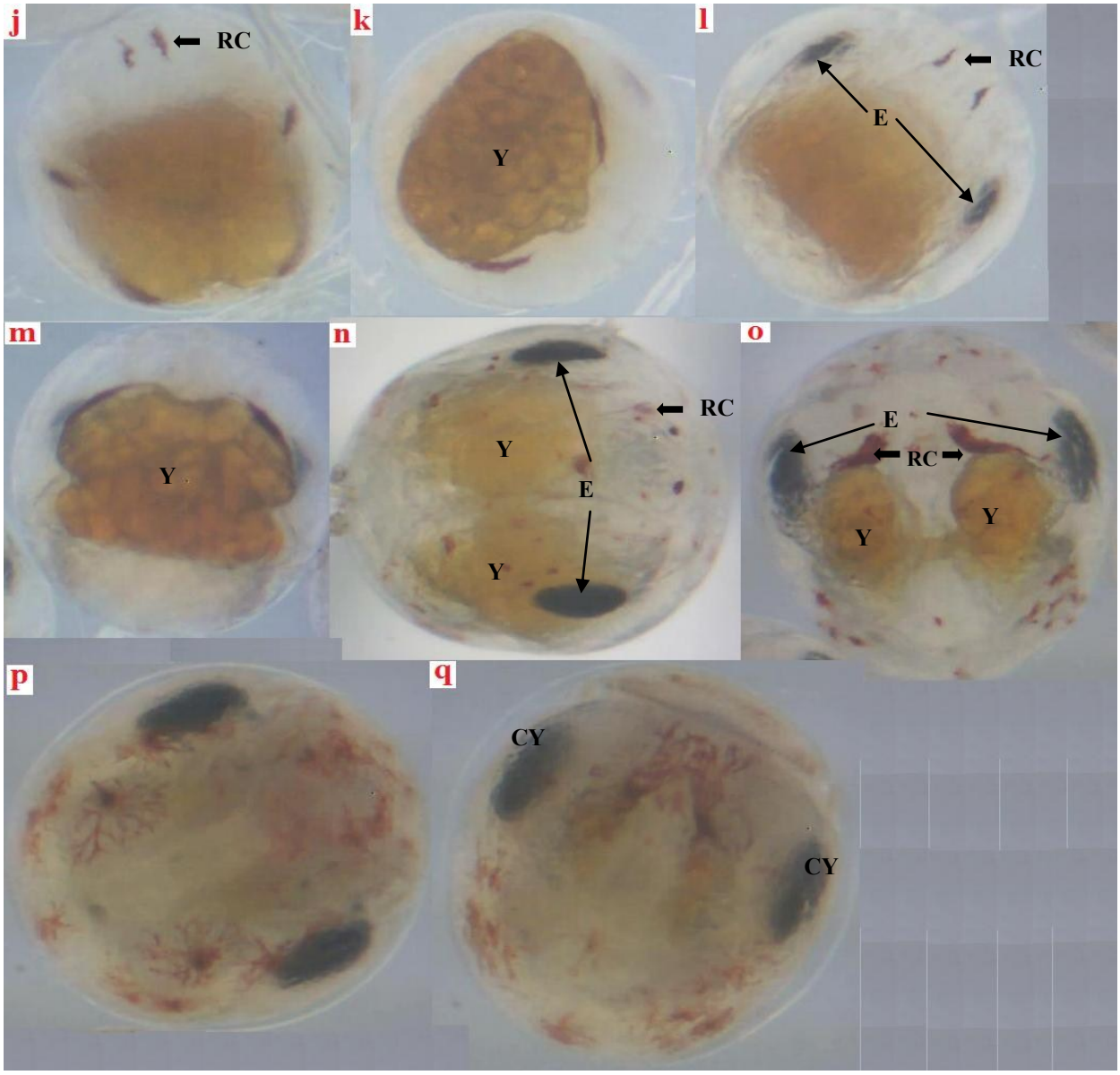

Figure 5. *continued. (j-k) stage IV: One quarter of the yolk cleared; the white band circles the yolk material which is now in the center; at the animal pole a periodic twitching is recognized; red pigment spots are seen at the edge of the yolk; colour of the egg mass is dull orange ;RC, red chromatophore; (l-m) stage V: One third of the yolk is utilized; two eye spots have made their appearance; red spots prominent and seen at the animal pole; colour of the egg mass very dull orange; RC, red chromatophore; (n-o) stage VI: Egg mass brownish orange in colour; eyes are well developed; yolk is found in the vegetal pole; two thirds of the yolk cleared; red pigments are spread all over the white space; RC, red chromatphore; (p-q) stage VII: Egg mass pale grey in colour; colourless yolk in the form of oil globules seen just below the two eyes as two pockets; heart beat prominent; embryo almost completely developed. CY, colorless yolk.*

crab, the telson extends past the optic lobes.

Stage 8 (Diapause)

Yolk is divided down dorsal midline into left and right halves, as well as distinct anterior (pinkish) and posterior (orange) lobes (Fig. 5n-o). The embryo is crescent-shaped and wraps three quarters of the way around the yolk, covering the entire surface in ventral view. Growth rate of eye length slows down, and eye width levels off. Heartbeat becomes distinct.

