Research Papers Submitted to the 25th Anniversary of Sagaing University of Education

Sagaing University of Education

FORWARD

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It has been producing thousands of teachers for Basic Education for 25 years. Teacher educators in Sagaing University of Education not only teach students to become well-qualified teachers but also upgrade themselves and do researches to find better means and ways for future education.

This is the compilation of research papers read at the ceremony of paper reading session in honour of Sagaing University of Education's 25th Anniversary.

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November, 2017.

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Contents

COMPARISON OF EMBRYONIC STAGES AMONG THE FRESHWATER PRAWNS *Macrobrachiumminutum* (J. Roux, 1917),*M. lamarrei* (H.Mile-Edward, 1837) AND *M. lanchesteri* (De Man, 1911)

Khin Khin Hla[1] & Nwe Nwe Khaing[2]

Abstract

A comparative study on the fecundity, morphology of eggs, incubation time and embryology of three species of *Merobrachium*, namely *Microbrachiumminutum*, *M. lamarrei* and *M. lanchesteri* was conducted.The incubation time was 18th days for *M. lanchesteri*, 16th days for *M. minutum* and 15th days for *M. lamarrei*, respectively. The rate of yolk metabolism varies from species to species according to time taken for embryonic development. Cleavage was meroblastic, and blastula regular. The embryonic development showed a sequence of morphological changes that passed through eight common embryonic stages. The embryonic characters were classified as naupilar (stage I to VIII). The longest time of the embryogenesis was recorded in *M. lanchesteri*. The post-naupliar period was also longer than the other two periods.

Key words: *Macrobrachiumminutum,Macrobrachiumlamarrei, Macrobrachiumlanchesteri*, Embryonic

Introduction

Freshwater prawns of the genus *Macrobrachium* (Decapoda, Caridea, Plamonidae) constitute one of the most diverse, abundant and widespread groups of crustanceans. They are known to be extensively distributeded across tropical and sub-tropical regions worldwide, and comprise over 200 described species (Murphy and Austin, 2004).

Studies of crustacean population fluctuation must include techniques for assessing environment impacts such as climate change on reproduction, including embryonic development rates, diapause and irregular embryogenesis. Previous studies of embryo development in decapods have relied on traditional visual technique to define development stages (Moriyasu and Lanteigne 1998; Yamaguchi, 2001).

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Crustaceans are particularly suitable research subjects because of their external and easily accessible embryos. Morphometry of embryos has been used to describe development stages in the freshwater prawn *Macrobrachiumborlii* (Lavrias et al., 2002).

Crustaceans with yolk eggs (centrolecithal eggs) present meroblastic or partial cleavage. The large amount of yolk triggers a delayed embryonic development that results in future structuring of embryonizednauplius (also called egg nauplius), with the formation of paired appendages, growth of caudal papilla and organization of appendage in the post naupliar region (Helluy and Beltz, 1991; Scholtz, 2000). Das (1935) and John (1947) having studied the development stages in *Palaemonlamarrei and Palaemoncarcinus* respectively.

The present study undertaken as part of a larger study on the embryonic development of three freshwater prawns (*Macrobrachiumminutum, M. lmarrei* and *M. lanchesteri*) during cultivation in the laboratory and studied based on embryonic character support a close affinity to malacostracan crustacean.

Materials and Methods

Study Area and Study Period

The prawn specimens were made available from Kandaw Gyi Lake, (Chan Mya Thar Zi Tonwhsip) Fig. 1. The specimens were transported to the laboratory of Zoology Department, University of Mandalay. The study period lasts from June 2011 to February 2012.

Fig. 1.Location Map of KandawGyi Lake

Preparation of Specimens

Males and female of each species were reared in the laboratory aquaria. They were identified based on Holthuris (1980) and Cai and Ng (2002) (Plate I). Males and females were released into the aquaria (120 ×90 ×70cm) in which constant aeration was provided and fed daily with pellet food to obtain the ovigerous females. Berried females, were detected, placed in separate glass aquaria (75 ×60 ×45cm) with continuous aeration and observed daily during incubation period. Temperature was recorded daily.

A. Macrobranchium mirnatum

B. Macrobranchium lamarri

C. Macrobranchium lanchesteri

Plate I (A, B, C)

Determination of Embryonic Development

Samples of eggs were obtained after spawning at 24h intervals. At least ten live eggs were removed from each berried females daily. Eggs were placed on a glass slide examined under compound microscope. Digital photographs of live embryos were taken with DP-12 Image Analizer throughout the incubation time. Egg features were observed and noted in live specimen based on nomenclature described by Anderson

(1982). A classification of the stages of embryonic development was determined by noting the quantity of the yolk, the space occupied by the embryo, and the appearance and subsequent growth of the eyes, appendages and other structures by staging method as proposed by Sandeman and Sandeman (1991). Embryonic development was divided into eight stages.

