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REPRODUCTIVE CYCLE IN *PARREYSIA FAVIDENS* (BENSON) : A FRESHWATER BIVALVE OF KOSI RIVER.

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INTRODUCTION

The freshwater bivalve *Parreysia favidens* forms an important shell fisheries of India. The freshwater mussel *Parreysia favidens* (Benson) belong to class Bivalvia, family Unionidae. It is widely distributed in fresh water. They are found in dhars and channels of river Kosi in North Bihar. They live in river bottom on sand, mud, with their ventral part of the shell valves remaining exposed. In the present case a detailed study was done on reproductive cycle, fecundity and development of gonads, particularly ovaries of *Parreysia favidens* collected from Burhi Gandak and dhars of Kosi river of North Bihar.

Sex in bivalves is a subject of great interest. They are either dioecious or monoecious. In some of them change of sex reversal also takes place. A perusal of literature reveals that the work on sex and seasonal gonadal changes has been done mainly with regard to oysters and a few other Pelecypods. There is little information on *Parreysia favidens*. Patil and Bal (1967) have worked on seasonal gonadal changes in adult freshwater mussel, *Parreysia favidens* Var. marcens collected from the Mula river, Kirkee (Poona). Patil and Bal (1976) also studied the seasonal changes in chemical composition of the freshwater mussel *Parreysia favidens* Var. marcens (Benson). Most of the studies on sex and seasonal gonadal changes have been made in relation to oysters *Venus mercenaria*. *Bloomer* (1930, 1931, 1934, 1935 and 1939) made observations on *Lamellidens marginalis* and *Anodonata cygnea* in respect of sex and gonadal changes. Agrawal (1980) made some collections from Gwarighat located near Jabalpur and studied the seasonal variation in the gonads of *Indonaia caerulea* (Lea).

Bloomer (1930) made note on the sex of *Anodonta cygnea* collected from

Bracabridge pool, Sutton Park. Healy and Lester (1991) worked on sperm ultrastructure in the Australian oyster *Saccostrea commercialis* (Iredale and Ronglley) collected from rocks and jelly pylons at Donnybrook (Pulmicestone Passage) southern Queensland Bauer (1987) worked on reproductive strategy of the freshwater pearl mussel *Margaritifera margaritifera*. According to the available literature (Hendelberg 1961, Smith, 1979) the pearl mussel was reported to be dioecious. Morton (1982, 1986) worked on some aspects of the population structure and sexual strategy of *Corbicula fluminalis* (Bivalvia ; Corbiculacea) from pearl river of Peoples Republic of China.

In the present study, the reproductive capacity, fecundity and development of gonads, particularly ovaries of *Parreysia favidens*, collected from Burhi Gandak of Siuri Ghat of Kosi river of North Bihar have been worked out.

MATERIALS AND METHODS

The live specimens of *Parreysia favidens* were collected by Ekamn dredge from the river Burhi Gandak at Siuri Ghat from 3' to 5' depth in water and kept in aquaria containing sand and tap water in the laboratory for a fortnight. They were fed with live Plankton twice in a week. For experimental purpose, the live animals were exposed in normal saline and the ovarian tissue was dissected out and fixed in "aceto-alcohol" overnight and then transfered to 70% alcohol. After fixation a small portion of the gonadal tissue was taken on a slide. If the tissue became hard and stiff the tissue was dipped into N/10 HCl for 20 to 30 seconds. After this the tissue was teased and isolated from the connective tissue and other interstitial substances that might have been present. The tissue was taken and put into the acetocarmine solution.

The macerated tissue was placed on a slide and covered with a thin glass cover-slip and was gently tapped with finger tips. The germ cells of the ovary got segregated. The margins of the cover slip was then sealed by nail polish or sealing wax.

The squash preparation of ovaries was then studied under light microscope, the diameter of ova was measured with the help of occular and stage micrometers (in um) uhder high magnification There were many ova of different sizes.

Fecundity : The number of ova or eggs present in the ovary of one species or individual is called fecundity. In other words, fecundity implies (i) the power of individual to multiply ; (ii) capacity to form reproductive element ; (iii) the number of eggs produced by an individual or a species.

