UNIVERSITI PUTRA MALAYSIA

EMBRYONIC AND LARVAL DEVELOPMENT OF Macrobrachium neglectum (de Han, 1905)

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BSc. AQUACULTURE

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UNIVERSITI PUTRA MALAYSIA BINTULU SARAWAK CAMPUS**

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EMBRYONIC AND LARVAL DEVELOPMENT OF *Macrobrachium neglectum* (de Han, 1905)

By

HASYIMAH BINTI ZAINAL ABIDIN

A Project Report Submitted in Partial Fulfilment of the Requirement for the Degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural Science and Forestry Universiti Putra Malaysia Bintulu Sarawak Campus

2022

Dedicated to

My parent, Rosiah Binti Salleh@Ibrahim

My brothers and sisters

Nik syubrey, Mohd Haika, Abu Urwah, Noorhidayu, Rohani, Amirul, Amin, Ain

My close friends

Koh Sooi Seng, Alvin, Mujib

all my lecturers and fellow friends.

ABSTRACT

Macrobrachium neglectus de Man, 1905 is one of the freshwater shrimp's species that found within the river systems flowing in Universiti Putra Malaysia Campus Bintulu, Sarawak. Adult prawns can normally grow up from 10cm – 15cm in size. The nutritional requirements have thus far not been studied and standardized. Thus far there have been no scientific studies on this species regarding embryonic development and total larval stages. The objective of this study is to observe the embryonic and early larval development of *M. neglectum* (de Han,1905). *M. neglectum* eggs developed in 15 days under laboratory conditions. The embryo occupies all the available space inside the eggs, with traces of yolk remaining in most embryos. By day 10, the clear region, which developed into the trunk and caudal portion of the embryo, had taken up about two-thirds of the embryo mass. The embryo's eyes grew larger and oval after 11 days. *M. neglectum* larvae go through 11 to 12 moults from hatching through transformation into juveniles. The freshwater prawn *M. neglectum* has a high fecundity proportional to the size of its female specimen. It incubates its eggs for 141 days at 28°C, which is shorter than other species such as *M. rosenbergii* and *M. olfersi*. *M. potiuma* eggs can hatch in 0 ppt. *M. neglectum* eggs' colour ranged from dark olive to light olive to yellowish until visible color before hatching. *Macrobrachium* spp. produce larvae with oceanic salinity requirements (30-35 ppt). *M. neglectum's* larval characteristics suggest a closer relationship with *M. rosernbergii* and *M. lar*, both subtropical species. Mating, fertilisation, embryonic development and larval development all occur in freshwater in *M. neglectum*. Eggs hatch in salinity-free, 100% freshwater. A larva takes 10 days to metamorphose into a post-larva. More research is needed to increase larval survival.

ABSTRAK

Macrobrachium neglectus de Man, 1905 merupakan salah satu spesies udang air tawar yang terdapat di dalam sistem sungai yang mengalir di Kampus Universiti Putra Malaysia Bintulu, Sarawak. Udang dewasa biasanya boleh membesar dari saiz 10cm – 15cm. Keperluan pemakanan setakat ini belum dikaji dan diseragamkan. Setakat ini belum ada kajian saintifik mengenai spesies ini mengenai perkembangan embrio dan jumlah peringkat larva. Objektif kajian ini adalah untuk memerhati perkembangan embrio dan larva awal *M. neglectum* (de Han,1905). Telur *M. neglectum* berkembang dalam 15 hari di bawah keadaan makmal. Embrio menduduki semua ruang yang ada di dalam telur, dengan kesan kuning telur yang tinggal dalam kebanyakan embrio. Menjelang hari ke-10, kawasan jernih, yang berkembang menjadi bahagian belakang dan ekor embrio, telah mengambil kira-kira dua pertiga daripada jisim embrio. Mata embrio membesar dan bujur selepas 11 hari. Larva *M. neglectum* melalui 11 hingga 12 peringkat persalinan kulit daripada penetasan melalui transformasi menjadi larva. Udang air tawar *M. neglectum* mempunyai kesuburan yang tinggi berkadar dengan saiz spesimen betinanya. Ia mengeram telurnya selama 14 hari pada suhu 28°C, yang lebih pendek daripada spesies lain seperti *M. rosenbergii* dan *M. olfersi*. Telur *M. potiuma* boleh menetas dalam 0 ppt. Warna telur *M. neglectum* berjulat dari zaitun gelap kepada zaitun muda hingga kekuningan sehingga warna kelihatan sebelum menetas. *Macrobrachium* spp. menghasilkan larva dengan keperluan kemasinan lautan (30-35 ppt). Ciri-ciri larva *M. neglectum* mencadangkan hubungan yang lebih rapat dengan *M. rosernbergii* dan *M. lar*, kedua-dua spesies subtropika. Persenyawaan, perkembangan embrio dan perkembangan larva semuanya berlaku dalam air tawar untuk *M. neglectum*. Telur menetas dalam air tawar tanpa kemasinan, 100%. Seekor larva mengambil masa 10 hari untuk bermetamorfosis menjadi pasca larva. Lebih banyak penyelidikan diperlukan untuk meningkatkan kemandirian larva.

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APPROVAL SHEET

I certify that this research project report entitled "**embryonic and larval development of *Macrobrachium neglectum* (de han, 1905)**" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Science in Aquaculture with Honours in the Faculty of Agricultural Science and Forestry, Universiti Putra Malaysia Bintulu Sarawak Campus.

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CHAPTER 1

INTRODUCTION

Macrobrachium neglectum (de Han, 1905) origin Palaemon (Eupalaemon) neglectus de Man, 1905 is one of the freshwater shrimp's species that found within the river systems flowing in Universiti Putra Malaysia Campus Bintulu, Sarawak. *Macrobrachium Neglectum* (de Han, 1905) adult prawns can normally grow up from 10cm – 15cm in size. The females are usually smaller than the males, ranging in size from 7cm to 10cm in size. The color in life is normally dark brown, in both the males and females. In general, some *Macrobrachium* species develop more quickly and to larger sizes, while others may grow more slowly and to lower sizes. In the culture of the *Macrobrachium* species, where the males develop considerably quicker than the females, the difference in growth between the sexes is very concerning. Male development differences have negative effects on both culture and mate choice. The embryonic and larval systems of crustaceans provide a special and useful tool for comprehending both physiological regulation mechanisms and developmental processes. During the period of development, palaemonid females carry centrolecithal eggs in an external brood pouch (Soundarapandian P et al., 2014a). The nutritional requirements of *M. neglectum* (de Man,1905) has thus far not been studied and standardized, but knowledge on the nutritional requirements of other species in the genus *Macrobrachium* has been extensively studied for practical uses over the last decade. Prawns can digest a wide variety of foods, both from plant and animal origins. The nutritional requirements of the genus *Macrobrachium*, the majority of which are freshwater and brackish water species, is less well understood than those of marine shrimps. The nutritional requirements of freshwater prawn and shrimp in terms of protein, lipid, carbohydrate, vitamins and minerals have been investigated under controlled and semi controlled culture conditions. Studies on nutrition and feeding were primarily conducted on prawn and shrimp larvae and juveniles. This prompted researchers to investigate the nutritional needs of freshwater prawns and marine shrimps (Prasad Paturi Assistant Professor, 2018). Recently, the embryonic and larval development of *M. rosernbergii* was investigated (Banu & Christianus, 2016). However, no one has ever conducted a study like this on the prawn *M. neglectum*. Determining information about the embryonic development of the edible, unexploited prawn, *M. neglectum*, was the goal of the current study.

