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Studies on ovarian development of freshwater prawn *Macrobrachium dayanum* (Henderson)

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Abstract
The paper deals with the ovarian development of *Macrobrachium dayanum*. The development of the oocyte into mature ova has been studied under two principal categories viz., pre-vitellogenic and vitellogenic phase. Other than oocytes, oogonia have also been seen in the ovaries during different months of the year. It has been observed that the ovaries of *Macrobrachium dayanum* possess maximum percentage of oogonia during the months of December to January and June to July which indicate that they are passing through immature stage. It was further observed that during the month of April and October to November ovaries were filled with primary, secondary and tertiary vitellogenic oocytes.

Keywords: Oogonia, vitellogenic oocytes, ovaries, *Macrobrachium dayanum*.

1. Introduction
Prawn fishery of India with an annual catch of over 100000 tons is second only to the united states and accounts for 18% of the world's population. Flourishing trades of exporting prawn pulp to Burma and Malaya from earlier times and frozen and canned prawns to USA and Japan has made Indian prawns a major foreign exchange earner (Jhingran 1991) [9]. The order decapoda contain the familiar shrimps, crayfish, lobsters, crabs and some of the most highly specialised crustaceans, the 8500 described decapods represent almost one third of the known species of crustaceans and they constitute the most important fishery of India. Most of the decapods are marine, but the crayfish, some shrimps and a number of crabs are the one that have invaded freshwater (Barnes, 1974). Holthius in 1980 listed 48 species of the genus *Macrobrachium* that are consumed as food and hence are commercially important. About half of these species have been examined for their potential suitability for aquaculture (Holthius 1980; New 1990, 1995; Brown 1991; Jhingran 1991; Jayachandran and Joseph 1992) [6, 2, 9, 7].
Apart from being a delicacy they are a rich source of protien and vitamin (A & D) most needed to combat malnutrition in human population of the world. They contain considerable quantities of glycogen and free amino acids in their muscles, imparting their flesh a sweet taste. As they contain a very little fat, they have become a favourite protein food for the weight conscious aristocrats (Kumar, 2003) [4].
Of the three freshwater species, *Macrobrachium dayanum* qualifies the criteria of edibility on the basis of its size and habitat from freshwater of Jammu. Except preliminary studies conducted on its morphology, nutritional status and reproductive cycle (Kailoo 1984, Malik 2004, Chalotra 2004, Samyal 2006, Samyal *et al* 2007 and Manhas 2012) [12, 21, 20] nothing is on record with regard to reproductive biology of *Macrobrachium dayanum*. Realizing this, therefore, presently studies on maturation and ovarian development were undertaken.

2. Material and Methods
Experimental organisms Prawn, *Macrobrachium dayanum* were collected from their natural habitat from a stream at Gho-Manhasan situated at 32˚ 67' Lat N; 74˚ 79' Long E located at a distance of 20 km north west of Jammu city. Monthly collections were made with the help of rectangular haul / sweep net with 1620 cm sq. mouth area (1mm mesh size) and 80 cm long during morning hours (8:00-12:00). Most of the live specimens were collected from Gho-Manhasan stream because of easy access and availability in abundance throughout the year. On the basis of sexual dimorphic characters, sex wise segregation was done in case of *Macrobrachium dayanum*. Fresh specimens of prawns were dried by wrapping in blotting paper and weighed (in grams) using electronic weight balance. Five to six specimen of prawn were

sacrificed and their gonads were removed and weighed on electronic balance.

Gonads (ovary) were fixed in freshly prepared Bouine's solution (Picric acid 70%, Acetic acid 25% and formalin 5%) for 12-24hrs. After fixation, gonads were washed 3-4 times with tap water, then processed through routine alcoholic grades from 70% (5-10minutes), 90% (5-10minutes), 100% (5-10 minutes) and finally cleared in xylene. The material was then transferred in xylol wax and kept overnight at 40 ˚c for effective impregnation of the wax. Blocks of gonadal tissue were then prepared for microtomy. 5-8µ paraffin sections were stained using Mallory's triple & Haemotoxyline-eosine. Finally the material was mounted with DPX and coverslips. Slides so prepared were then studied under Nikon Ys 100 microscope and photographed with the help of SDC-313-Camera.