A total of six live animals were used. The ovaries along with the mesentries of

each animal were dissected out. The weight of the ovarian mass was taken and discharged into 3ml of distilled water. The suspension was made homogeneous and sieved through a metallic net or mesh (size 160 μm) to exclude the unwanted tissues. A pinch of eosin powder was added to stain the ova present in the suspension. The stained suspension was taken in RBC counting chamber of Neubauer slide with the help of a pipette and the counting was done at 400 fold magnification. For each animal the counting of ova was made in 5 small squares on a diagonal line for 5-times (RBC counting chamber has 25 small squares). The ova gave an average count of ova in 0·1 mm^2 volume (0·1 μl) of media. Mean count was obtained from average counts from 6 animals.

The data generated from these counts were arranged in three groups and then averaged (group average). The average value being the mean count of ova present in 1 ml of preparation whereas the sum of three groups was the total number of ova present in 3ml of suspension of the whole ovary. From the mean values of ova of all animals the average value of mean count of ova/animal was determined.

RESULTS

The sexes are separate in *P. favidens*.

The smallest sized ova were found in the month of August to December, 0·0-75 µm (about 94%). During the months of April to July, the largest size of ova were encountered (Fig. 1). The results were compiled and the percentage frequencies of different size categories were tabulated. The values vary in different months of the year (Table-1).

With gradual increase in temperature of the atmosphere and water during February and March, the ova gained in size and were in the size range of 0·75 and 1·50 to 2·25 µm diameter. During the month of April the smallest size of ova found were ; 0·075 µm 33%, 1·50 µm 43%, 2·25 µm 16% and the 3·00 µm 8%. With gradual increase in temperature of the atmosphere as well as water in the month of May, the ova of different sizes were observed viz. 0-0·75 µm (1·0%), 1·50 µm—(10%), 2·25 µm—(20%), 3·00 µm—(30%) and the 3·75 µm—(19%), 4·50 µm—(11%) and 5·25 µm—(9%) the largest size of ova.

During the months of May and July the ova start increasing in their volume. Therefore, the average frequency of enlarged size ova was evident in May and July. This increase in volume was quite naturally due to the accumulation of yolk, fat, etc. in the

TABLE I

Ova frequency of different size as present in the ovaries of *Parreysia favidens* in different months during one and half year (June, 1993-Nov. 1994)

Stage	Diameter of Ovum	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov
Stem cell I	0-0·75	23	38	96	97	98	98	100	94	83	60	33	1	24	39	94	95	96	99
	0·75-1·50	19	11	4	3	2	2	—	5	15	28	43	10	18	12	6	5	4	1
Oogonia II	1·50-2·25	17	12	—	—	—	—	—	1	2	12	16	20	16	9	—	—	—	—
	2·25-3·00	15	13	—	—	—	—	—	—	—	—	8	30	15	13	43	—	—	—
Pri. Oocyte III																			
	3·00-3·75	10	10	—	—	—	—	—	—	—	—	—	19	11	10	—	—	—	—
Sec. Oocyte IV																			
	3·75-4·50	11	10	—	—	—	—	—	—	—	—	—	11	10	12	—	—	—	—
Ova Mature V																			
	4·50-5·25	5	6	—	—	—	—	—	—	—	—	—	9	6	5	—	—	—	—
		100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