1.1 Justification

Macrobrachium neglectum (de Han,1905) is a species from the family Palaemonidae and genus *Macrobrachium*. It is a medium sized prawn that occurs in many freshwater streams of Borneo. Thus far there have been no scientific studies on this species regarding embryonic development and total larval stages of *Macrobrachium neglectum* (de Han,1905).

1.2 Objectives

The objective of this study is

(i) to observe the embryonic and early larval development of *Macrobrachium neglectum* (de Han,1905).

CHAPTER 2

LITERATURE REVIEW

2.1 Biology of Freshwater Prawn

A medium-sized freshwater prawn is known as *Macrobrachium neglectum* (de Han, 1905) males can grow to a total length of 11 cm (from the tip of the rostrum to the tip of the telson), whilst females can grow to a maximum of 9 cm. Typically, females are smaller than males and have fewer spines on the second pair of pereiopods. Their incubation chamber is generated by the abdominal pleura arching and lengthening, and they brood their eggs on the pleopods (Maciel & Valenti, 2009). The body is typically brown to dark brown, with light brown pigmentation covering the whole cephalothorax. Like all species of decapods, prawns have a body made up of two different parts: the cephalothorax and abdomen (Sharma, 2005). The smooth, rigid cephalothorax is covered by the carapace. This species has a relatively thin rostrum. 14–15 teeth are present on the dorsal rostrum, and 9–10 teeth are present below. The anterior carapace border has the hepatic spine. The cephalothorax region has five pairs of pereopods, or real legs. The abdomen is extremely clearly segmented, in contrast to the cephalothorax. This body has six segments, and each segment has a pair of swimmerets or pleopods on the ventral side. The telson functions as the tail fan, and the swimmerets of the sixth abdominal somite are firm and rigid. The telson is conical in shape and tapers gradually to a sharp tip. The very small posterior bristles on the telson do not protrude past the tip. The carpus of the second pair of pereopods extends beyond the scaphocerite, making them relatively lengthy. The dactyls are bristle-covered and 3/4 the length of the chelae. The chelae are long and have spinules along them that are widely spaced apart (Maciel & Valenti, 2009).

2.2 Growth and Development of Freshwater Prawn

A population's growth of individuals is typically quite random. Various genetic, social, and environmental factors are involved in the reasons. Variation in growth is a key factor in determining an individual's fitness because body size frequently influences reproductive output and survivability. The evaluation of life cycle strategies will depend heavily on how intraspecific interactions influence growth variation. Since it affects the catch in fisheries and aquaculture systems, variation in aquatic animal growth is also of practical interest (Ra'anan et al., 1991). According to the study, healthy broodstock can mature and be used for breeding within six months of being raised (Mohanta, 2000). Like other arthropods, shrimp grow by first releasing the exoskeleton's bonds with the living mass, or exoskeleton, and then fast consuming water to enlarge the new elastic cuticle and quickly harden it for body structure, motility, eating, and defence. According to individual size, shrimp undergo a diecdysis moulting cycle, which differs with the anecdysis cycle observed in crabs and lobsters, which exhibits longer intervals. Moulting has a reproductive purpose in addition to being a necessary step for growth throughout a shrimp's lifespan, allowing fecundation and the concomitant maturity of the gonads (reproductive versus non-reproductive growth-related moult). Moulting is necessary for growth as well as to get rid of scars, parasites, infections, damaged portions, and limb loss (Lemos & Weissman, 2021). The crustacean moulting cycle is typically divided into four phases: metecdysis, which is the time immediately following ecdysis; anecdysis, which is a time of tissue growth and food storage; proecdysis, which is a time of active morphological and physiological changes in preparation for the next moult; and ecdysis, which is the shedding of the old cuticle (Kamaruding et al., 2018).

2.3 Background of breeding

Among all species that successfully colonise new habitats are crustaceans. Crustaceans make up about 28% of the alien species found along North American coastlines (Ruiz et al., 2000). These animals' small size, especially in the juvenile stages, and their exoskeleton, which shields them from damage during transportation and other potential sources of physical injury, are only a few traits that have contributed to their success (Ruiz et al., 2000). Many organisms can also withstand significant changes in salinity and temperature. Additionally, because of many crustaceans' economic value, they have been purposefully introduced into several countries for commercial farming. Freshwater shrimps of the genus *Macrobrachium* have been prized as food for hundreds of years. Freshwater species with many of the characteristics listed above include the giant river prawn, which is traditionally referred to as *Macrobrachium rosenbergii* de Man, 1879. This species is a classic illustration of one that has spread throughout the world due to its success in commercial aquaculture (Iketani et al., 2011). For many years, catches of *Macrobrachium* from the family Palaemonidae have supported small local fisheries in Indo-Pacific coastal areas (Dugan & Publisher, 1975). In 1956, Thailand made the first attempts to artificially increase *Macrobrachium rosernbergii* production. It is comparatively well known how giant river prawn aquaculture has spread around the globe. The species' modern aquaculture started in the 1960s. Shao-Wen Ling found in 1961 that the larvae of this species needed brackish water to live while working at the Marine Fisheries Research Institute in Penang, Malaysia (Iketani et al., 2011). Young juvenile *Macrobrachium rosernbergii* were collected from the wild and stocked in earthen ponds in these early experiments. The yields from these early culture attempts were very low, but the results demonstrated that prawns of the genus *Macrobrachium* had good potential for aquaculture.

