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### Fecundity and Embryonic Development in Three *Macrobrachium* Species

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Fecundity of three *Macrobrachium* species (*M. rosenbergii*, *M. malcolmsonii* and *M. lamarrei*) was estimated through random sampling followed by egg counting where highest fecundity was observed in *M. rosenbergii* (1408±709) and lowest in *M. malcolmsonii* (32±7). Fecundity per individual, per unit body length and per unit body weight was higher in summer (in *M. rosenbergii*). In all three species, length vs. fecundity relationship was found to be significant but weight vs. fecundity was insignificant in case of *M. malcolmsonii* and *M. lamarrei*. Relative fecundity (/cm) vs. total length relationship of *M. rosenbergii* was insignificant in winter but significant in summer, insignificant for *M. malcolmsonii* and significant for *M. lamarrei*. Whereas, relative fecundity (/gm) vs. total weight relationship was insignificant in all the species. Comparatively larger eggs were found in *M. lamarrei* (0.55mm) and smaller in *M. rosenbergii* (0.39mm). In the same time, a microscopic study was conducted to observe the embryonic development of selected species where successive stages of embryonic development was visualized from developing eggs of same individual indicated the asynchronous fertilization.

**Keyword:** Fecundity, Body parameters, Embryonic development, *Macrobrachium* spp.

#### 1. Introduction

Freshwater prawn farming plays an important role in global aquaculture, in terms of quantity and value <sup>[1]</sup> and also in the national economy of Bangladesh. Bangladesh has 7 families, 67 species of prawns of which genus *Macrobrachium* includes 14 species. Present study includes the fecundity and embryonic development of three important *Macrobrachium* species (i.e., *M. rosenbergii*, *M. malcolmsonii* and *M. lamarrei*). Fecundity was defined as the number of eggs laid per hatching that was found to adhere to the female pleopods <sup>[2]</sup>. It is important for a species to maintain and increase the population density. It is also very important in

estimating the reproductive potential of brood prawns which in turn helps management strategies of prawn hatcheries; estimating the number of spawners required for producing desired quantity of seeds. The prawn has four phases in its lifecycle: egg, larva, post-larva and adult. Fertilization is external and takes place when the eggs are extruded. The eggs, like bunches of berries in the brood chamber are carried by the brood during the whole incubation period and the mother prawn moves the pleopods back and forth intermittently to provide sufficient aeration to the eggs. In the meantime, the first pair of the thoracic legs is busy cleaning the eggs of any foreign matter. In general, the initiation of

a new individual begins with fertilization, the fusion of sperm and egg, followed by embryo development. The newly fertilized eggs are homogeneously granulated. During embryogenesis, eggs increased in diameter and moisture content while organic dry mass

declined<sup>[3]</sup>. During the present study, an attempt was taken to measure the fecundity, its relationship with body parameters (length, weight) and to find out the subsequent developmental stages of embryogenesis.

**Table 1:** Fecundity of three *Macrobrachium* species

Species name		Mean					
		total length (cm)	total weight (gm)	fecundity (/individuals)	fecundity (/gm)	fecundity (/cm)	egg diameter (mm)
<i>M. rosenbergii</i>	Winter	9.33±1.38	8.64±2.63	1316±583	151±43	139±52	0.39 (0.34-0.45)
	Summer	8.88±1.10	6.31±2.84	1500±847	230±46	162±71	
	Average	9.11±1.20	7.47±2.90	1408±709	190±59	150±61	
<i>M. lamarrei</i>		5.59±0.29	1.44±0.25	119±020	84±16	021±03	0.55 (0.5-0.6)
<i>M. malcolmsonii</i>		5.18±0.32	0.93±0.20	32±007	36±08	006±01	0.45 (0.4-0.5)