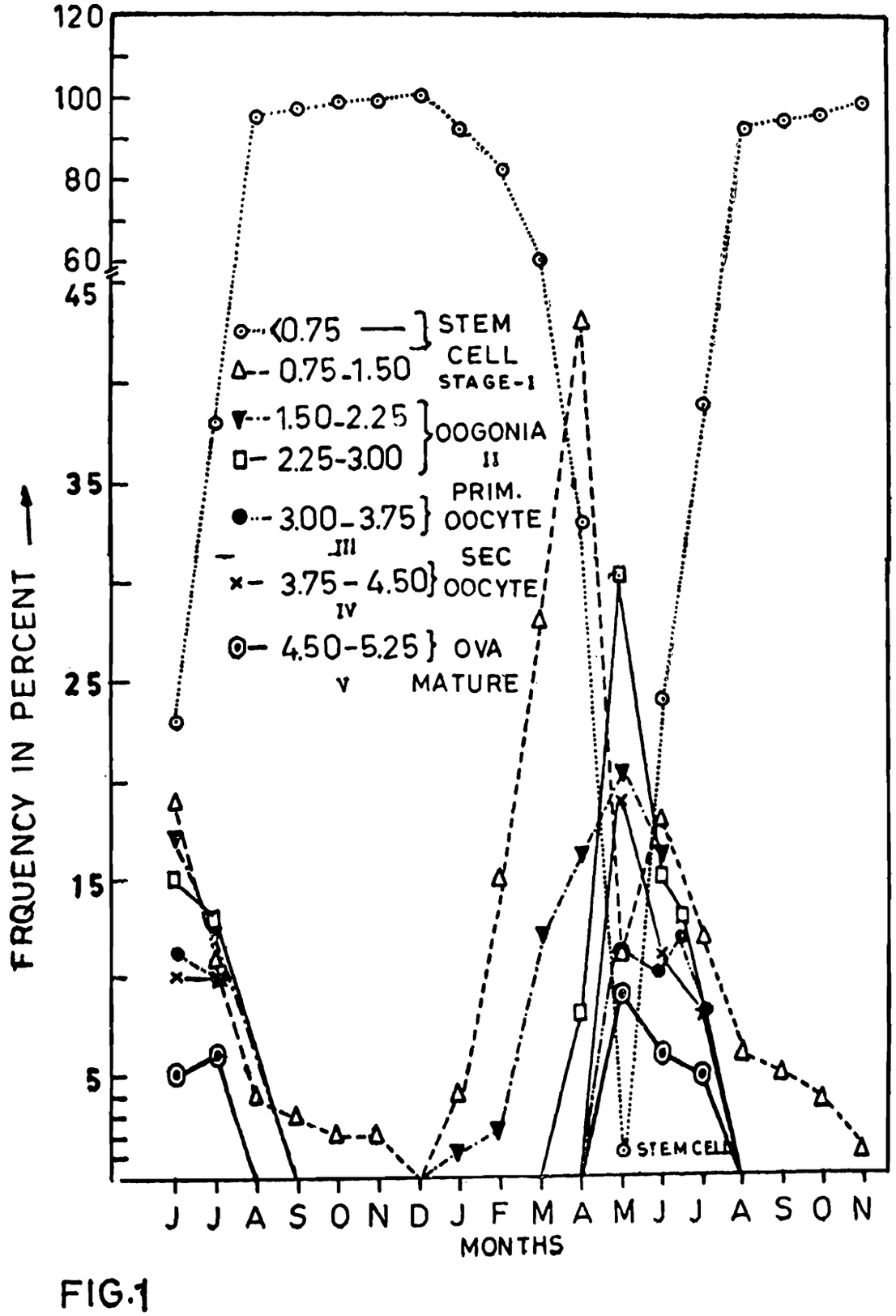


FIG.1

Fig. 1: Graph showing monthly variation in the Frequency of different size groups of *Parreysia favidens* during June'93-November, 1994.

cytoplasm of matured ova. The sizes observed in June were 0 075 μm—(23%), 1·50 μm—(19%), 2·25 μm—(17%), 3·00 μm—(15%), 3·75 μm—(10%), 4·50 μm—(11%) and the 5·25 μm—(5%). In the month of July the ova were observed to be 0·075 μm—(38%), 1·50 μm—(11%), 2·25 μm—(12%), 3·00 μm—(13%), 3·75 μm—(10%), 4·50 μm—(10%) and the 5·25 μm—(6%). After the release of the matured ova by the end of May and July, the ovary was left out with only small sized ova (stem cells). (Table-1).