2.4 Aquaculture of Freshwater Prawn

In terms of determining the dynamic and structure of aquatic habitats, *Macrobrachium* species are crucial both commercially as a highly prized human food and ecologically (Ahmed et al., 2021). Generally, the fisheries sector has attracted considerable attention because of its huge export potential. Unfortunately, the freshwater prawn fishery statistics remain intermittent, incomplete and in many cases inaccurate, since the fisheries sector surveys do not distinguish between prawns and shrimps. There are more than 100 species of freshwater prawns in the genus *Macrobrachium*, which are widely spread throughout the tropical and subtropical zones. Although certain species, including *M. amazonicum* and *M. dayanum*, can finish their life cycle in inland salt and freshwater lakes, this genus mainly needs estuary conditions during the larval stages. Young *M. rosenbergii* move upstream in search of freshwater. Large adults are found in rivers as well as lakes, bogs, and irrigation canals, frequently far from the sea (New, 1990). One of the most important aspects of aquafarming is water quality management. It is critical to understand the different extremes and optimum levels of water quality parameters such as temperature, pH, dissolved oxygen, salinity, nitrogenous compound, hardness, hydrogen sulphide, and chlorine for the prudent management of freshwater prawn culture (Michael B New, 1995).

2.5 Nutritional Requirement

Nutrition. The primary determinant of how developmental processes in *P. vulgaris* are carried out was thought to be food. The organisation of developmental processes into a hierarchy based on how much dietary energy is used for maintenance activities, mainly for moulting processes at the expense of growth and morphological development. Additionally, under the impact of external conditions, the direction of energy flow may alter, potentially having various effects on the hormones considered to regulate moulting, growth, and morphological development (Dugan & Publisher, 1975). In general freshwater prawns of the genus *Macrobrachium* are omnivorous and coprophagous by nature. These prawns have been shown to prefer natural food over artificial feeds. Feed in the aquaculture of freshwater prawns under standard practice is initially given at 5-8 percent of the body weight per day for grow-out culture. The feeding rate decreases as the animals grow, peaking at 1.5-2 percent body weight gain per day when the animals reach about 20 g in size. Farmers typically feed the farmed prawn twice a day with diets containing protein levels of 20 to 35 percent. However, in ponds with sufficient natural food, the species thrives well even with feeds containing 15% dietary protein. Diets containing approximately 35-40% protein, a gross energy level of approximately 3.2 kcal/g diet, and a protein:energy ratio of approximately 125-130 mg protein/kcal are suitable for the growth of *Macrobrachium* in clear water systems devoid of natural foods. Broodstock raised in ponds with natural food (benthic micro- and macrofauna) require approximately 30% protein in their diet. Many commercial grow-out feeds contain 24-32 percent crude protein. A protein/starch ratio of 1:1 has been shown to improve feed efficiency and growth rate. The prawn requires the same ten essential amino acids as other crustacean and fish species, but the quantity required has not been determined. The prawn muscle's amino acid composition can be used to provide guidance values in feed formulation.

CHAPTER 3

METHODOLOGY

3.1 Study location

The research site is near a flowing river in UPM Campus Bintulu, Sarawak, Malaysia. The research was carried out at the stream with coordinates (3°12'30.3"N 113°05'32.7"E).

Figure 1. Map of UPM Campus Bintulu, Sarawak, Malaysia and study location.

3.2 Sampling procedure

Except for Taiwan, where broodstock is cultured by getting healthy egg-bearing females from hatcheries, most hatcheries use berried prawns that are captured in the wild (Mohanta, 2000). The sampling was done at the stream in UPMKB by using shrimp net with 8 holes. The commercial pellets were used as a bait. The samples will be collected the next day of sampling. The specimens that were collected were ranged from 5 to 15 cm in length. Totally 20 specimens were collected and transported to the virology laboratory of Department of Animal Science & Fisheries UPM Campus Bintulu in live condition by keeping them in bag bucket containing freshwater and aeration. After reaching the laboratory they were washed carefully with distilled water to remove the dust and algal particles. Figure 2 and 3 below shows the differences between male and females of *Macrobrachium neglectum*.

Figure 2. Adult male *Macrobrachium neglectum*.

Figure 3. Adult female *Macrobrachium neglectum*.

3.3 Laboratory procedure

3.3.1 Broodstock maintenance

The collected broodstock were placed in the fiber glass tanks (60 x 30 x 20 cm) filled with aerated tap water. Optimum temperature (27-28ºC) and dissolved oxygen (5ppm) was maintained in the brood tank. For about a month, mature male and female fish were kept together in a tank in a 1:3 ratio. Every day, commercial shrimp pellets were fed to the broodstock and the excess feed, excreta and shed out skin were siphoned out. Water parameters were measured on a weekly basis. Temperature was measured with a graduated thermometer, hidrogen ion concentration (pH) with a pH test kit (Habashy et al., 2012), and ammonia with an ammonia test kit. After a few weeks, the detected gravid females were separated and placed in a glass tank aquarium with water at 28°C and continuous aeration.

3.3.2 Embryonic development

The development of the eggs was closely observed every day. The colour and size of the prawn eggs were used to determine oocyte maturity. 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. Three oocytes that were extracted from berried female prawns using forceps at 24-hour intervals was examined under a microscope at 40x magnification. The oocytes' colour and morphology were observed and recorded. 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 stage. The gradual changes in the embryonic development and increase in the size of the eggs were recorded to understand the different developmental stages (Soundarapandian P et al., 2014b). The colour was dark olive the first week, and the colour began to change from olive to bright yellow and colourless embryo the second week.

3.3.3 Larval rearing

The tank was prepared before the larval was separated from the broodstock. The tank used for larval rearing was a (60 x 30 x 20 cm) tank with non-chlorine water, aeration, heater, thermometer, and filter to maintain water quality, with the filter components being zeolite, activated charcoal, and sponge to absorb ammonia contents in water. Once the first stage of larvae inside the eggs was fully developed, the larva was ready to come out of the eggshell to start active life (Soundarapandian P et al., 2014b). The process of hatching and development of larva was studied through 40X magnification of compound microscope. After the newly hatched larval were placed in the tank, 30% of the water was changed daily with aerated tap water, and newly hatched artemia and artificial feed were fed to the larval before changing the water. Temperature, pH, ammonia, nitrite, and nitrate levels were also measured daily and every 2-3 days. *Macrobrachium neglectum* larval stages were recorded daily until they metamorphosed into the post larvae (PL) stage.