Results

Pre-naupliar Period

Stage I: Day 1 and Day 2

The fertilized eggs of *M. minutum*, *M. lamarrei* and *M. lanchesteri* were globular in shape and filled with yolk mass (YM). The embryonic structures were not visible on the surface of the egg and different yolk granules were observed on day 1.On day 2, some egg yolks may start to split into small droplets (SD) where cleavage can occur. The cleavage furrows (CF) were found on the surface of the egg. They yolk occupied the central position in the blastula (Plate IIA, D and G).

Stage II: Day 3 and Day 4

On day 3, blastomeres (BL) were clearly seen on the surface of the egg. The cleavage furrow do not reach the central yolk mass. These furrows were deeper in *M. minutum and M. lamarrei*, but shallow cleavage furrow appeared in *M. lanchesteri*. The rudiment of embryonic region increased in size namely, a stout cephalic lobe infront and a narrow and elongated thoracio-abdominal lobe behind with prominences of appendage bud was well developed in *M. minutum* and *M. lamarrei* (Plate II B and E). These structures were not developed in *M. lanchesteri*, (Plate II H).On day 4, gastrulation took place on the light patch of egg. A germinal disc was formed and the six primordial structures (PS) were clearly seen in *M. minutum*and*M. lamarrei*(Plate II C and F). However, these structures were slightly prominent in *M. lanchesteri* (Plate II I).

Naupliar Period

Stage III: Days 5 and Day 6

On day 5, a total of eight prominent embryonic structures were clearly seen on the embryonic region. The first and second maxillae (MX)

and maxillepeds (MXP) were formed between the mandible and the thoracico-abdomenal lobe (Plate III A and D). But eight embryonic structure well not clearly seen on *M. lanchesteri* (Plate III G).On day 6, in both *M. minutum* and *M. lamarrei* optic lobe appeared very conspicuously and antennulae and antennae were directed posteriorly (PlateIII B and E). However *M. lanchesteri* was not clearly seen on optic lobe (Plate III H).

Stage IV: Day 7 and Day 8

On day 7 and 8, the embryo of *M. minutum* and *M. lamarrei*superficially grows and follows the long axis of the egg. The optical lobes (OL) are prominent features in the embryo and the eye pigment became darker (Plate III C and F).The optic lobe are prominent but the eye pigment slight darker in *M. lanchesteri*. (Plate III I). On day 8, the carapace (C) has developed and naupliar appendages were elongate and tubular. The caudal papilla (CP) was curved upward (Plate III C, F and I).

Post-naupliar period

Stage V: Day 9 and Day 10

On day 9, in *M. minutum* and *M. lamarrei* eye became oval in shape and larger. The antennule and antenna have become more tubular, elongated backwards and lie parallel to each other (Plate IV A and D). *M. lanchesteri*eye became slightly oval in shape (Plate IV G).On day 10, the heart vessel formed and pulsated at regular intervals. The yolk has diminished to more than half its original volume. Abdominal somites become visible. The thoracic leg rudiments increased in size (Plate IV B and E). But *M. lanchesteri*eye became slightly oval in shape and its yolks diminished to nearly 65% its original volume. (Plat IV H).

Stage VI: Day 11 and Day 12

On day 11, *M. lanchesteri*cannot clearly seen the margin of this optic lobe. The eye of *M. minutum* and *M. lamarrei* appeared thin, dark red pigment line appeared in the margin of the optic lobe. Pigmented granules scattered around the eye pigmented area. On day 12, the post-naupliar appendages grew with setae and were projected on the caudal papilla in *M. minutum* and *M. lamarrei*. (Plate IV C and F).The rudiment post-naupliar appendages were found at the abdominal somites and the heard pulsated at regular intervalon*M. lanchesteri*(Plate IV I).

Stage VII: Day 13 and Day 14

On day 13, the yolk highly reduced and decreased to 25% of the egg volume of *M. mirutum* and *M. lamarrei*. The eye was almost spherical. Telson has extended beyond the anterior end of the embryo and curved upwards M. *lanchesteri* decreased to 40% of at volume On day 14, the eyes became larger and more oval, protruding beyond the cephalo-thorax. Thoracic appendages folded towards abdominal region with distinct chelae (Plate V A and C). In M. *lanchesteri*, the heart beat continuously and regularly Appendages suddenly spasm.

