9. RESULT

9.1 Observation of Behavioral patterns in *B. bengalensis*

In this present observation breeding behavior was studied in *B. bengalensis*. Where special emphasis was given towards the type of breeding, production rate and sexual maturity level.

9.1.1 Observation of breeding behavior

At the time of stocking, mature male and female of *B. bengalensis* was identified and then as per sax ratio (1∶1), they were kept in the earthen pot. The male snail was differ from female snail, due to the presence of curved, modified right tentacle which served as its copulatory organ another point was there female was longer and heavier than male of same age. group (Monzon,1985).The avg. size of female male were 31 mm and 26 mm respectively. It was seen that after 2 to 3 days, when they acclimatized with the tank environment, they started to graze over the algal mass. The idenfication of the algal parts was done with aid of BSI, Howrah, W.B.. After identification of those algal mass the dominant specimens were detected mainly *Spirogyra* sp, *Leptolyngbya tenue,, L. valderianum,* etc (Table 16). In the gut content observation only mud and undigested algal parts were observed (Plate no. 38 to 39)

It was noticed that after 5 to 7 days of stocking, they started to breed. In *B. bengalensis,* viviperiod type of breeding behavior was observed, which means they laid (directly) tinny juveniles. It was seen that in every year (from February 2010 to January 2013) the breeding season started within the middle or last week of February. Peak breeding period were observed from starting of April or late April to August. Some time, it was last up to of September (mainly in 2012).After this peak breeding period, the rate of breeding was observed to have slow decreasing trend and it was lasts up to November. But in 2012 it was continued up to December. Here the "Peak breeding period" means the intensity to produce young juveniles.

9.1.2 Morphometric and behavioral study of Juveniles

The appearance of the new born snails were not entirely similar to that of the adults (Plate no. 35 to 36). In contrast to the fact that, shell-height exceeds shell-width in the adults. But about in case of new-born snails, their shell-height smaller than shell-width. The new-born individuals were measured in avg.1.85 mm in height and 2.11mm in width. At that time, they were transparent to look (shell colour was milky white) and irregular blackish patches was observed within their flesh. This blackish patches denoted about their accumulation of chromatophores pigments in the flesh. But at this stage sexual dimorphism could be not possible to distinguish (Plate no. 36 to 37).

9.1.3 Observation of parental care in *B. bengalensis*

At first, the juveniles (less than 2 mm in height) when coming out from the embryonic pouch of their mother, they attached with their mother's outer hard shell. At that time, it was carefully noticed that, the juvenile snails got nutrition from their mothers' outer hard shell. After minute observation, it was noticed that microscopic or very small sized algae, which grew on the outer hard shell of the mother snail, that helped to provide nutrition to the tinny juveniles.

9.1.4 Maturation study in *B. bengalensis* through biometric data

However, when the tinny juveniles rised in size over 3.5 to 5 mm, at that time they started to graze over the algal mass of experimantal cultured tanks. After 1.5-2.5 week, when that juveniles were over 8 mm (approx.) they were transferred into the prepared juvenile tanks (from **MLBT 1, MLBT 2** and **CBT** to **MLJT 1, MLJT 2** and **CJT** respectively). At that time shell of *B. bengalensis* was thin, shell breadth was larger than its length and deep brown shell colouration was observed. But here it can be mentioned that at this stage, starting of shell development was noticed. After 2.5 to 3 months (after 10 to 12 week), they were noticed to attain maturity (Ma *et.al*, 2010). At this time, their shell lengths were noticed to have 16±1.21 mm for **MLJT 1** tank , 16±0.96 mm for **MLJT 2** tank and 14±0.61 mm for **CJT** tank**.** In this period, they were transferred to **MLBT1a , MLBT2a** and **CBTa** from **MLJT 1, MLJT 2** and **CJT** respectively, means they were transferred to 2nd set of brooder tank, from the 1st set of juvenile tank.

As per the analysis of biometric data it was observed that, generally, females were longer wider and heavier than the males. Sub-adult and adult snails mostly had four whorls, while in juvenile it was three. Most of the snails with a shell length of 18 .0 mm and shell width of 16 .0 mm were gravid. Dissection of gravid females revealed that more developed young (with shell) were located anterior to midportion of the pallial oviduct while embryos/ova enclosed in white translucent capsules occupied the mid-portion to posterior region of the pallial oviduct. The V-shaped oviduct is located anterior part of ventral region . The oviduct appears white for most of its length, and frequently pink to rise in the distal region. The faintly translucent, white ovary (often difficult to discern in living animals) is located in the apical whorl along the digestive gland. Uterine young were found to be wider than they were long. They were usually three-whorled (Plate no. 37 to 38).