3. Results and Discussion

The development of the oocytes into the mature vitellogenic ova have been studied under following two principal categories. Other than oocytes, oogonia have also been seen in ovaries during different months of year.

I) Pre-vitellogenic phase.
II) Vitellogenic phase.

A brief description of these stages is as follows;

Oogonia: These are small spherical cells with centripetally located nucleus. Oogonia possess homogenous cytoplasm. These have been observed to be present either near the germinal zone attached to the germinal epithelium or freely lie in the ovocoel in the months from December to March and May to July. Percental occurrence of these oogonia varied from 59.13% to 63.18% during December to January and 54.17% to 62.03% during months of June to July (Table 1) (Figure 1). These oogonia start declining from the month of February and August onwards to touch as low as 4.73% in the month of May and 5.13% in the month of November (Table 1).

Stage I or Previtellogenic oocytes: The cytoplasm of these oocytes have been observed to be homogenous as all the oocytes lacked yolk. The nucleus appeared vesicular and nuclear material stained black with haemotoxyline stain. Pre-vitellogenic oocytes are comparatively larger in size and lie free in the ovocoel (Figure 2). The oocytes which range in diameter from 0.140- 0.189 mm showed great variation in size. Based on size variation, these have been categorized into previtellogenic oocytes-I (PVOI) (0.140-0.156 mm), pre-vitellogenic oocytes-II (PVOII) (0.158-0.172 mm) and pre-vitellogenic oocytes-III (PVOIII) (0.172-0.189 mm) with a mean oocyte diameter of 0.152mm, 0.166mm and 0.184 mm respectively (Table 2 and Figure 2). Percental occurrence of all three types of pre-vitellogenic oocytes have been observed to vary from 33.12- 38.19% during the months of December to January and 37.18-43.14% during the months of June to July with maximum percental occurrence of 43.14% in the month of June. These oocytes start declining in the ovaries from the months of January and June onwards when their contribution is just 4.17% in the month of October and 3.27% in the month of April (Table 2).

Vitellogenic Oocytes: On the basis of size of oocytes, nuclear changes, appearance of yolk vesicles and yolk accumulation, vitellogenic oocytes have been categorized into three sub-stages. I) primary vitellogenic oocytes (S-II), II) secondary vitellogenic oocytes (S-III) and III) tertiary vitellogenic oocytes (S-IV) / mature oocytes. This is the phase when synthesis of yolk takes place. Follicle cells start appearing during this phase and marked changes in the nucleus, nucleolus and ooplasm have been observed.

Stage II or Primary vitellogenic oocyte: Ooplasm of these oocytes is granular, small yolk vacuoles have been observed to make their appearance in the periphery of ooplasm of the oocytes. Size of the oocytes as observed ranges in diameter from 0.196-0.214mm. Nucleus stain bluish black with haemotoxyline, is centrally located, increases in size and had a wavy nuclear membrane. During this stage percental occurrence of these small size primary vitellogenic oocytes have been observed to vary from 34.16 - 41.12% & 36.21-47.38% during the months of February to March and August to September respectively (Table 2) (Figure 50). Small round follicular cells around oocytes have been observed.

Stage III or Secondary vitellogenic oocytes: In secondary vitellogenic oocytes, small amount of yolk get deposited in vacuoles, small unstainable vacuoles fuse together to form large yolk vesicles. Small eosinophilic yolk granules start accumulating in the periphery region of oocortex. The follicle cells remain confined to the periphery as was characteristic in the earlier phase. With advancemement in this phase of vitellogenic oocytes, the perinuclear ring disappear. Nucleus in these oocytes increases further in size and nucleoli start appearing in the nucleoplasm. In some oocytes towards the end of this stage the nuclear membrane become indistinct. The diameter of secondary vitellogenic oocytes vary from 0.198-0.242mm. Their occurrence in the ovaries have been recorded to be in the range of 49.05%- 54.03% & 48.31- 57.13% during the months of April to May as well as during the months of September to October respectively (Table 2) (Figure 3).