Stage VIII: Day 15 to Day 18

On day 15, embryo of *M. lamarrei*increased in size and remaing yolk occupied dorsally. Eyes were completely differentiated into cornea and oval-shaped retina. The abdominal region has five distinct segments, the last one being the longest. At this stage the embryo was ready for hatching (Plate VD). In *M. mirutum* the heart beats regularly and all appendages showed jerky movement. Embryo occupied 80% of egg in this period. On day 16, embryo occupied 90% of egg space. The yolk granules were found inside the mid gut. The chromatophores were present in cephalic and abdominal region. The embryo hatches on this day in *M. mirutum*(Plate VB).

On day 17,the yolk of *M. lanchesteri*was highly reduced. Two anterior and two posterior lobes appeared on the dorsal portion of egg. The yolk mass decreased to 30% of the egg volume (Plate V E). All the embryonic characters were completely developed and the yolk of embryo reduced to 25% of egg volume. The embryo showed occasional jerky movements which become more frequent near the time of hatching. The embryo hatches on 18 day in *M. lanchesteri* (Plate V F).

Comparison of embryonic development among three *Macrobrachium* species

Microbrachiumminutum

The egg takes 16 days for development. The yolk area about 100% and dark green in color on one day in the egg. The colour turned pale transparent during the late stage. Eye pigmented appeared as crescent on day 6 and the heart vessel pulsate on 10 days was found.

Microbrachiumlamarrei

The egg takes 15 days for development. The yolk area about 100% and dark green in color on one day. The color turned pale transparent during the late stage. Eye pigmented appeared as crescent on day 6 and the heart vessel pulsate on 10 day was also found similar to *M. minutum*.

Microbrachiumlanchesteri

The egg takes 18 days for development. The yolk area about 100% and dark green in color in one day in the egg. The color turned pale transparent during the late stage. Eye pigmented appeared as crescent on day 7 and the heart vessel pulsate on 12 day was found.

A. *M. minutum* (2 day)

B. *M. minutum* (3 day)

C. *M. minutum* (4 day)

D. *M. lamarrei* (2 day)

E. *M. lamarrei* (3 day)

F. *M. lamarrei* (4 day)

G. *M.lanchesteri* (2 day)

H. *M.lanchesteri* (3 day)

I. *M.lanchesteri* (4 day)

Plate II (Pre-naupliar Period)

A. *M. minutum*
(5 day)

B. *M. minutum*
(6 day)

C. *M. minutum*
(8 day)

D. *M. lamarrei*
(5 day)

E. *M. lamarrei*
(6 day)

F. *M. lamarrei*
(8 day)

G. *M. lanchesteri*
(5 day)

H. *M. lanchesteri*
(6 day)

I. *M. lanchesteri*
(8 day)

Plate III (Naupliar Period)

A. *M. minutum*
(9 day)

B. *M. minutum*
(10 day)

C. *M. minutum*
(12 day)

D. *M. lamarrei*
(9 day)

E. *M. lamarrei*
(10 day)

F. *M. lamarrei*
(12 day)

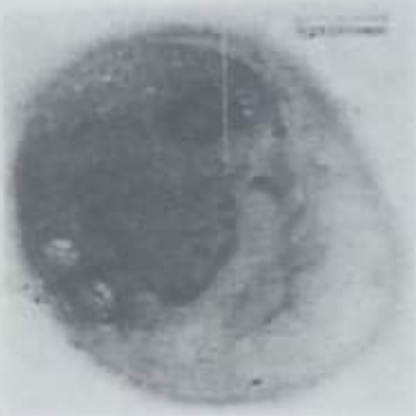
G. *M. lanchesteri*
(9 day)

H. *M. lanchesteri*
(10 day)

I. *M. lanchesteri*
(12 day)

Plate IV (Post-naupliar Period)

A. *M. minutum*
(14 day)



B. *M. minutum*
(16 day)

C. *M. lamarrei*
(14 day)

D. *M. lamarrei*
(15 day)

E. *M. lanchesteri*
(17 day)

F. *M. lanchesteri*
(18 day)

Plate V (Post-naupliar Period)

Discussion

The quantity and disposition of yolk in the eggs of different crustacean species is closely related to cleavage and embryonic development patterns (Anderson, 1982 and Fioroni, 1992).

In this study, the incubation period of the three prawns studied was different to the species. *M. lanchesteri* showed the longer development time than the other two species, mainly due to the longer post-naupliar period. Moreover the amount of yolk present in *M. lanchesteri* was more than other two species at stage VIII according to Helluy and Beltz (1991), the large amount of yolk trigger a delayed embryonic development time.

