# OVARIAN DEVELOPMENT IN THE FRESHWATER PRAWN MACROBRACHIUM JOHNSONI RAVINDRANATH, 1979 (DECAPODA, PALAEMONIDAE)

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## Abstract

A study on the characterization of the ovarian development in the freshwater prawn *Macrobrchium johnsoni* was conducted. The specimens were from Ayeyarwady segment of Myinmu Township. A total of 1621 specimens (550 ovigerous females and 1071 non-ovigerous females) of *M. johnsoni* were collected. A total of 20 female prawns were randomly chosen to study the reproductive biology. Four stages of ovarian development were recorded; Stage I ovary (Predeveloping), Stage II ovary (Developing), Stage III ovary (Maturing) and Stage IV ovary (Ripening) according to both morphological and histological observations. The results indicated that freshwater prawn *M. johnsoni* can spawn the whole year round according to the record of occurrence of ovigerous females, the fluctuation of GSI values and occurrence of oogonia and maturing oocytes throughout the study period. It was assumed that the reproduction of the study species may be continuous type. The present study provides important information on the reproductive biology of crustaceans in general and also can serve as a significant foundation for aquaculture of a freshwater prawn species.

Keywords ovarian development, ovigerous female, GSI, HSI, reproductive

# Introduction

The prawns, *Macrobrachium* species belonging to the family Palaemonidae are decapod crustacean of high economic importance world-wide and have been subjected to intense aquacultural practices especially in Asia and America. Moreover this genus is particularly important among palaemonid prawns including species of major scientific and economic interest. Therefore, understanding the reproductive periodicity of this group is imperative to develop management and culture programs (Arimoro & Meye, 2007).

The reproductive process is an essential part of the biology of the species. In addition an understanding of the gonadal development and reproductive cycles is of fundamental importance for the conservation of natural stocks and for culture purposes (Chellappa, *et al.*, 2005).

White, *et al.*, (2003) also stated that a more precise estimate of spawning season may be determined from microscopic gonad stage. Therefore, histological analysis of gonad should be investigated in order to confirm the sexual maturity. Determination of oocyte diameter with histological tools provides basic information on classification of ovarian development (Revathi, *et al.*, 2012).

Present study is to investigate ovarian developments of the fresh water prawn *M. johnsoni* such as spawning period, morphological and histological observation of ovary development, GSI and HSI from Ayeyarwady river segment of Myinmu Township.

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# **Materials and Methods**

# **Study Area**

The study area was Ayeyarwady River Segment of Myinmu Township, situated in Sagaing Region on the northern bank of Ayeyarwady River.

# **Study Period**

The study was carried out from April, 2019 to March, 2020.

# **Developmental stages of the Ovary**

After sampling and dissection, ovaries of female *M. johnsoni* were observed. The gonadosomatic index and hepatosomatic index were determined according to (King, 1995).

 $GSI = \frac{Gonad weight \times 100}{Body weight}$  $HSI = \frac{Liver weight \times 100}{Body weight}$ 

#### Determination of the developmental stages of ovary

For histological examination, 20 prawns at different ovarian developmental stages and five individual of each ovarian stage were used. Respective stages of each ovarian tissues were fixed in 10 volumes of 5 % formalin for 12-24 hours. Afterwards the tissues were dehydrated in alcohol and cleared in xylene, embedded in paraffin and sectioned at 7  $\mu$ m. The sections were stained with haematoxylin - eosin and mounted permanently in Canada balsam for microscopic analysis. The developmental stages of germ cell were determined according to (Huang, *et al.*, 2010).

#### Analysis of Ovigerous Female

Ovigerous females were separated from other females. A total of 550 ovigerous females (Berried female) were observed and gathered data for analysis. The data referring to the total length and weight of the females were noted.

# Results

After sampling and dissection, ovaries of female *M. johnsoni* were classified by their external morphology and were further examined histologically. The ovary of *M. johnsoni* was a two lobed organ situated above the hepatopancreas and below the pericardial sinus and the heart. The anterior and posterior ends of the ovaries are touching each other and leaving a gap at the middle in immature ovary. The colour, shape and size of the ovaries vary according to developing stages. In this study, four ovarian developmental stages were initially classified based on the external morphology, including the size, shape and colour.

# Morphological appearance of developmental stages of ovary

**Stage I (Pre- developing):** creamy white, thin, small, and its anterior end remained close to the stomach and extending to the posterior border of hepatopancreas (Plate 1, A).