Like all germ tissues in the present investigation, there as stem cell population which proliferates to produce a new crop of stem cells plus the maturing ova. As such, the smallest sized ova were present during the whole year which represent the stem-cell population. The maturing intermediate cells (Oogonia, primary oocyte, secondary oocyte etc.) were variable during April to July. By the end of July the mature cells were ready to be fertilized (Fig. 1).

DESCRIPTION OF THE MORPHOLOGY OF OVA AT DIFFERENT STAGES OF MATURITY

Five stages of development of ova were observed : PLATE-I (Figs. 1-6)

Stem Cells : The stem cell population is present throughout the year. Upon division it produces two daughter cells, one of which remains as stem cell. The other daughter cell undergoes differentiation to produce mature ova after passing through intermediate stages of oogenesis.

The stem cells were observed as very-minute bodies having poorly distinct nucleus and scanty cytoplasm. Usually circular in outline, but sometimes oval or ellipsodial stem cells were also observed.

Intermediate I Stage : The ovum of this stage was very small. It is surrounded by vitelline membrane. The cytoplasm is almost clear with small yolk granules and a nucleus.

Intermediate II Stage : The ova of this stage appeared to have an external semi-transparent vitelline membrane of slightly wavy nature. This coat is separated from the egg by perivitelline space. Cytoplasm of the ovum is full of yolk particles. The anterior region of the ovum had a poorly distinct nucleus with chromatin network. Dispersed in the internal "medullary" portion of the ovum were seen a few yolk/fat granules and droplets.

The "cortical region" of the stage II was not traceble. It was quite likely that

the "cortical region" might be temporary stage of the raw materials which was consumed during vitellogenesis.

Intermediate III stage : It represents a dividing stage of ovum wilh perivitelline membrane. A nucleus was often present in the centre of the cell which was circular in outline. Very small yolk particles in granular form were fairly present. Bifurcation of the shell takes place at this stage.

Intermediate IV stage : In this stage the mature ovum is seen with rod-shaped particles and yolk granules. The egg is surrounded by a semi-transparent gelatinous sheath of slightly fibrous nature.

Stage V : The large sized end cells were encountered in cell population.

A full mature ovum was fairly of large size and was developing into a larva wlth shell and adductor muscle. A well developed glochidium larva is surrounded by shell valves with adductor muscle and byssus thread.