## 2. Materials and Methods

Experimental prawns were collected from Kaptai Lake (*M. malcolmsonii*), Satkhira (*M. rosenbergii*) and Sirajgonj (*M. lamarrei*, *M. rosenbergii*). *Macrobrachium* species were identified according to classification proposed by Gomes-Correia<sup>[4]</sup> and Holthuis<sup>[5]</sup>. Ovigerous females were preserved individually in plastic bags in order to preserve the egg mass as intact as possible. In the present communication, the term fecundity was referred to the number of eggs born in the brood pouch during a single spawning act. All developmental stages were considered for the analysis. To estimate fecundity, all eggs were removed from the pleopods and were counted using a stereo microscope to facilitate visualization and counting. The data referring to total length and weight of the females and number of eggs, weight and coloration of gonad were noted. The total length (rostral trip to tail tip) was measured with a measuring board fitted with meter scale. The weights of the specimens were measured on an electronic balance. The trends of relationship between fecundity and body parameters (length and weight) were estimated by the formula:  $F = a + bX$ . For body measurements i.e.,  $X$  (L or W). Where,  $F$  = fecundity,  $L$  = body

length,  $W$  = body weight,  $b$  = slope and  $a$  = constants. The coefficient of correlation ( $r$ ) of each of the relationships was also assessed.

In addition to the above studies, the relative fecundity was also calculated by using the following formula:

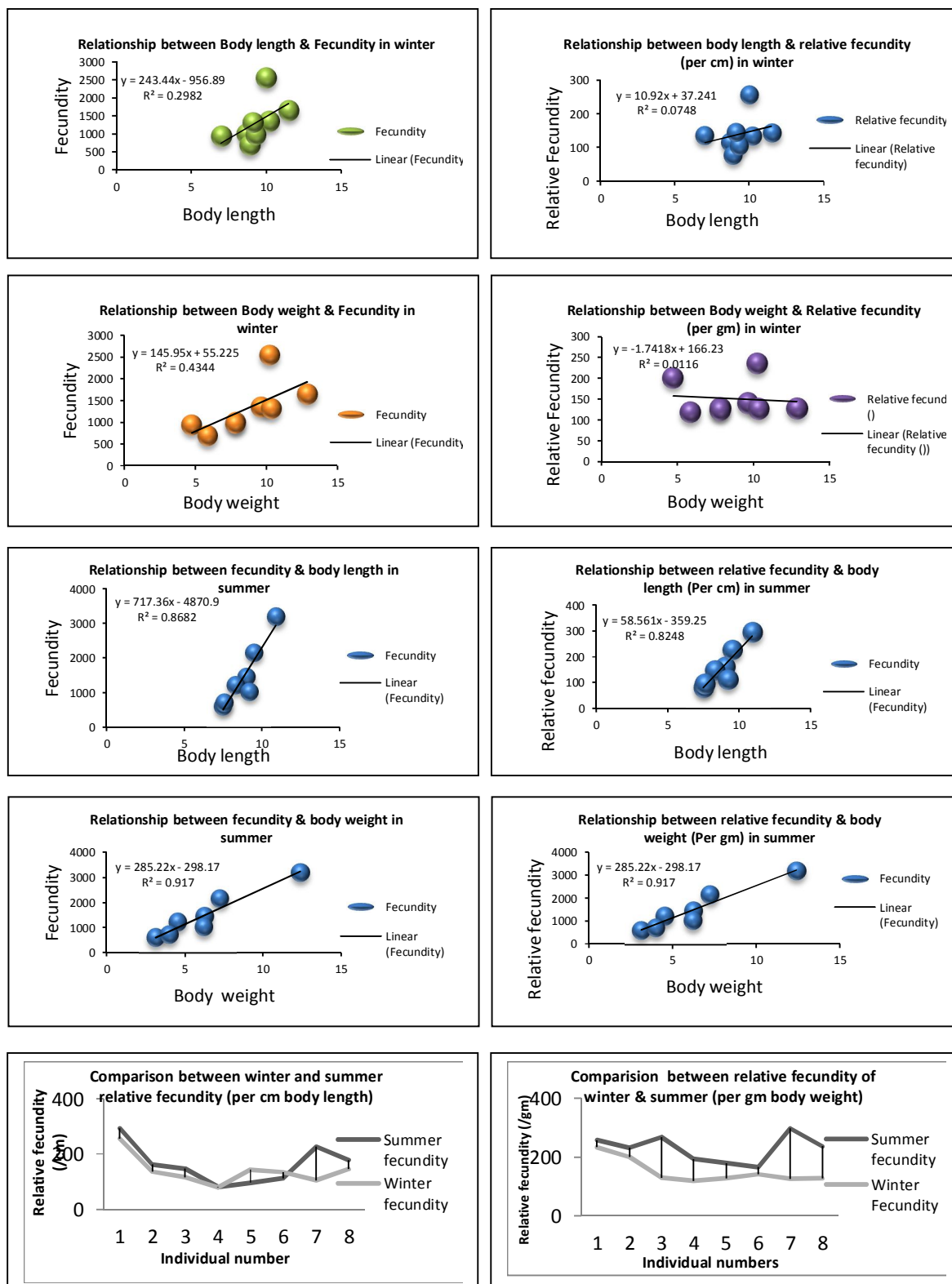
$$\frac{\text{Total number of eggs in the brood pouch}}{\text{Total length/weight of prawn}}$$