In the course of the present investigation, a sharp sexual dimorphism was observed between male and female snail. The male snail was differ from female snail, due to the presence of curved, modified right tentacle which served as its copulatory organ. It was mainly black with transverse bands of grayish yellow in an irregular pattern. Another point was that female was longer and heavier than male of same age . Thus, the single measurement of heights were taken into consideration.

Mature females were pregnant throughout the year while the number of embryos possessed by each individual varies in different season. To study about the maturation as well as developmental stages of *B. bengalensis,* direct count procedure was followed and that is why the females were taken to sacrifice. Generally, females were longer wider and heavier than males. Most of the snails with a shell length over 19 mm. Dissection of gravid females revealed that more developed young (with shell) were located anterior to mid portion of the oviduct while embryos/ova enclosed in white translucent capsules occupied the mid-portion to posterior region of the oviduct. Uterine young were found to be wider than they were long. Here it was observed that from the end of the February to August the % of young in the oviduct were maximum and after the end of August, the % of young were shown in declining condition and it become lowest in December. It was studied that in viviparous group, after development of uterine wall, the percentage of mature young started to increase (Van Cleave, 1932).

The maximum number of embryo was found from April to June (Fig 18), and this was recognized as peak period for their breeding. Beside this it was also observed rate of producing uterine ova was highest in January and lowest in September (Fig 18).Here it also observed in (Fig 18), that a sharp ascending and descending peak of both avg. percentage of uterine ova and avg. percentage of uterine young were observed which denotes about the month wise stages of maturation and gonadal development of *B. bengalensis.* Production rate of juvenile snail (per year) was observed, when they were aged. The average number of per year production of juveniles, for a female *B. bengalensis* was estimated (through scarified process, counting mature batch) i.e. 81±5.8 (aged) and 23±2.6 (at the age of three months) for the tank **MLBT1** and **MLBT1a** respectively. For the tank **MLBT2** it was 79±5.2 (aged) and for **MLBT2a** it was 28±0.5 (after 3 month). For **CBT** and **CBTa**, tanks the number were 43.1±3.2 (aged) and 15±2.6 (after 3 month) respectively. Along with it can be said that, to attain fully developed state (or aged stage) it took about 8-9 months and then, then the avg. production rate of a snail became over 70-75 nos. of juveniles per year mainly for MLBT1a and MLBT2a tanks. Fecundity were observed batch by batch, this means when the matured batches were developed within a uterus of snail, they were released and next immature juveniles were retained by mother snail to be matured. The number of matured young within the uterus (ready to released) was observed 15-20 (avg. range). But this number was varied according to the seasonal change.

The average total mortality of juvenile for **MLJT 1+ MLJT 1a, MLJT 2 +MLJT 2a** and **CJT + CJTa** tanks were noted 14.63%, 18.24% and 47% respectively. First, after stocking in the **MLBT 1, MLBT 2** and **CBT** tanks the avg. mortality rate were observed 3% , 3.29% and 5% respectively (Table 16). During the experimental period of time (February 2010 to January2013) the total average mortality rate in brooder tank i.e. **MLBT1+MLBT 1a** , **MLBT 2 + MLBT 2a** as well as **CBT+CBTa** were observed 14%, 16% and 51% respectively. In the control tank, in absence of proper physico-chemical parameters and desired food materials, relatively high mortality rate was observed. Side by side relatively lower fecundity and late maturity rate were also observed. Here it can be mentioned that the life span of male was observed 1 yr or hardly 1.5 yrs, while female poses over 3 yr to 3.5 yrs. The female was seen to breed throughout their life span but after 2-2.5 yr. low rate of producing of young juveniles were observed.

9.1.5 <u>Study of water quality parameters of the experimental tanks</u>

From the water quality point of view it was noted that, among the required parameters such as pH, DO, Hardness, Alkalinity were in accepted limit mainly for MLBT1, MLJT1, MLBT1a, MLJT1a and MLBT2, MLJT2 ,MLBT2a , MLJT2a tanks (Table 18). But for the control group of tank, such as CBT, CJT, CBTa, CJTa, the required parameters were not in accepted limit. For the three sets of tanks the Ammonium (NH_4^+) level was not found. However regarding lack of proper water quality parameters the control group of tank was observed to have very less production of algal mass, high mortality, less maturity etc. Here it is needed to mention that in this captive environment mainly pH was the very important required water quality parameters for *B. bengalensis*.