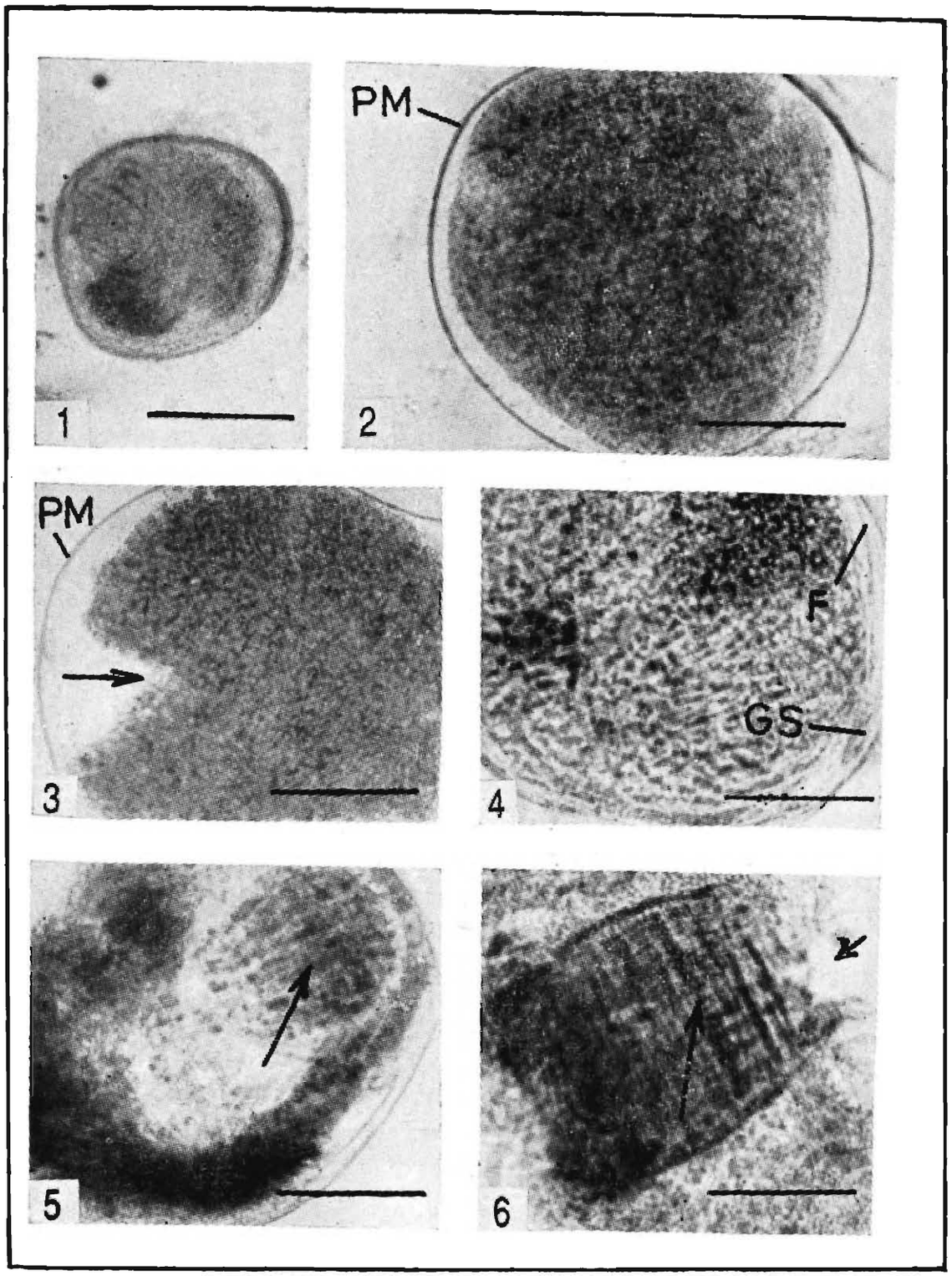
April to July is the breeding season of *P. favidens*. In the last week of July one to two size groups of the matured ova are discharged. During this period the gills become thickened, hundreds of eggs are discharged in the mantle cavity of the body and are carried through the ostia into water-tubes of the outer and inner gill lamina.

TABLE-2

Distribution of Ova count in the Ovary Suspension (3ml of water) of *Parreysia favidens*

S. No.	Weight of the Ovary (In mg)	Mean Count of Ova (Y) (In 0·1 ul)	$Y \times 10^4$ (in 1 ml)	Total Count in 3 ml	Standard Deviation	Standard Error
1	100	109·6666	1096666	3290000	13·57699	9·60035
2	180	100·0000	1000000	3000000	13·89244	9·82644
3	175	111·3333	1113333	3340000	12·8582	9·09212
4	120	177·6666	1776666	5330000	42·35957	29·95274
5	100	115·3333	1153333	3460000	19·29594	13·64429
6	90	99·6667	996667	2990000	14·74222	10·42433
Grand Total			118944	3568333	294478·1	131694·6

PLATE I



Figs. 1 : Fertilised ovum with nucleus, vitelline membrane and very small yolk granules. 2 : Larger stage of ovum with cytoplasm full of yolk particles enclosed within perivitelline membrane (PM). 40×4. 3 : Dividing stage (arrow) of ovum with perivitelline membrane 40×4. 4 : Showing mature ovum with condensed fibrilleae and yolk granules. The egg is surrounded by a semi-transparent gelatinous sheath (GS) of fibrous (F) nature. 40×4. 5 : Small developing larva with arrow shell and adductor muscle. 40×4. 6 : Developing glochidium larva with byssus thread (BT) (arrow) and shell with adductor muscles (arrow). 40×4.