Normal development of prawn embryos were observed with a stereomicroscope (2X, 4X & 10X) fitted with a camera at various times after removing eggs from different gravid females to obtain a description of embryonic development.

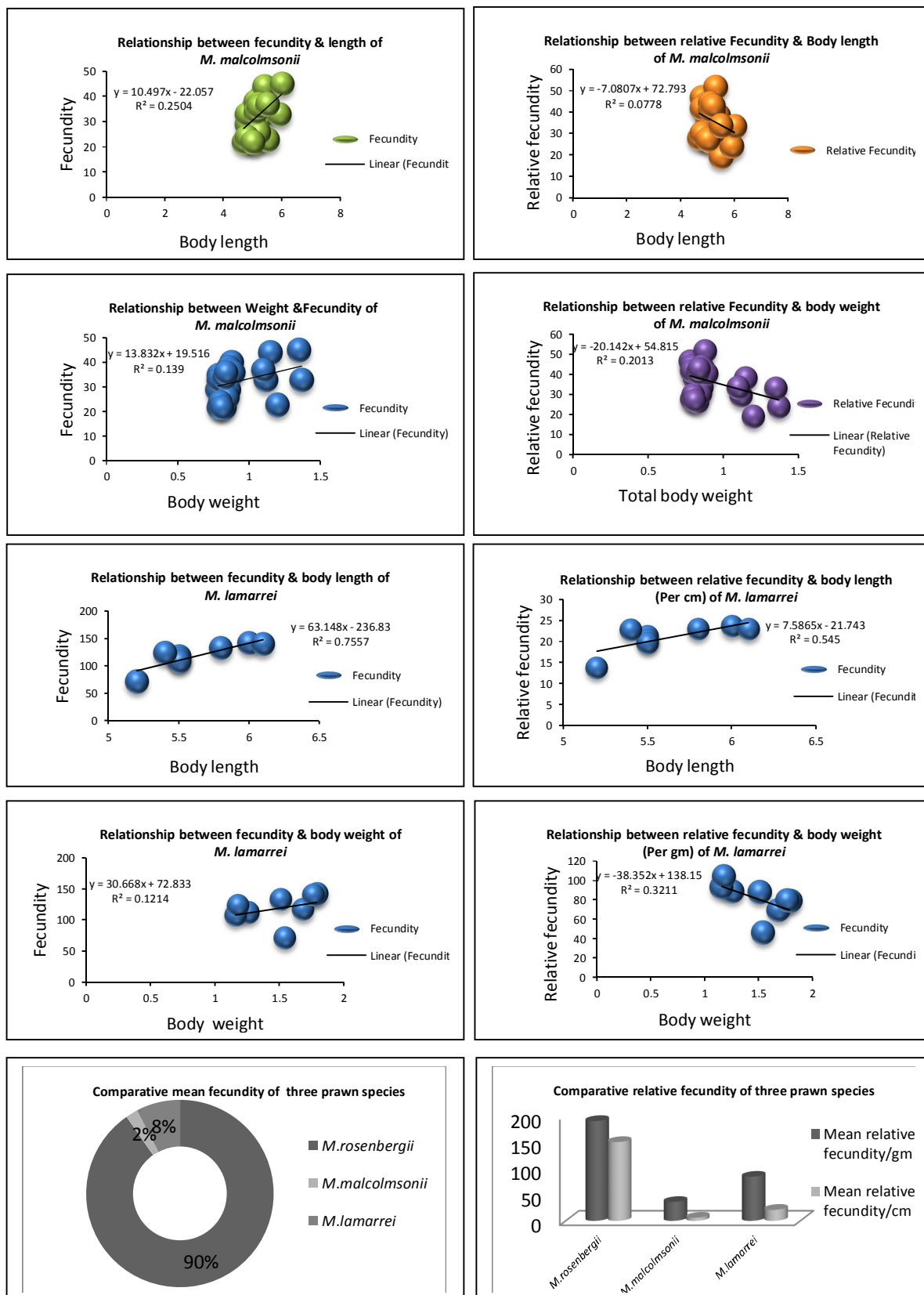
## 3. Results and Discussion

### 3.1 Fecundity

The mean total length and weight, mean fecundity, mean relative fecundity (per cm & gm) and egg diameter of three *Macrobrachium* species were shown in table 1. Fecundity of *M. rosenbergii* was found to be higher in comparison with *M. lamarrei* and *M. malcolmsonii*. The number of eggs shed by different species of the prawn may vary considerably<sup>[6, 7, 8]</sup>.



**Fig 1:** Relationships between length & weight with fecundity, length & weight with relative fecundity and comparative relative fecundity of winter and summer in *M. rosenbergii*.



**Fig 2:** Relationships between length & weight with fecundity and length & weight with relative fecundity of *M. malcolmsonii* and *M. lamarrei*.

In case of *M. rosenbergii* it was also noticed that relative fecundity both in terms of per gm body weight and per cm body length in summer was higher than the winter which might due to seasonal variation or physio-chemical properties of aquatic bodies or geographical differences as the summer prawns were collected from different sources. Variation resulted primarily from species adapting to changing environments <sup>[9]</sup> and high intraspecific variation developed as populations adapt independently to local habitats which could ultimately result in genetic differentiation <sup>[10,11,12]</sup>. Sharma Subba <sup>[13]</sup> reported that the fecundity of *M. lamarrei* ranged from 82-308 for females of 5.7-7.4 cm, *M. rosenbergii* ranged from 24225 to 191092 for females of 14.3 to 23.5 cm and *M. malcolmsonii* ranged from about 3500 to 94000 for females of 5.4 to 16.5 cm that was higher than the present study in all three species. It indicated that these prawns were variable in size depending on the geographical position and nature of food available <sup>[9]</sup>. Previous study on fecundity showed that fecundity of a species was under both genetic and environmental control and was a measure of fitness