Stage IV or Tertiary vitellogenic oocyte / Mature oocytes: Tertiary vitellogenic oocytes further increase in size and attain their utmost size of 0.198-0.264mm during this phase. Yolk vesicles are distributed in the entire cytoplasm and nucleus is displaced towards the periphery which acquires eccentric position. Yolk deposition is almost complete in all these oocytes and whole of the ooplasm upto perinuclear region gets occupied by yolk globules. These oocytes are now designated as mature ova. The entire cytoplasm becomes eosinophilic. Percental occurrence of tertiary vitellogenic oocyte vary from 42.86%-64.15% & 43.16-61.73% in the months of May as well as during the months of November to December. (Table 2) (Figure 4).

Fully mature oocytes called, mature ova, ovulate and get deposited in the brood pouch of the ovigerous prawns in the 1st, 2nd & 3rd pleopods where the ova not only get fertilized but start further development also. The maximum preponderance of mature stage IV oocytes during the months of May to June as well as November to December signifies that prawn *Macrobrachium dayanum* is a biannual breeder.

The present viewpoint find support from the findings of Subaramanium (1963) [22]; Ryan (1965) [19]; Jegla (1966) [8]; Kulkarni (1992) [13]; Kailoo (1984) [12]; Malik (2006) [15]; Samyal *et al*. (2006) [21] and Samyal (2007) [20]. Concerning different stages during developement of oocytes into ovum, present author's view point get strengthened from the observations recorded by a number of authors on *Macrobrachium dayanum* (Adiyodi and Adiyodi,1974;

Subaramanium, 1979; Kailoo, 1984; Malik, 2006; Samyal *et al.* 2006 and Samyal, 2007) [1, 23, 12, 15, 21, 20] as well as those who have worked on different species (Joshi, 1980 on *Peaneus stylifera* and *Penaeus hardwickii*; Mirajaker, 1980 on *Macrobrachium kistensis*; Garimella, 1982 on *Macrobrachium lamarrei*) and Chakarvarty (2003) [3] who too reported similar stages in the development of oocytes into ovaries of giant freshwater prawn *Macrobrachium rosenbergii*. Observations made by these workers are in line with present observations too wherein they have recorded four different stages in the ovarian development cycle of respective prawn species.

Table 1: Maturity stages, percental occurrence, oocyte diameter colour of ovary and macroscopic characteristics of ovary of *Macrobrachium dayanum*

Maturity Stage/ Month	Most prevalent oocytes development stage with its percental occurrence	Oocyt diameter	Colour of ovary	Macroscopic characteristics of ovary
Immature undeveloped phase/ oogonial stage (Dec-Jan), (June-July)	Oo = 59.13-63.81%. Oo = 54.17 - 62.03% S-I = 37.18 – 43.14% S-II = 33.12 – 38.19%	SI = 0.140-0.189mm. SII = 0.196-0.214mm	Translucent	Ovary is small, translucent and difficult to distinguish. Oogonia cells are aggregated, as seen through the microscope.
Previtellogenic phase/ Developing phase (Feb-Mar), (Aug-Sep)	SIII = 7.05- 9.95%. Rest were SI & SII.	SIII = 0.198- 0.242mm.	Yellowish	Oogonia cells have advanced into oocyte stage. Developing ovaries are larger, opaque, and yellowish, with scattered melanophores over the surface. Pre-vitellogenic oocytes appear. Ovaries are yellow is in colour.
Vitellogenic phase/ Developed phase. (April), (Oct-Nov)	SIII = 49.05- 54.03% SIV = 38.11- 45.35%. Rest were SI & SII.	SIV = 0.198- 0.264mm.	Dark orange	Vitellogenesis shown by eosin staine oocytes. Nucleus appears. Vesicular bodies appear with chromatin clumps. Ova are nearly ripe. Ovaries are dark orange in colour.
Mature phase. (May-June), (Dec).	SIII = 38.05- 47.34% SIV = 42.86-64.15% Rest were SI & SII. Oo = 4.73% & 5.13%	SIV = 0.242- 0.264mm.	Dark green	Post vitellogenic stage is characterised by shrunken and irregularly shaped cells observed after ova release. Ovary is dark green in colour.