Regarding *B. bengalensis* alkaline water was mostly essential, in absence of which several abnormalities were observed in *B. bengalensis* specially in control sets of tanks. Such abnormalities were slow growth rate, less maturity level, the survivility rate of juveniles became lees, decalcification in the outer hard shell of *B. bengalensis* etc. This decalcification in hard shell, turned it into the soft to very soft in nature (likely to breakage condition).Here it can be mentioned that, after observing the pH and productivity level (generation of algal biomass) liming and manuring were done respectively. Those process were executed only for the first and second group of experimental tanks.

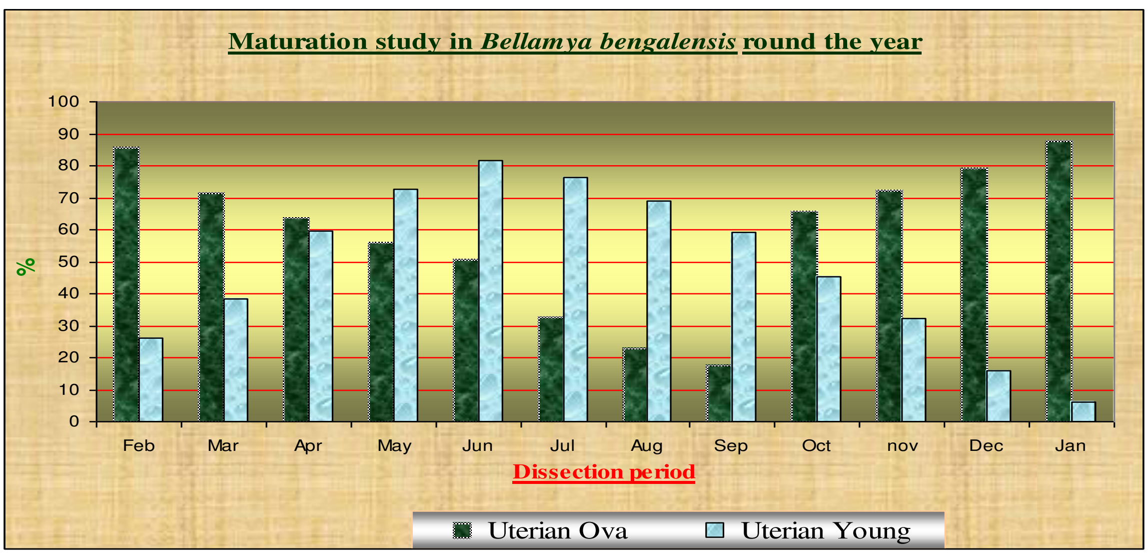


Fig. : 18

Table 17: Behavioral study of *B. bengalensis* during February 2010 to January 2013