which was affected by specific features of different environments <sup>[14]</sup>. This approach might compromise the accuracy of our understanding of species evolution and species coexistence <sup>[15]</sup>. In crustacean, fecundity was reported to be a measure of the reproductive fitness <sup>[15]</sup> and clutch size was highly correlated with the size of individuals <sup>[16]</sup>. However, individual of the same species produced varying number of eggs depending on their age, length, weight and environmental condition <sup>[17]</sup>. Graziani et al. <sup>[18]</sup> explained that in *Macrobrachium* species the fecundity was extremely associated with the female age and that could increase while the female becomes mature. Jee and Kok <sup>[19]</sup> found decreasing fecundity in *M. rosenbergii* during egg incubation which might due to unfertilized eggs dropping off and some eggs being eaten by the brooders during the incubation period <sup>[17]</sup>. Fecundity could be as high as 80,000 to 100,000 eggs in mature females while first brood stock might be around 5,000 to 20,000 <sup>[20]</sup>. In larger females, the absolute fecundity was found to be

increased, reached the maximum value, and then decreased again in the largest females <sup>[21]</sup>. The fecundity varied with the length and body weight in freshwater crab species, *Cancer pagurus* <sup>[22]</sup>.

To estimate the fecundity, study was carried out during November-December (2009) and April-May (2010) which was odd time to get enough gravid females. The breeding season of *M. rosenbergii* was found to be July to September on account of the available monsoon as well as suitable temperature <sup>[23]</sup>. The breeding period of *M. malcolmsonii* extends from the end of June to the beginning of October, when the river is in full flood <sup>[24]</sup> but the present study was conducted in winter which might also be the cause of less fecundity. *M. acanthurus* exhibited significant differences in fecundity, mean size of ovigerous females and egg size, according to the time of year <sup>[25]</sup>. Variation might result from differences in the amount of resources available, or from environmental factors such as temperature <sup>[26]</sup>.