Stage 9 (Eye Enlargement)

Embryo takes up >50% of egg in side view. Posterior lobe of yolk is visibly reduced, relative to anterior lobe (Fig.5p-q). Yolk lobes are clearly separated in dorsal view.

Stage 10 (Rapid Growth Phase)

Area, length and width of embryo increase rapidly. Dorsal edge of yolk is separated from the perimeter of the embryo case (Fig.5r-s).

Stage 11 (Yolk Depletion)

Area of yolk decreases rapidly, as other dimensions increase during this period of rapid growth. Maxillipeds are well defined and pigmented (this not pronounced in *H. pacifica*) (Fig.5t-u).

Stage 12 (Hatching)

Hatching starts. Embryo length and width, and eye length and width reach maximum values. Ommatidia develop a greenish yellow fringe, producing a "halo" effect around eye. Anterior and posterior regions of yolk become distinct, and the latter is reduced to individual lipid globules.

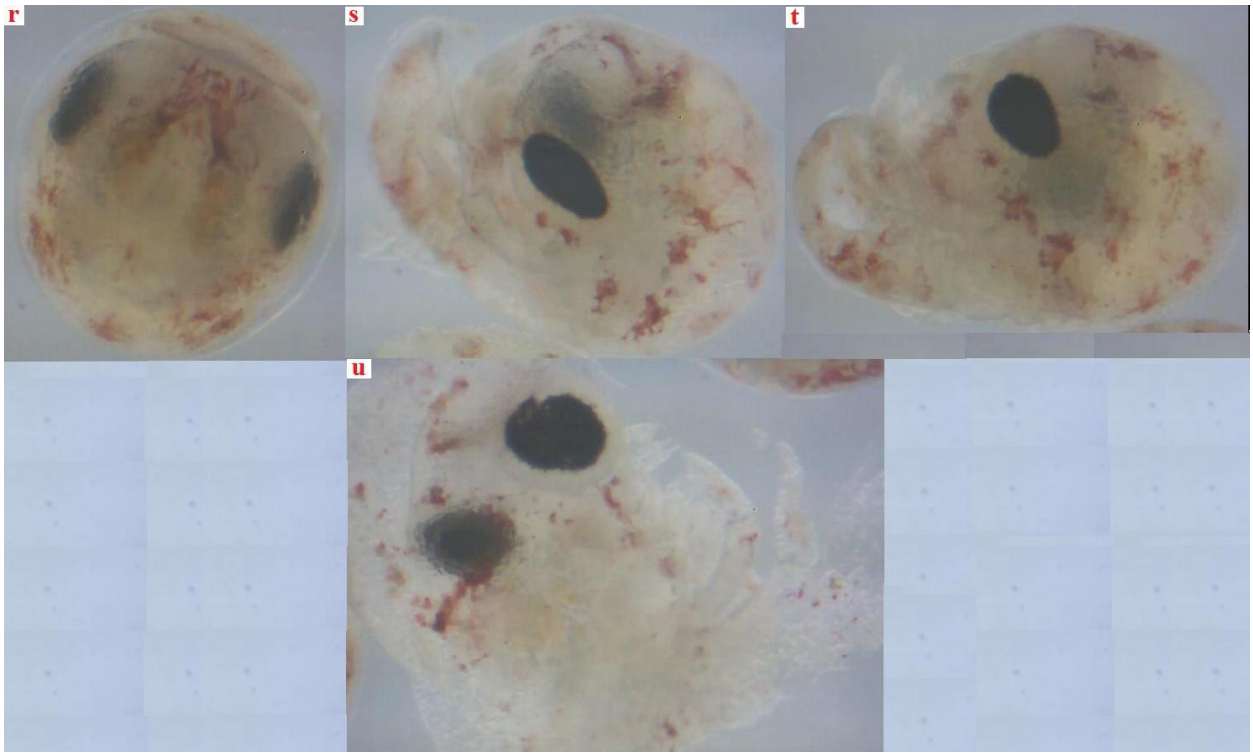

Figure 5. *continued. (r-t) stage VIII: Egg mass pale grey in colour; colourless yolk in the form of oil globules seen just below the two eyes as two pockets; heart beat prominent; embryo almost completely developed; (u) stage IX: Embryo fully formed; egg mass white in colour; no yolk globules seen; about to be released.*

4.0 Conclusions and Recommendations

The eggs of *H. Pacifica* Dana are isolecithal and filled with evenly distributed yolk mass. The cleavage is holoblastic. Egg development was classified into eight stages and a synchronous process as shown by the eggs that are from the same stages of development. Early egg developmental stages of *E. asiatica* and *H. pacifica* are almost the same. However, there are differences when developmental stages of *H. pacifica* were compared to that of the *P. playtus.* This may be due to the different environment where the embryos were grown. More studies are needed to determine the factors that can affect the egg development of this organism. Proportion of the various stages in the egg development of the berried females during different months of the year must also be studied. The reproductive cycles using more reliable methods such as gonad, digestive gland, and egg mass indices, as described by Subramoniam (1979) must be investigated. Furthermore, artificial insemination may be done to observe the egg development in the laboratory and to establish a standard criteria of its development.

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