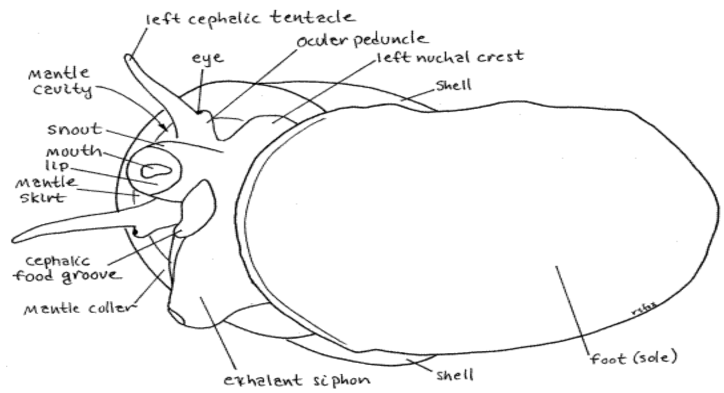
Sl No.	Item Name / Parameter		Result (February 2010 to January2013)		
			For the tank MLBT 1, MLJT 1 ,MLBT 1a & MLJT 1a	**For the tank MLBT 2, MLJT 2 ,MLBT 2a & MLJT 2a**	**For the tank CBT, CJT, CBTa, CJTa**
1.	Breeding season starts		End of February	End of February	End of February
2.	Peak breeding period		April to July	April to July	April to July
3.	Minimum breeding rate		End of November	End of November	End of November
4.	Feeding (Algae)		*Spirogyra* spp, *Leptolyngbya tenue, L. valderianum, Microcystis aeruginosa, Oscillatoria* sp these are dominating algae. Beside these *Lyngbia* sp, *Rizoclonium* sp, *Merismopedia* sp group were found	*Spirogyra* spp, *Leptolyngbya tenue, L. valderianum, Microcystis aeruginosa, Oscillatoria* sp these are dominating algae. Beside these *Lyngbia* sp, *Rizoclonium* sp , *Merismopedia* sp group were found	Very few cluster of algae (*Merismopedia* sp, *Spirogyra* spp) were found, but sometimes it was automatically disappear.
5.	Juvenile production by each snail. (per Year)		Fully adult 81±5.8 & 23±2.6 (after 3 month)	Fully adult 80±8.6 & 28±0.5 (after 3 month)	Fully adult 43.1±3.2 & 19±2.6 (after 3 month)
6.	Maturity		After 2.5 to 3 months when shell length 16±1.21 mm	within 2.5 to 3 months when shell length 16±0.96 mm	After 3 months when shell length 12±1.5 mm
7.	Mortality	After Stocking (avg.)	3%	3.29%	5%
		Juvenile (avg.)	14.63%	18.24%	47%
		Rearing period (avg.)	14%	16%	51%

Values are mean ± *SD*

Table-18 Water Quality parameters (Pre & Post stocking) during Feb. 2010 to Jan. 2013

Sl No.	Parameter		Result (February 2010 to January2013)		
			For the tank MLBT 1, MLJT 1 , MLBT 1a & MLJT 1a	**For the tank** MLBT 2, MLJT 2 , MLBT 2a & MLJT 2a	**For the tank** CBT, CJT, CBTa, CJTa
1.	Temperature (^{0}C)	Pre stocking	26.8	26.8	26.8
		Post stocking	31.3±9.8	31.8±9.5	31.6±9.2
2.	pH	Pre stocking	8.5	8.5	7.0
		Post stocking	8.5±0.21	8.5±0.15	5.5±0.19
3.	Dissolve Oxygen (ppm) (DO)	Pre stocking	7.6	7.2	7.5
		Post stocking	6.2±0.56	6.8±0.61	4.9±0.26
4.	Alkalinity (ppm)	Pre stocking	193.8	191.5	110.2
		Post stocking	188±3.9	182.3±4.2	102±2.8
5.	Nitrate (ppm)	Pre stocking	0.39	0.42	0.08
		Post stocking	0.35±0.09	0.36±0.06	0.03±0.02
6.	Phosphate (ppm)	Pre stocking	0.40	0.40	0.19
		Post stocking	0.26±0.02	0.28±0.07	0.16±0.01
7.	Ammonium (NH_4^+) (ppm)	Pre stocking	Nill	Nill	Nill
		Post stocking	Nill	Nill	Nill
8.	Hardness (ppm)	Pre stocking	226	229	172
		Post stocking	219.6±2.58	222.0±2.12	173±0.85

Values are mean ± *SD*



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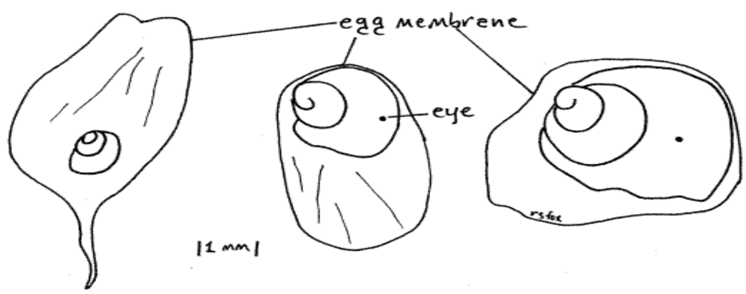