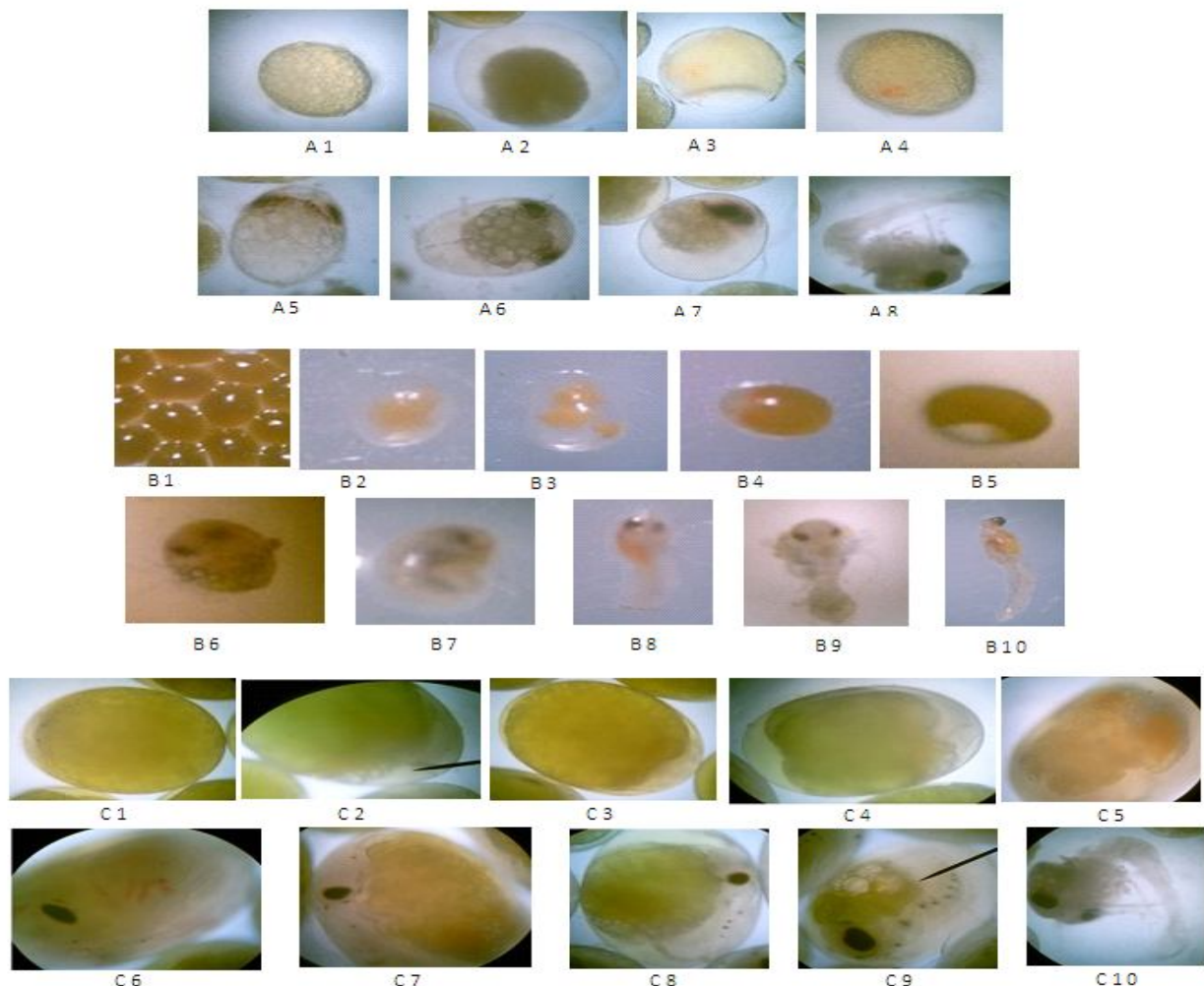
In present study, the eggs per gram body were higher than the eggs per millimeter body length (Table 1) that was very similar to the finding of Sharma and Subba <sup>[13]</sup> in *M. rosenbergii* and *M. lamarrei*.