CHAPTER 4

RESULTS

4.1 Egg coloration and morphology during embryonic development.

All developmental stages were considered for the analysis. *M. neglectum* eggs developed in 15 days under laboratory conditions (28.5 ±0.45 °C, 6.2 ±0.438 mg/l dissolved oxygen, 7.8 ±0.302 pH, and 0.33 ±0.045 mg/l ammonia). The egg volume of *M. neglectum* increases during development. In contrast to the colourless embryo, the yolk mass of the centrolecithal eggs was olive. The embryonic development of *M. neglectum* took 14(±1) days on average. The morphological features of the 15 days development were represented in figure 4 below. The eggs are slightly ellipti chal in shape and dark olive to bright olive in colour at first, then gradually change to bright yellow and colourless embryo a few days before hatching (figure 4). The transparent chorion securely encloses the oval, fertilised egg. There are no visible embryonic structures on the egg's surface. The dark olive, granular yolk mass is firm. The cell number of the fertilised eggs increased in the first two days after spawning. The blastopore area, which appeared on the egg surface, was observed to have high cell density. There are no other differentiated structures in (fig 4 day 1 and day 2). The appearance of several blastomeres on the surface of the egg is the first indication of embryonic development. The blastomeres are all the same size and have prominent nuclei. As the cleavage furrows deepen, they become more distinct. The number of blastomeres grows as cell divisions are repeated. When the blastoderm is organised, cell size gradually decreases (fig 4 day 3). On day 4, a distinct region at one pole of the embryonic mass was clearly visible (fig 4 day 4). On day 6, the clear region was lengthwise extended, forming the trunk of the developing embryo (fig 4 day 6). On the yolky mass, a pair of small dark eye spots appeared after 7 days (fig 4 day 7).

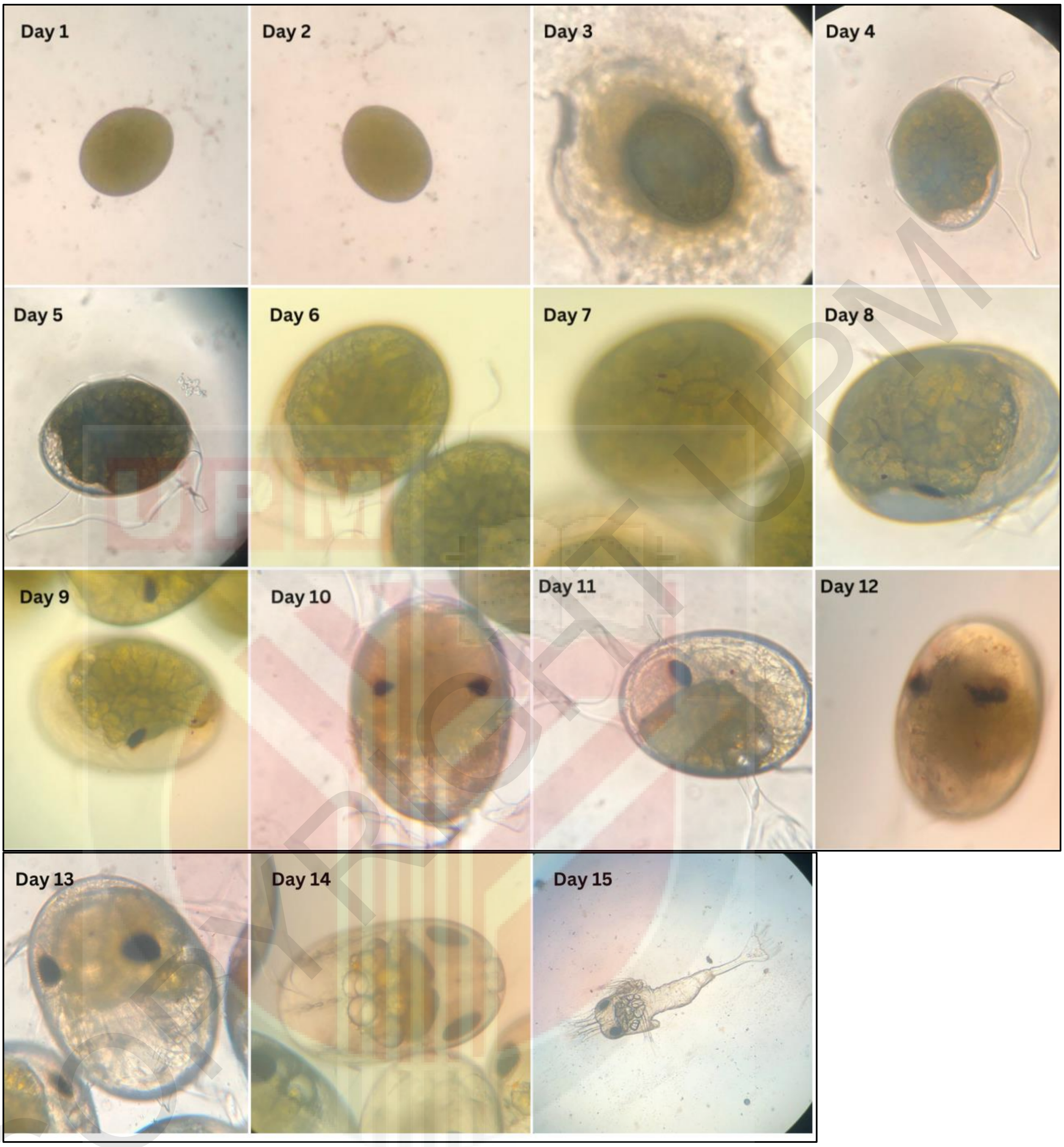

Figure 4. Embryonic developmental stages of *M. neglectum* observed under microscope.

By day 10, the clear region, which developed into the trunk and caudal portion of the embryo, had taken up about two-thirds of the embryo mass, and the caudal papilla had extended as a lappet of tissue across the median portion of the eggs (fig 4 day 10). The embryo's eyes grew larger and oval after 11 days. On a 12-day-old embryo, the appendages form beneath the clear trunk region and the eyes enlarge, become oval, and are surrounded by striation (Fig 4 day 12). On a 13-day-old embryo, the eyes were dark and rounded, striation was visible, and the translucent globules grew and occupied most of the dorsal area of the yolky mass, and the embryo was discovered to occupy all the available space inside the eggs, with traces of yolk remaining in most embryos. Appendage rudiments were also visible. On a 14-day-old embryo, telson and uropod rudiments unfolded, and a segmented abdomen appeared (fig 4 day 14). The eggshell breaks and the newly hatched larvae appear after hatching, which normally occurs at night and may last for 2-3 days (fig 4 day 15).