**Stage II** (**Developing**): pale yellow, oval shaped and its anterior end of ovary reached just behind the stomach and the posterior end extended to the anterior end of the first abdominal segment of the prawn (Plate 1, B).

**Stage III** (**Maturing**): orange in colour, distinctly expanded laterally and its anterior end of the ovary covered stomach and the posterior end distinctly bi-lobe and extended to the anterior end of the first abdominal segment of the prawn (Plate1, C).

**Stage IV** (**Ripening**): deep green in colour, and characterized by the great increased size and the presence of visible oocytes. Anterior end of the ovary became more elongated, extending beyond the penultimate spine of dorsal rostral teeth (Plate1, D).

Table 1	Total ovary length,	weight, gonadosomatic	index (GSI), hepa	atosomatic index (	(HSI)
	and oocytes size of	each ovarian stage of M	. <i>johnsoni</i> from N	lyinmu Township	)

Ovarian	NS	Total length (mm)	Body weight (g)	Ovary Weight (g)	GSI	HSI	Oocyte Length (mm)	Oocyte width (mm)
Stage I	114	39 - 64	0.86 - 5.15	0.01 - 0.2	1.61 - 12.95	2.02 - 10.49	0.05 - 0.65	0.0025 - 0.45
		$(51.34\pm4.66)$	$(\ 2.55 \pm 0.67)$	$(0.04\pm0.05)$	$(1.54\pm2.35)$	$(5.66\pm1.72)$	$(0.14\pm0.19)$	$(0.09\pm0.12)$
Stage II	31	44 - 61	1.65 - 4.48	0.02 - 0.2	0.51 - 8.87	1.84 - 8.33	0.005 - 0.64	0.0025 - 0.44
		$(53.55\pm4.33)$	$(2.80\pm0.76)$	$(0.05\pm0.05)$	$(1.90\pm1.77)$	$(4.96\pm1.47)$	$(0.16\pm0.18)$	$(0.09\pm0.1)$
Stage III	60	39 - 64	1.46 - 5.13	0.01 - 0.25	0.30 - 14.93	1.75 - 10.68	0.005 - 1.05	0.0025 - 0.55
		$(52.73\pm4.98)$	$(2.68\pm0.81)$	$(0.08\pm0.67)$	$(3.55\pm3.53)$	$(5.10\pm1.93)$	$(0.25\pm0.31)$	$(0.14\pm0.17)$
Stage IV	35	37 - 58	1.64 - 4.3	0.02 - 0.3	1.01 - 8.66	1.77 - 8.47	0.25 - 0.8	0.15 - 0.61
		$(50.57\pm4.87)$	$(2.48\pm0.67)$	$(0.1\pm0.06)$	$(4.42\pm1.68)$	$(4.63\pm1.60)$	$(0.56\pm0.16)$	$(0.38\pm0.14)$



A. Stage I



B. Stage II



C. Stage III





Plate 1 Morphological appearance of developmental stages of ovary

# Histological appearance of developmental stages of ovary

**Stage I:** Histologically the ovaries consisted of oogonia (99.84%), previtellogenic oocytes (0.11%) and vitellogenic oocytes (0.06%). The oogonia had a well- defined nucleus with clear nucleoplasm and surrounded by ooplasm. In previtellogenic oocytes, the nucleus was large and surrounded by large and apparent cytoplasm. In vitellogenic oocytes, nucleus was smaller than that of oogonia and previtellogenic oocytes. The oocytes had a few lipid droplets and yolk granules were present in cytoplasm of vitellogenic oocytes. The oocytes with thick follicle cells were also noted (Plate 2, A).

**Stage II**: The ovaries composed of oogonia (99.31%), previtellogenic oocytes (0.41%), vitellogenic oocytes (0.25%) and mature oocytes (0.03%). In mature oocytes, nucleus was small and cytoplasm with lipid droplets and yolk globules. The mature oocytes were surrounded by thick follicle cells (Plate 2, B).

**Stage III**: The ovaries composed of oogonia (96.86%), previtellogenic oocytes (0.64%), vitellogenic oocytes (1.41%) and mature oocytes (1.10%). In this stage, the nucleus of mature oocytes became vestigial or disappeared and surrounded by thick follicle cells (Plate 2, C).