* bar=500 μm

They are held by the mucous secretion of gills and are then fertilized by sperms. The fertilized eggs are retained in the gills which develop into the glochidium larva. The outer and inner gill lamina get greatly enlarged due to the accumulation of a large number of embryos. The gills act as breeding pouches "marsupia". For housing the fertilized eggs remarkable changes take place in the gills structure (Munshi and Pandey, 1991). Females are easily recognized at this stage by the swollen appearance of their outer gill plates.

Glochidia remain entangled by their "byssus thread and are nourished with the mucous secreted by the gills which act as brood pouches. When sufficiently mature, they come out through exhalent siphon into the water where they slowly sink to the bottom or get scattered by water current.

The data generated from counts were arranged in three groups and then averaged (group averaged); the average value is the mean count of ova present in 1 ml of preparation, whereas the sum of three groups is the total number of ova present in 3 ml of suspension of the whole ovary. Then from the results of combined mean values of all animals the average of ova produced by one individual was computed.

The total count of ova produced per animal was 3568333 S.E.$\pm$1·31694. (Table-2).

Discussion

According to Bloomer (1931) the follicles show the presence of globules in *Lamellidens marginalis*. Patil and Bal (1967) observed the seasonal gonadal changes in adult mussels. The spawning starts in the month of March and continues up to October indicating a prolonged breeding period. After the starting of spawning, lipid globules appear in the lumen of the follicles. In the male, spermatic *morulae* appeared in male individuals during the breeding season, no indeterminate sex condition or hermaphroditism was noticed. The sexually inactive stage (resting stage) was very short. In the present work the reproductive capacity and fecundity of *Parreysia favidens* were determined. Patil and Bal (1976) have found seasonal variation of chemical components of ovary of *Parreysia favidens*. Proteins, glycogen and lipid and inorganic constituents vary in different seasons. These variations were correlated with the gonadal changes of the mussel.

Agrawal (1980) observed in the gonads of *Indonaia caerulae* (Lea) partial spawning in the month of March that gradually rose till July and August. The

species ts considered to be a continuous breeder throughout the year, having a peak of spawning in the months of October and November. After the onset of spawning lipid globules appear in the lumen of the follicles. The spermatocytic *morulae* appeared after the start of the spawning.

Bauer (1987) postulated reproductive strategy of long extended period of reproduction in the freshwater pearl mussel, *Margaritifera margaritifera.* Due to the extended reproductive period populations are less vulnerable to fluctuations of environmental condition. The high fertility was independent of age and absence of a postreproductive period, resulting in a single female producing 200×10^6 glochidia during its reproductive life span of 75 years.

The breeding season of *P. favidens* is similar to the findings of Nagabhushanam and Bidarkar (1990).

The breeding season in *P. favidens* falls between April to July as shown by size frequency study of ovaries. It may be concluded that the months of April to July comprise the breeding season of mussel, *P. favidens.* The spawning takes place during the last week of May and is continued till first week of July.

SUMMARY

The reproductive cycle of a freshwater bivalve *Parreysia favidens* (Benson) of Kosi river has been investigated by determining the frequency percentage of different size ova present in the ovary in different months of the year.

In December the ova were of very small size (0-0·75 um). They increased in size from February onwards. Three size groups of ova were encountered of which 15% were in the size range of 0·75-1·50 um. In April four size groups of ova were encountered of which 6·7% ova were in the range of 2·25-3·00 um. In May seven size groups of ova were found of which 13% were in the size range of 2·25-3·00 um, and 19.5% were in the size range of 3·00-3·75 um. The largest sized ova were in the range of 4·50-5·25 um. The small size groups of ova were in the range of 0·0-0·75 um.

An individual animal with body weight 30·1g and having ovary mass of 120 mg produces 53,30,000 (Ftfty three Lakh and thirty thousand) ova.

April to July is the breeding season of *P. favidens.* In the last week of July the mature ova are discharged. During this period the gills become thickened, thousands of eggs are discharged in the mantle cavity of the body and are carried through

the ostia into water-tubes of the outer and inner gill laminae. The fertilized eggs are held by mucus secreted by gills which develop into the Glochidium larvae.

Key words : Reproductive cycle, *Parreysia favidens.*

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