4.2 larval development

The larvae go through 11 to 12 moults from hatching through transformation into juveniles. Each moult from the first to the fifth produces a new larval stage; however, following the sixth stage, development becomes erratic, and each moult does not produce a new stage. Consequently, moulting without morphological change is possible (P. C. CHOUDHURY, 1969). Every day, the differences were identified under a compound microscope with a magnification of 40X. It takes 10 days for the larvae to mature into post-larvae. The morphological and different stages were recorded. There were six stages for early larval development of *Macrobrachium neglectum.*

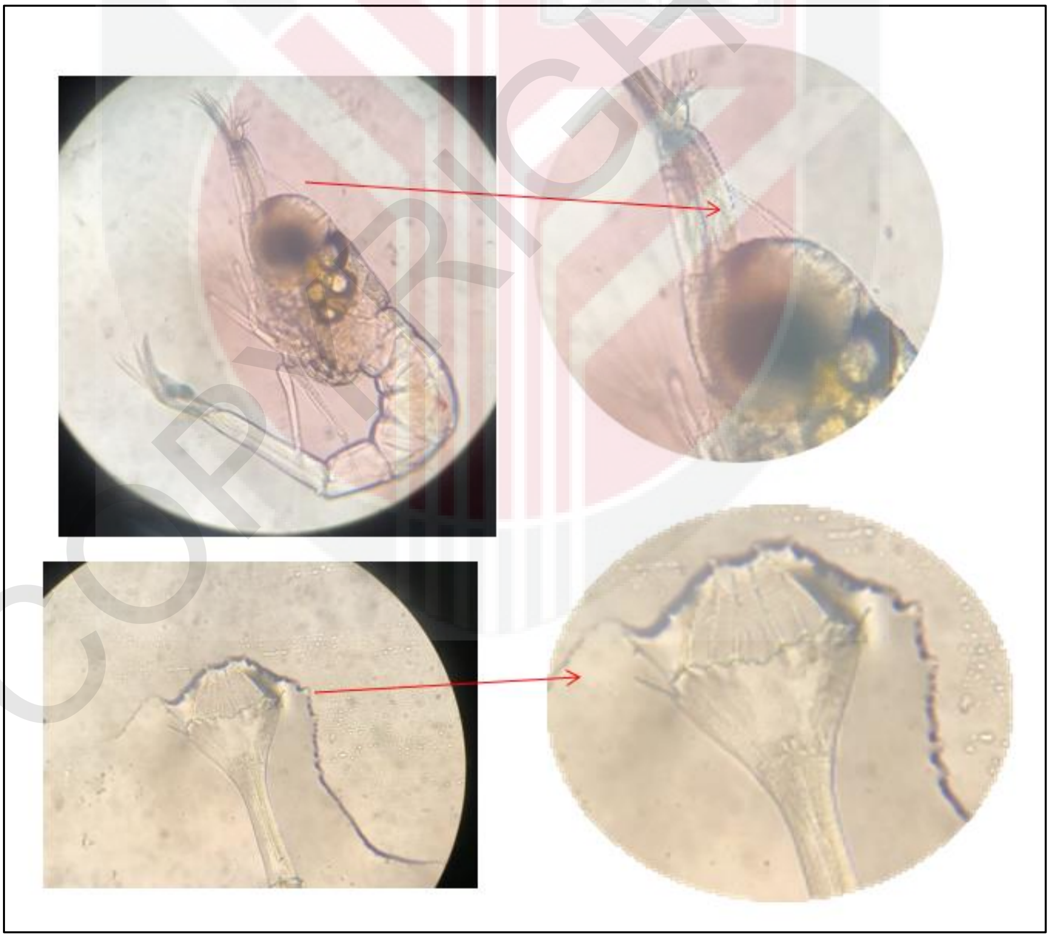

Figure 5. Larval stage: stage I.

Stage I

The larva's body was transparent and straight. On the antennular peduncle, there are three to four little red chromatophores surrounded by bluish pigment. On the front of the eye, there is a single little red chromatophore with lengthy dendrites. There is a large, dark red chromatophore with long dendrites on the back of the eye, where the eye meets the carapace. Abdominal segment 3's dorsal side is home to two pairs of tiny red chromatophores with tiny dendrites, the central pair being particularly close together. Blueish pigment is dispersed all throughout these chromatophores. The antennal peduncle and base of the second maxilliped both have light orange pigment. *M. neglectum* larvae have a short, straight rostrum that is toothless. The telson lacks uropods, has a roughly heart-shaped shape, and does not articulate, forming a solid join with the sixth abdominal somite. This stage lacks fully formed walking legs (pereiopods), with the first two pairs only present as buds. The first three pairs of maxillipeds are present, as are the eyes, which are sessile and located on the anterior half of the cephalothorax. The body is highly transparent, with lipid globules visible in the foregut and midgut. Antennule, peduncle unsegmented; flagellum unsegmented with 1 long plumose and 1 short no-plumose apical seta; exopod with 4 or 5 annulations on distal hal, 9 long plumose setae on inner side, 1 short seta on tip of outer margin, 1 plumose and 1 non-plumose setae on outer margin. Mandibles; left mandible with 1 large and 1 small tooth on the incisor part, 4 or 5 small teeth on molar part, right mandible with 1 large and 1 small tooth on the incisor part and 2 very small protuberances on molar part. Maxillule was very small palp prominent with 2 small spines at apex, proximal lacinia with 4 setae at tip, distal lacinia twice as large as proximal, 2 large and 2 small teeth at apex. Maxilla, scale with 5 long plumose setae on outer edge, proximal seta largest, endopod small with 1 long terminal plumose seta, 2 setae on the proximal half, protopod divided into 3 lobes, proximal lobe with 4 setae, 2 setae on each of the middle and distal lobes, all setae with 4 plumose setae at apex. Maxilliped 1, base almost round with 3 setae on upper margin, endopod very small, finger-shaped, with 3 terminal setae, exopod long with 4 plumose setae at apex. Maxillipeds 2 and 3, endopod 4 segmented, 1 long, finely serrated claw and 2 setae at apex, exopod long, 4 long plumose setae at apex. Pereiopods 1 and 2 was biramous buds. The telson triangular, posterior margin broad, slightly concave, notched in the middle, 7 pairs of stout plumose (2 lateral pairs plumose only on inner edge), many small stout spines in between setae. Most larvae did not consume feed on the first day. Most of the individuals were seen feeding on the second day after hatching. Freshwater prawn larvae are planktonic and swim tail first, ventral side uppermost.