A linear relationship was obtained between the body length & fecundity (*M. rosenbergii*,  $r = 0.55$  &  $0.932$ ,  $t = 1.631$  &  $6.31$  in winter and summer respectively; *M. malcolmsonii*,  $r = 0.05$ ,  $t = 2.64$ ; *M. lamarrei*,  $r = 0.83$ ,  $t = 4.19$ ), body weight & fecundity (*M. rosenbergii*,  $r = 0.66$  &  $0.96$ ,  $t = 2.152$  &  $8.4$  in winter and summer respectively; *M. malcolmsonii*,  $r = 0.37$ ,  $t = 1.84$ ; *M. lamarrei*,  $r = 0.32$ ,  $t = 0.95$ ) which indicated the significance of relationships (fig.1-2). In all three species length vs. fecundity relationship was significant but weight vs. fecundity relationship was insignificant in *M. malcolmsonii* and *M. lamarrei*. In other words the fecundity was directly dependent on the body length of the prawns, i.e., fecundity increased with the increase of body length. The summer result of *M. rosenbergii* was highly significant as it fits to 0.1%. Fecundity in all species was found to be



directly related to total length, standard length and total weight [27, 28]. The fecundity of *M. malcolmsonii* was found to be more closely related to the weight than its length. Similarly the

number of eggs and the length of female body were found to be positively correlated in the case of *M. dayanum* [29].



**Figure 3.** Embryonic developmental stages of *M. rosenbergii* (Plate A1-A8, 4X), *M. malcolmsonii* (Plate B1-B10, 2X) and *M. lamarrei* (Plate C1-C10, 4X) observed under microscope.

Relative fecundity (/cm) vs. total length relationship of *M. rosenbergii* was insignificant in winter ( $r = 0.27$ ,  $t = 0.687$ ) but significant in summer ( $r = 0.91$ ,  $t = 5.37$ ), insignificant for *M. malcolmsonii* ( $r = 0.232$ ,  $t = 1.09$ ) and significant for *M. lamarrei* ( $r = 0.66$ ,  $t = 2.49$ ). Relative fecundity (/gm) vs. total weight relationship was insignificant (*M. rosenbergii*,  $r = -0.107$  &  $0.43$ ,  $t = -0.265$  &  $1.17$  in winter and summer

respectively; *M. malcolmsonii*,  $r = -0.45$ ,  $t = 2.32$ ; *M. lamarrei*,  $r = 0.10$ ,  $t = 0.3$ ) in all the species.

### 3.2 Embryonic development

Embryonic developments of the prawns were studied observing mature eggs in their brood pouch (Fig. 3). In present study eight morphometric parameters were used viz. total volume of egg or embryo, yolk area, average

diameter of embryo, eye length and width, eye pigmentation, rostrum formation, body segmentation and appendages formation in a bird's eye view. Embryonic development has been described for few decapods, and no standard exists for defining developmental stages<sup>[30]</sup>. Embryonic development in *M. borellii* was divided into seven stages based on major morphological characteristics<sup>[31]</sup>. However, using only egg coat, yolk and eye shape and diameter, a researcher can identify a developing stage with mean accuracy<sup>[31]</sup>. Present investigation was carried out with the help of a stereomicroscope to obtain descriptions of embryonic development followed by Guerrero, et al.<sup>[32]</sup>. Embryonic development (egg stages) was divided into 3 stages based on yolk content following Guerao and Ribera<sup>[33]</sup>:

Stage I: Vitellus occupying more than  $\frac{1}{2}$  of the egg volume, non-eyed eggs (Plate-A2, B2 & C2).

Stage II: Vitellus occupying less than  $\frac{1}{4}$  of the egg volume, non-eyed eggs (Plate-A3, B3 & C4).

Stage III: Vitellus occupying less or equal of the egg volume, and decreasing progressively until hatching, eyed eggs (Plate-A4-7, B5-9 & C5-9).

The mature eggs were strongly ovoid and measured 0.34-0.45 mm in *M. rosenbergii*, slightly elliptical and 0.4-0.5 mm and 0.5-0.6 mm in *M. malcolmsonii* and *M. lamarrei* respectively on their long axis (Table 1). Early fertilized homogeneously granulated eggs were yellowish green in color in *M. rosenbergii* (Plate-A1), dark green in *M. malcolmsonii* and *M. lamarrei* (Plate-B1 & C1). The yellowish green color of eggs turned into bright yellow and then again turned into dark brown before hatching (Plate-A2-8). The dark green color eggs of *M. malcolmsonii* and *M. lamarrei* turned into light green and then again turned into transparent white (Plate-B2-3, C2-4). A "V"-shaped embryo became apparent (Plate-A3, B4 & C4), followed by rapid appendage development and shape of the embryo changed from elliptical to strongly ovoid.