Wear (1974) stated that the order of appearance of embryonic larval appendages and other morphological features did not vary from species to species, although there was some variation between species in the percentage of the original yolk volume metabolized relative to the degree of development of the embryo. At constant temperature, the rate of yolk metabolism during the greater part of embryonic development varies only slightly in the eggs.

Rajyalakshmi (1960) indicated the main points of different embryonic history of *Palaemonrudis*, *P. mirabilis* and *P. scabriculus* were indicated. The complete incubation period was 17-19 day for *P. murabilis* at 25 to 26°C. The ventral plate and embryonic rudiment appeared by fourth and fifth day. The pulsation of heart vesicle can be seen on the 10th day and the eye streak appeared on 9th day, respectively. Muller *et al.* (2004) studied the different incubation time of four species of family Palaemonida maintained at 26°C, such as 14 days for *M. olfersi*, 12 days for *Palaemon argentines*, 13 days for *P.pandaliformis* and 21 days for *M. potuna*, respectively.

This finding agrees with the present study, the different incubation times were obtained from spawning to hatching, such as 16 days for *M. minutum*, 15 days for *M. lamarrei*, and 18 days for *M. lanchesteri* at 26-28°C. The germinal disk and primordial embryonic rudiment were found on 4th day in *M. minutum* and *M. lamarrei* but these structures were also found on 5th day for *M. lanchesteri*. The streak of eye pigment became visible on 6th day for *M. minutum* and *M. lamarrei*, however, *M. lanchesteri* appeared on 7th day. Regular pulsation of heart can be seen by the 10th day in *M. minutum* and *M. lamarrei*.

In this study, the cleavage furrows was observed on the surface of the whole eggs, however the furrow were shallow and do not reach the central yolk mass in three studied species. The cleavage furrow and blastomers was seen in stage II, followed by the organization of embryonic nauplius appendages such as antennae, antennulae, antennulae, mandible and the formation of caudal papilla in stage III and IV. In *Macrobrachium* species, including *M. americanas*, all structures appeared invariably in a similar sequence and follow matching patterns (Garcia-Guerrero and Hendrickx, 2009).

The next stage was the post-naupliar period, which is far longest period on studied species. The slow beating of heart occurred in stage V and VI. The bending of caudal papilla, eye with dark pigmentation, yolk highly reduce, naupliar and post-naupliar appendage with setae become appeared in stage VII. The formation of abdominal segment, visualization of ommatidian and chromatophore can be seen in stage VIII. This result is similar to Muller *et al.* (2004), who studied the external embryonic development of four species of Palaemonid prawns, the prenaupliar and naupliar periods are faster due to the organization of less complex embryonic structure. The post naupliar period is longer because the structure have to be finalized and to acquired functionaly before hatching. Wear (1974) observed that in each species, yolk is metabolized more slowly during early embryonic development (egg percentages) and a little more rapidly during the last few days before hatching.

The embryonic development of the three species considered was similar, with yolk cleavages followed by invagination, gastrulation, formation of a tissue cap and subsequent stages recognized as nauplius and metanauplius both relegated to embryonic life as in all Decapoda and hatching as a zoeal stage in development (Gurney, 1942). Therefore, it is assumed that no significant difference would be expected in the embryonic development of all *Macrobrachium* species.

Many workers have divided the crustaceans egg stages based on the appearance of distinctive morphological features such as the eye, heart beat and appendage formation. However, such morphological characters only being to appear mid way during embryonic development (Samuel and Sourdarapandian, 2009). With respect to the changes in egg volume during incubation by Mashiko (1982) stated the stage of eye pigmentation in the embryo was lower than 20% of the initial value measured soon after

spawning. This degree of increment before eye pigmentation was approximately in most of the other *Macrobrachium*species.

The eye stage as crescent was found in *M. minutum* and *M. lamarrei*on day 6 whereas *M. lanchesteri* on day 7. Appendage formation start to appeare on day 7 found *M. minutum* and *M. lamarrei* but, *M. lanchesteri* was also found on day 8. The heart vessel pulsate appeared on day 10 was found in *M. minutum* and *M. lamarrei* however, *M. lanchesteri* was also found on day 12 espectively. These findings agreed with those of authors cited above.

The external description of the embryology of *Macrobrachiumminutum*, *M. lamarrei* and *M. lanchesteri* could be useful for future studies dealing with its ontogeny, including the use of histological techniques that will provide information on the development on internal organs.

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