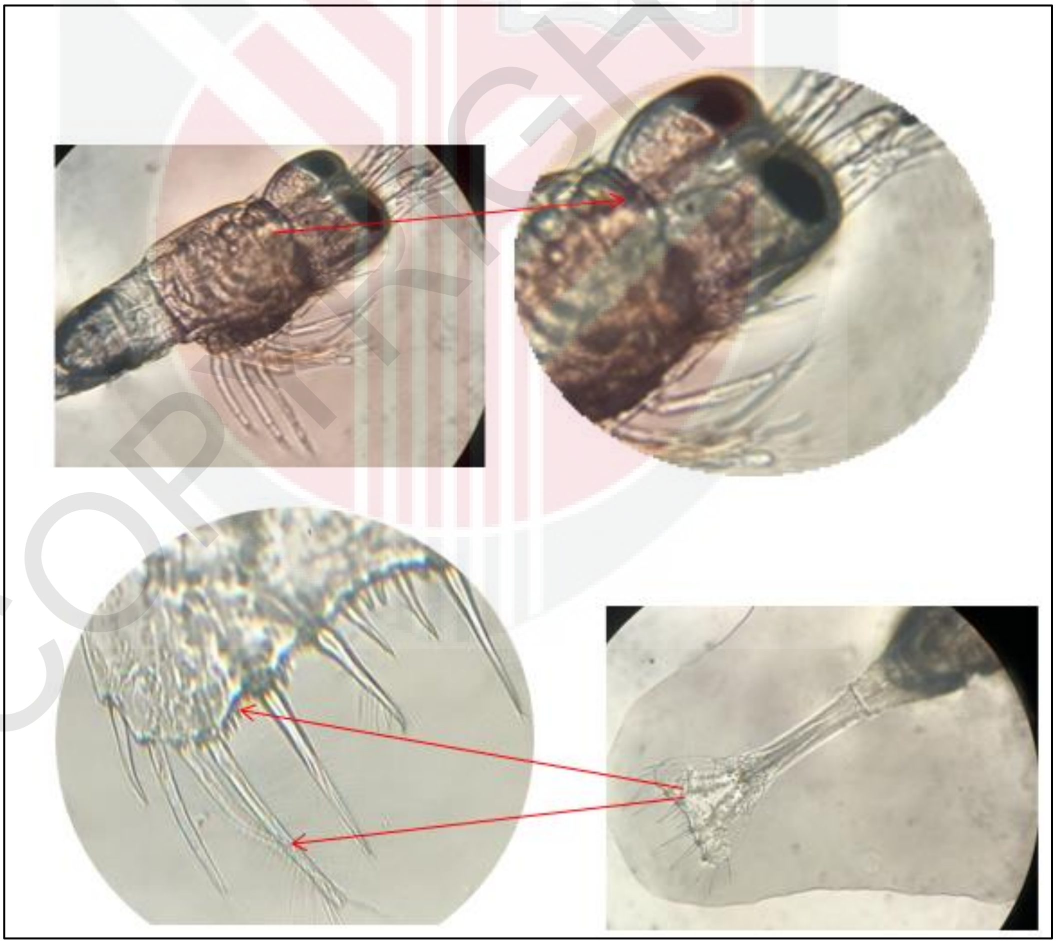

Figure 6. Larval stage: stage II

Stage II

The rostrum has remained largely unchanged since day one. The carapace develops supraorbital and branchiostegal spines also visible in this stage. The presence of stalked eyes, but not fully stalked, is one of the most noticeable features of this day. Also present but unrecognisable is the dorsal median spine. A join begins to form between the sixth abdominal somite and the telson, allowing partial articulation, and rudimentary uropod exopods may be seen forming within the telson, which will appear in the next stage. One additional pair of short plumose setae present at centre of the posterior edge, lateral pair 2 plumose on both edges of the telson. Although the antennal flagella are present, they are not segmented. For antennule, two-segmented antennular peduncle present. The first two pereiopods have emerged and resemble the third maxilliped. 1^{st} and 2^{nd} periopods become biramous and the 4^{th} exopod segmented but endopod unsegmented. The left mandibles with 2 large and 3 small teeth on incisor part, innermost tooth curved and serrated, molar part with 4 or 5 short, pointed teeth. Right mandible with 3 strong and 2 slender teeth on incisor part, innermost slightly serrated, molar part with 5 small, pointed teeth. Maxillule, proximal lacinia with 4 long teeth, 1 plumose seta a nd 1 non-plumose setae. For maxilliped 1, basal lobe with 6 setae, endopod with 3 terminal and 2 lateral setae. Figure 6 also show the rudiments of telson, and uropod presents.

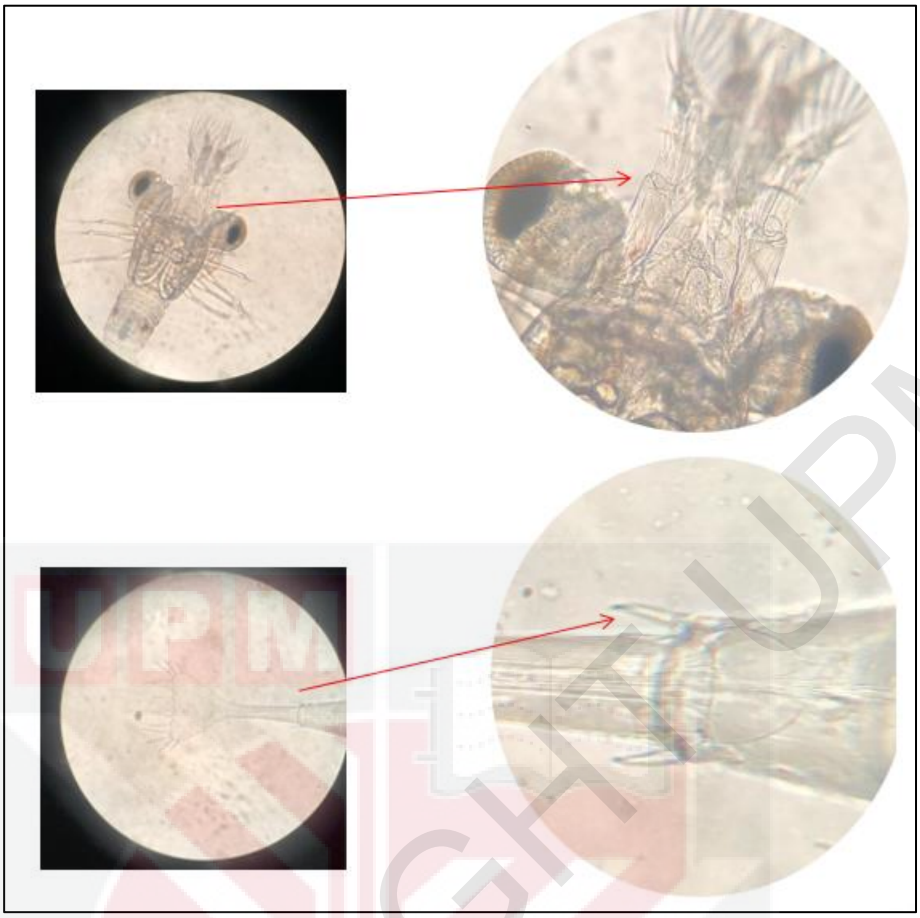

Figure 7. Larval stage: stage III

Stage III

The first tooth on the rostrum appears on the dorsal carina, immediately behind the eyes, and the pterygostomian spine develops two distinct points. One epigastric spine present behind the base of rostrum. Prominent red and yellow chromatophores with long dendrites present on dorsal side of eye stalk and on anterior margin of eye. The stalked eyes extended slightly. Antennular peduncle 3-segmented, middle segment with a bright red chromatophore. Antennal exopod bears 12 or 13 setae, 3 or 4 annulations confined to distal end, antennal flagellum 3-segmented with 3 setae at apex. Large uniramous buds of pereiopods 5 and small biramous buds of pereiopods 3 present. The telson more elongated, articulation between telson and abdominal segment 6 distinctly visible, lateral and central pairs of setae not plumose. Ventral spine of abdominal pleura was developed during this stage (figure 7).