The diameter of the ova of *M. lamarrei* were obtained to be  $0.36 \pm 0.15$  mm in average and that of the berried eggs ranged from 0.6 to 1.2 mm<sup>[34]</sup>. Sharma and Subba<sup>[13]</sup> reported that *M. lamarrei*

eggs were slightly elliptical and mature eggs measured about 0.54-0.64 mm in long axis, whereas, *M. malcolmsonii* being much bigger than other species possesses ova of 0.1 to 0.6 mm in diameter<sup>[35]</sup>. Eggs of *Macrobrachium borellii* were ovoid with a maximum diameter that varies from the moment of oviposition to the time of hatching from 1.5 to 2.0 mm<sup>[31]</sup>. According to Ling<sup>[23]</sup>, the eggs of *M. rosenbergii* were slightly elliptical, long axis measures 0.6-0.7 mm, bright orange in color, until 2-3 days before hatching when they become grey-black. The color of the eggs showed contrast from *M. lamarrei* which were dark green when immature and turned into light green and then transparent at the time of hatching<sup>[13]</sup>.

Newly fertilized eggs occupied huge yolk contents (Plate-A, B1 & C1). Egg coat and eye variables significantly increased as the embryo develops while all yolk variables decreased as the embryo consumed the vitellus. When the organ development initiated then the volume of egg again increased up to hatching. The major yolk area decrease was observed between stages A1-7, B1-6 and C1-9, which was coincident with a marked increase in the catabolism. The mature ovum contains oil droplets that were evenly distributed over the yolk surface similar to the observation stated by Martyn, et al.<sup>[36]</sup>. Stevens<sup>[30]</sup> observed that embryo area declined from an initial value of 0.95 mm<sup>2</sup> on day 1 to 0.83 mm<sup>2</sup> on day 72 and then increased to 1.28 mm<sup>2</sup> on day 388. Egg dry mass, lipid and carbon content all decreased during development as reserves were utilized; at the same time both water and mineral ash content increased<sup>[37]</sup>. When compared to orange eggs, yellow eggs were slightly heavier (6%), but grey eggs were significantly heavier by 31.6% which can be explained by the elongation of fully formed organs and appendages by about 17 days post fertilization<sup>[19]</sup>. ANOVA results showed that mean egg size was significantly different between early and late embryonic developmental stages of all shrimp species ( $P < 0.05$ )<sup>[28]</sup>. This was due to increased water content and changes in the biochemical composition during embryonic

development [26]. At the end of the incubation period, the growth of egg volume was an important feature of the embryos and the hatching of the larvae [15, 38].

First cell division was not apparent, after which divisions occurred until the blastopore appeared (Plate-B5). Cleavage at the animal pole produces cell mass which was clearly visible in contrast with white transparent vitelline membrane in *M. lamarrei* and at the gastrulation stage subsequent movement of cell layers was observed (plate-C4) after which beginning of eye formation was noticed (plate-C5). At blastula stage of *M. rosenbergii* the vitellus layer decreased notably (plate-A3) and after that some of the cells became pigmented and started to form eye spot (plate-A4).

The transparent eggs possessed two black spots of eye (Plate-A5, B6 & C6) which were clearly visible externally before hatching (Plate-A8, B10 & C10). Eye formation was visualized as a blackening of aggregated cells and in subsequent stages the eye spots became darkens and at later stages eye stalk formed. The middle-eyed embryo had fully pigmented eyes whereas, in the late-eyed embryo, the choroid covered most of the retina.

After appearing eye spots body formation began (Plate-A6, B7 & C7). Formation of internal systems such as digestive system, nervous system, circulatory system was noticed (plate-C8-9). Ganglions were visualized as red spots (plate-C8-9). Formation of rostrum began after optic cup stage and completed before appendages development (plate-C7-9). In the meantime body segmentation starts to be separated (plate-C9). Formation of appendages such as pleopods, first thoracic legs, tail bristles etc. was observed at later developmental stages (Plate-A5-8, B7-10). A hard carapace formation was noticed in embryo just before hatching (Plate-A8, B10 & C10).

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