Oo = oogonia, S-I = Previtellogenic oocytes, S-II = Primary vitellogenic oocyte, S-III = Secondary vitellogenic oocyte & S-IV = Terttiary vitellogenic oocyte,

Table 2: Maximum percental occurrence and oocyte diameter of previtellogenic and vitellogenic oocytes observed during the year

Stages		Oocytes			Months
		PVO I	PVO II	PVO III	
	Oocyte diameter	0.140mm - 0.156 mm	0.158mm- 0.172mm	0.174mm- 0.189mm	
Pre-vitellogenic Stage (S-I)	Percental occurrence	33.12 – 38.19			December – January
		37.18 – 43.14			June – July
		4.17			October
		3.27			April
Primary vitellogenic stage (S-II)	Oocyte diameter	0.196 mm - 0.214mm			
	Percental occurrence	34.16 – 41.12			February – March
		36.21 – 47.38			August – September
Secondary vitellogenic stage (S-III)	Oocyte diameter	0.198mm – 0.242mm			
	Percental occurrence	49.05 – 54.03			April – May
		48.31 – 57.13			September – October
Tertiary vitellogenic Stage (S-IV)	Oocyte diameter	0.198mm – 0.264mm			
	Percental occurrence	42.86 – 64.15			May
		43.16 – 61.73			November - December

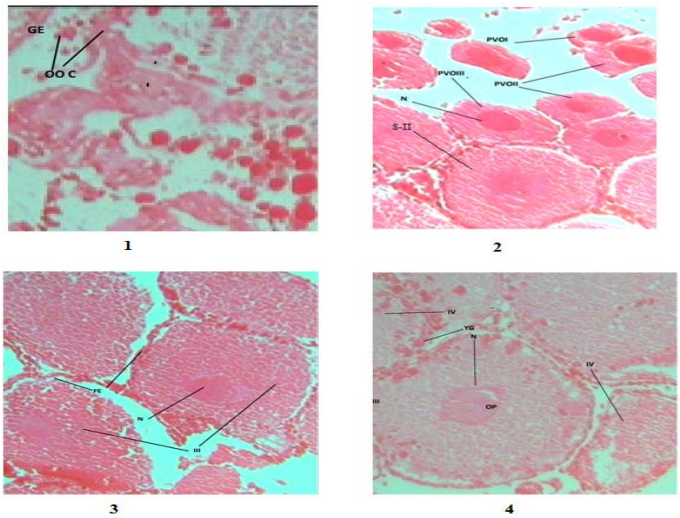

Fig 1: Microphotograph of T S of ovary showing oogonia attached to germinal epithelium in the ovocoel.

Figure 2. Microphotograph of T S of ovary showing three types of previtellogenic oocytes.
PVOI = Pre-vitellogenic oocyte substage –I, PVOII = Pre-vitellogenic oocyte substage –II, PVOIII = Pre-vitellogenic oocyte substage –III, N = Nucleus, III = Stage –III vitellogenic oocyte

Figure 3. Microphotograph of T S of ovary showing primary vitellogenic oocytes.
F E = Follicular epithelium, N = Nucleus, II = Stage –II vitellogenic oocyte

Figure 4. Microphotograph of T S of ovary showing secondary & tertiary vitellogenic oocytes.
III = Stage –III vitellogenic oocyte, IV = Stage –IV vitellogenic oocyte, N = Nucleus, Y G = Yolk globules
C P = Cytoplasm

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