Figure 8. Larval stage: stage IV

Stage IV

The rostrum more developed with rudiment of one dorsal tooth. The eyes also more extended. The fifth pereiopod becomes biramous and the setae developed at the ends.

This stage is often noticeably pigmented,with chromatophores distributed over various parts of the body. Antennal exopod with 15 or 16 plumose setae and 1 spine, no annulations on the distal end of exopod. Maxilla bears 8 pulmose setae on exopod. Pereiopods 5 uniramous, largest, segmented, with 1 long, curved terminal spine. Pereiopods 3 and 4 biramous buds, buds of pereiopods 3 larger than those of pereiopods 4.

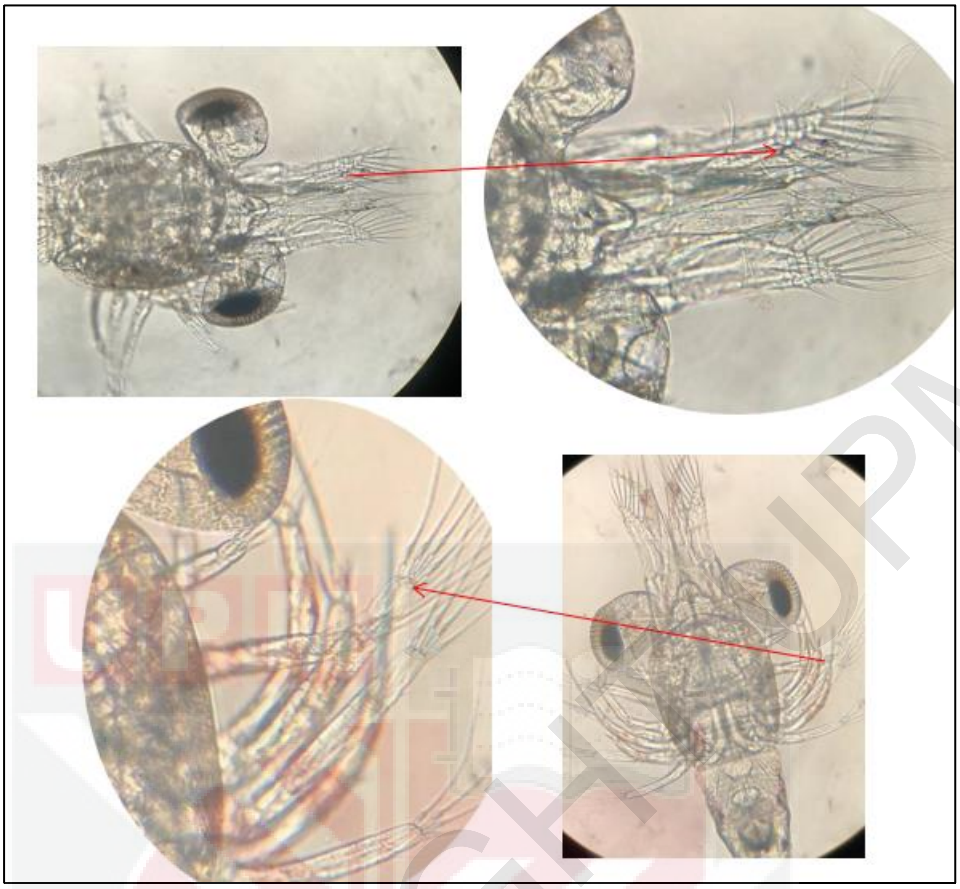

Figure 9. Larval stage: stage V

Stage V

There is no distinct change for carapace, rostrum and the eyes. Chromatophores on pereiopods 5 very prominent. Long red dendrites present in central pair of chromatophores on dorsal side of abdominal segment 3. Lateral pair on the same segment with yellow dendrites. Inner flagellum of antennulae elongated but non-segmented. Exopod of maxilla bears 9 plumose setae, middle lobe of protopod with 3 plumose setae. Endopod of maxilliped 2 with 4 segments and serrated claw at apex. Pereiopods 3 biramous like 1 and 2, pereiopods 4 biramous buds, pereiopods 5 largest and remain uniramous. The telson narrows, uropod partially developed.