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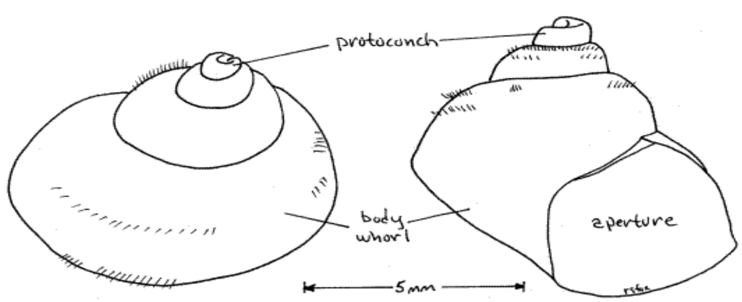

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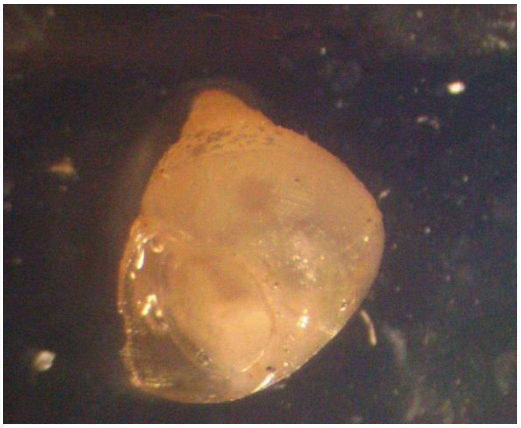

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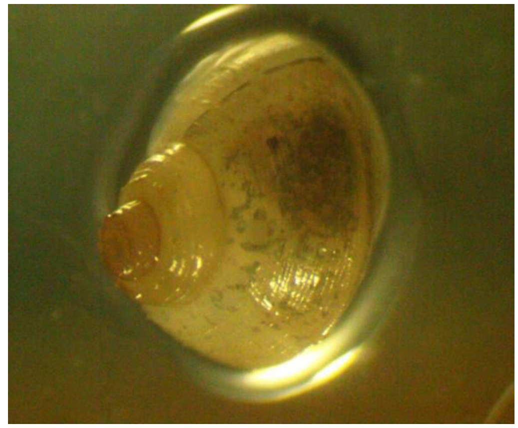

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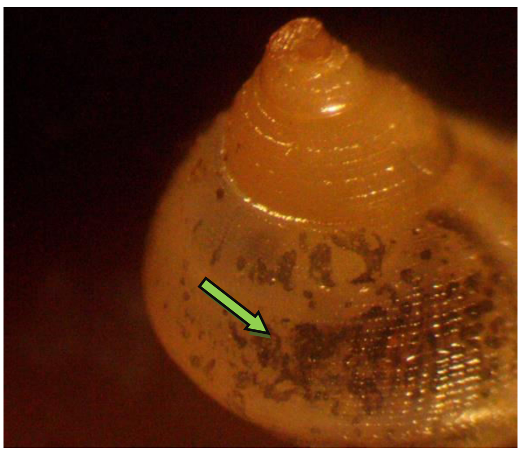

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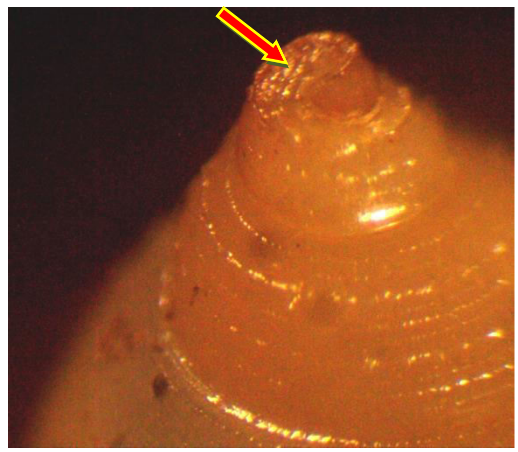

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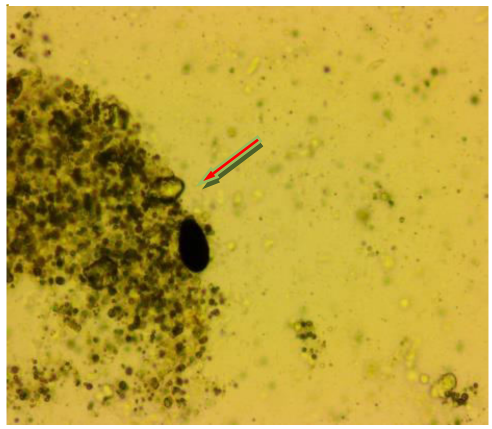

Plate No. 38

Plate No. 39