**Stage IV**: The ovaries composed of previtellogenic oocytes (2.78%), vitellogenic oocytes (1.89%) and mature oocytes (78.33%). The major feature of mature oocytes was the enlarged size, which was the maximum compared to other type of germ cells. Large lipid droplets and yolk globules were distributed in the cytoplasm of mature oocytes. Follicular cells became very thin or disappeared. If present, it was fully elongated and adhered tightly to the surface of the oocytes (Plate 2, D).



A. Cross section of Pre-developing stage



C. Cross section of maturing stage



B. Cross section of Developing stage



D. Cross section of ripening stage

Plate 2 Histological appearance of developmental stages of ovary

Diameter of oocyte size varied according to the different stages of development, from 0.005 mm to 1.05 mm in length and 0.0025 mm to 0.61 mm in the width. In female, the range of total length was (37-64 mm), body weight (0.86-5.15 g), gonadosomatic index (0.30-14.93), and hepatosomatic index (1.75-10.68) (Table 1).

The results showed that GSI increased to significant (p< 0.01) level during the development of the ovary from stage I-IV. The stage IV ovary showed higher GSI values than other stages. The results exhibited significant increase in oocytes diameters from stage I to IV. Conversely, HSI values decreased significantly (p<0.01) during the development of the ovary through the stage I-IV (Figure 1).

## **Occurrence of Ovigerous Female**

Ovigerous females (berried female) were observed in all month of study periods. The lowest and highest percentage of berried females were 0.56% in November and 74.58% in other months.

### **Sexual Maturity**

A total of 240 females were examined to determine the length at first maturity stage. The smallest female carrying the eggs was found to be 37 mm in length while nonovigerous females with mature gonads (Stage IV ovary) were at a maximum size of 58 mm in total length (Table 1).

### **Spawning Season**

Ovigerous females were observed throughout the year during the study period, the highest percentage of ovigerous females prawns occurred in May (74.58%). Moreover females with stage III ovary were observed throughout the year.



Figure 1 Ovary weight and gonadosomatic index (mean  $\pm$  SD) of prawns (N = 20) with each ovarian developmental stages of *M. johnsoni* 

## Discussion

The present study was conducted on the ovarian development of the freshwater prawn *Macrobrachium johnsoni* from Ayeyarwaddy river segment of Myinmu Township. In the present study, ovarian development was categorized into four stages, based on external morphological differences (colour, position and size of the ovary). In the ovarian development of the other species of *Macrobrachium*, such as *M. amazonicum* and *M. rosenbergii*, was divided into four to six stages (Chang & Shih, 1995).

Similarly, the ovarian developments could be histologically classified into four stages according to the presence and amount of nucleus, yolk globules, yolk vesicles, follicle cells and oocyte sizes. Oogonia and yolk vesicle stages of oocytes were observed throughout the study period. In Stage I, ovaries and oogonia were present mostly in the germ layer.

Gonadosomatic Index (GSI) and Hepatosomatic index (HSI) are common tools used as quantitative methods to verify the gonadal development and represent the percentage of these organs to the total weight of the animal. Gonadosomatic index (GSI) is the most common index used to define reproductive cycling in animal population (Magalhaes, *et al.*, 2012).

Khin Khin Lay, (2007) recorded that the mean GSI values of both males and females of *M. malcolmsonii* were high from March to August. According to Win Mar, (2007), the highest GSI value of females *M. palaemonoides* was found in March and continued to increase between January to April.

According to above results, it was determined that higher GSI values occurred during the hot season but fluctuated in other months. Nevertheless, it can be assumed based on the data collected during the study that *M. johnsoni* is a continuous breeder.

Four developmental stages of ovaries could be discerned in *M. johnsoni* based on both morphological and histological considerations. Ovigerous females were observed throughout the study period. As reproducing females (female with mature ovary and carrying eggs) were found throughout the year, so that *M. johnsoni* is regarded as a continuous breeder, and thus recommended as a prospective candidate for aquaculture practice.

## Conclusion

The present study elucidated the ovarian development of freshwater prawn *M. johnsoni* from Ayeyarwady River of Myinmu Township. A total of 240 females were examined for ovary condition, and mean ovary size increased in accordance with stages I, II, III and IV. From the histological observation, the mature oocytes had been found in the stages of II, III and IV. Moreover, the gradual increase of GSI values was observed from stage I to IV in this study. Females with mature ovaries (Stage III) occurred in all months in this study. So, it was assumed that females spawned the whole year round.

This study contributes to the reproductive biology of freshwater prawn *M. johnsoni* and the findings will be useful for the culture of the prawn. It is further recommended that reproductive biology of other freshwater prawn species from the same study area should be investigated.

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