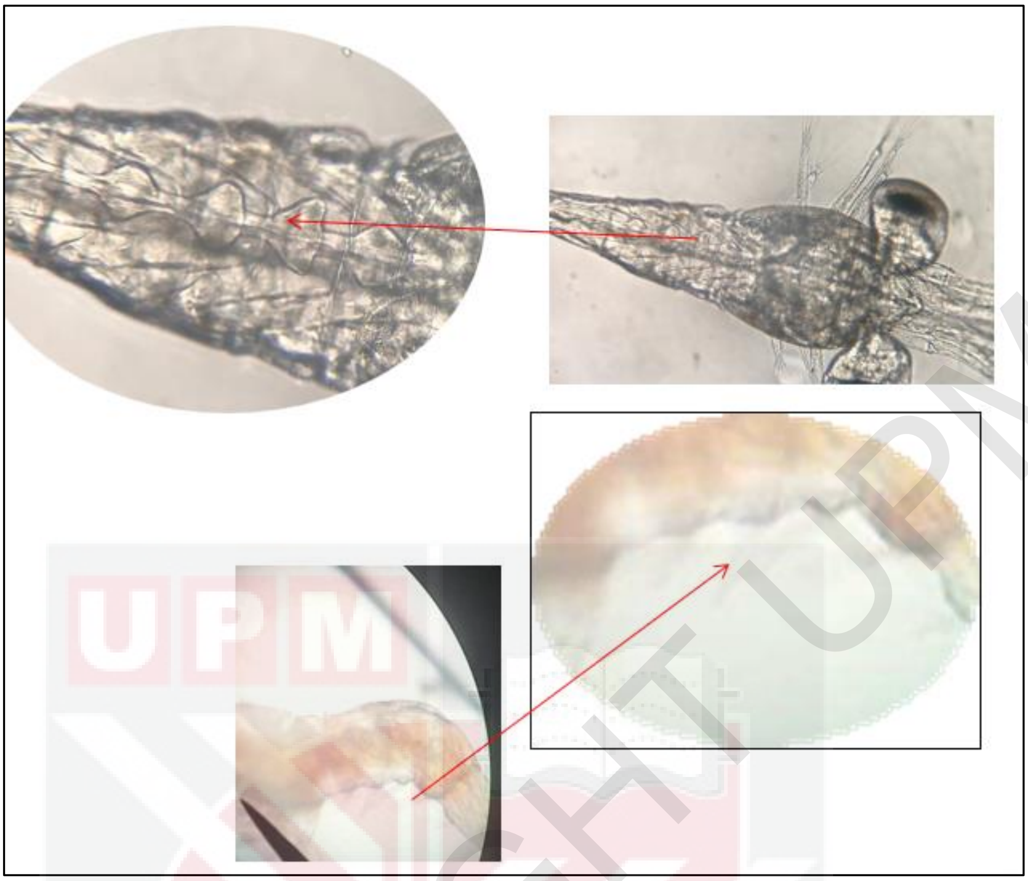

Figure 10. Larval stage: Post-larvae (PL)

Post-larvae (PL)

Characteristic of bright chromatophore with long dendrites present on segment 4 of pereiopods 5. Red chromatophore present on mid-ventral abdominal segments 3 and 4. Pleura of abdominal segments show up of six segments. The rostrum developed rudiments of 2nd dorsal tooth. The eyes slightly enlarged. Inner flagellum of antennule elongated, remains unsegmented and finger-like, outer flagellum splits at apex, inner part with 3 aesthetes at tip and the outer part with long non-plumose seta. Antennal flagellum as long as scale. Exopod of maxilla with 11 or 12 plumose setae, 2 setae pointed backward. Maxilliped 1 with 1 epipodite at the base. All 5 pairs of pereiopods present, pair 4 smallest, all biramous except pereiopods 5. Pleopod buds present as small protuberances, pairs 2, 3 and 4 more developed than pairs 1 and 5.

CHAPTER 5

DISCUSSION

5.1 Embryonic development

The females of *Macrobrachium spp*. incubate their embryos. It occurs frequently in several decapod crustaceans and is carried out by pleopods (swimmerettes) of the abdomen till hatching. The embryo is protected from physical and chemical stimuli at this time by its investment coats (egg coats, egg envelopes), which also help to maintain the interior environment. Due to its initial exposure to the aquatic environment, the outer investment coat is crucial in this capacity (Soundarapandian P et al., 2014b). The current study's findings provide information on the embryonic and larval development of the freshwater prawn *M. neglectum* in captivity. *M. neglectum* has a high fecundity proportional to the size of the female specimen. This variation in fecundity can be linked to female age and reproduction capacity (Graziani et al., 2003). However, the female fecundity of *M. neglectum* was lower than that of the majority of *Macrobrachium* species. This is most likely due to *M. neglectum*'s small size, which allows less space in their cephalothoracic cavity for ovocyst growth during ovarian maturation. The mean incubation time for *M. neglectum* eggs (141 days at 28°C) was shorter than that of other *Macrobrachium* species such as M. rosenbergii (20 days at 28.5°C) (Habashy et al., 2012) and *M. lar* (29 days at 28°C), but most common with *M. olfersi* (14 days at 26°C) (Yara Müller, 2004). This is because their eggs are smaller in size than those of other *Macrobrachium* species, and the embryonic development duration is determined by factors such as height. Furthermore, the difference in egg incubation time between *Macrobrachium* species may be due to the temperature of the incubation medium (Habashy et al., 2012). The eggs grew during the embryonic process, and their volume increased as embryos developed. This increase in egg volume could be related to osmotic water absorption to ensure cell mobility, structural organisation, and biochemical composition of eggs. In terms of hatching rates, the eggs can hatch in 0 ppt, indicating that they do not require salinity to hatch like other *Macrobrachium* species like *M. rosernbergii* and *M. macrobrachion.* According to (Soundarapandian P et al., 2014b), *M. idea* eggs' colour ranged from greenish opaque to light green to brownish yellow to dull whitish. The egg's colour altered during development from brown to grey as the yolk was consumed and the embryo's outline became visible, while *M. neglectum* eggs colour ranged from dark olive to light olive to yellowish until visible color before hatching. Egg sizes and development times are comparable *among M. olfersi, P. pandaliformis, and P. argentinus* species. On the other hand, *M. potiuma's* large eggs allowed for a more extended embryogenesis, which leads to the development of more complex structures. *M. potiuma* also had the longest time of development (Yara Müller, 2004). The length of the embryonic periods in this investigation were identical, demonstrating the need for a set amount of time to organise the embryo's traits.

5.2 Larval development

Even in the presence of aeration (without light), the larvae exhibited a more benthic habit, in contrast to *M. rosernbergii*, where healthy larvae without aeration remain near the water surface. This is consistent with the findings of (Lal et al., 2014), who stated that larvae occupied the upper portion of the water column but were not directly associated with the surface. This could be related to predator avoidance and the use of subsurface currents for larval transport out of coastal waters during dispersal, and it could be important for providing feed in culture where larvae can easily access it. Cannibalism was not observed during this study, though it cannot be ruled out completely, whereas *M. rosenbergii* has been documented for this (Graziani et al., 2003). Several *Macrobrachium* spp. produce larvae with oceanic salinity requirements (30-35 ppt). *M. neglectum*, on the other hand, can hatch and produce larvae at 0 ppt and can survive until it becomes a post-larva. *M. neglectum*'s larval characteristics suggest a closer relationship with *M. rosernbergii* and *M. lar*, both subtropical species. The first stage of these species is morphologically like that of uniramous pereiopods, biramous pleopods, and rounded telsons. Individual variation in developmental patterns has previously been written off as a simple laboratory artefact. However, this phenomenon has now been observed in a wide range of taxa of Decapoda and other crustaceans, and it has been linked to genetic or hormonal factors, as well as fluctuations in environmental variables (in these situations, phenotypic plasticity) (Marco-Herrero et al., 2019).

CHAPTER 6

CONCLUSION

Mating, fertilisation, embryonic development, and larval development all occur in freshwater in *M. neglectum*, but the eggs only hatch in salinity-free, 100% freshwater. With a few exceptions, such as variations in egg colour, embryonic development took 15 days until hatching, as it does in the majority of *Macrobrachium* genus species. According to the findings of the study, a larva takes 10 days to metamorphose into a post-larva, and more research is needed to increase larval survival and shorten the time it takes for development to occur in order to ensure feasibility. However, this study offers the first ever account of the full larval development of *M. neglectum* along with morphological descriptions, both of which are essential resources for its and maybe other related species' successful larvae rearing.

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