Asian Journal of Biological and Life Sciences | Original Research

Gametogenic phenology in freshwater Molluscan species; *Lamellidens marginalis* and *Parreysia corrugata.*

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Submitted : 23.01.2013 Accepted :22.02.2013 Published : 30.04.2013

Abstract

Annual reproductive cycle of the freshwater uninoid bivalves *Lamellidens marginalis and Parreysia corrugata* from Panchganga river, Maharashatra, India were studied for period of June, 2010 to May, 2011. Monthly sample of 10 individuals of both the species were collected and subjected to germinal cell count along with shell size distribution and evaluation of sex ratio. Histological observations of gonadal cells were conducted, to understand the gametic physiology. Gametogenic proliferations in male and female bivalves were quantified to determine gonadal maturation in the species. Germinal cells were correlated with the environmental parameters, to understand its impact over reproductive mechanism in freshwater uninoid bivalve molluscs. Comparative data of phenological alterations in physiology of reproduction was analyzed and quantified statistically using ANOVA. Phenological gametogenic cycle of *Lamellidens marginalis* showed advancement in breeding behavior, in comparison to prolonged gametogenetic behavior of *Parreysia corrugata*.

INTRODUCTION

Day by day aquatic ecosystem develops as challenging environment for sustainability of animals, due to unstability created by changing its physicochemical properties. In natural ecosystem, molluscs comprises second largest group of invertebrates, after insects and considered as most suitable phyla, to tolerate such a great peril of freshwater instability. Freshwater molluscs are economically important group of animals and world widely utilized as a food source, b'coz of its high proteinacious meat and negligible amount of fat content. Although, molluscs having such tremendous commercial importance, they receive very little attention due to which some of the species come under endangered zone in the animal Kingdom [1]. Among freshwater molluscs, bivalves are well classified, where family Uninoidae comprises large group of bivalve species. The developmental strategies of freshwater Uninoids showed variation from typical marine Pelecypods. The general reproductive cycle, developmental history of Uninoids was not only grossly studied but also well-documented [2-6].

Coe (1943) [7], documented difference in sexual features of molluscan Uninoidae species. Some freshwater Uninoids are tachytichtic, while most are bradytichtic in reproductional mode. Heller (1993) and Montenegro (2010) [8-9], has remarked hermaphrodatism as a regular mode of reproduction, whereas Morton, (1991) [10] criticized it as occasional mode of reproduction in strictly gonochoric bivalves. Matteson (1948), Schalie (1963), Yokley (1972) and Heard (1975) [11-14], recorded the variety of reproductive strategies on the base of qualitative analysis.

Researchers have focused on quality indices, quantitative assessment, its management and tissue differentiation as a tool of practical application for better understanding of reproductive phenomenon. Thomson (1995) [15], has studied reproductive biology of *Cyclonaias tuberculata* by quantitative assessment of reproductive cells. Whereas, Alexander (1995) [16], has carried out work on sexual differentiation of reproductive tissues of *Mytilus galloprovincialis*. Recently Eliana, (2009) [17], recorded quality indices of gonadal and somatic tissue of Pearl oyster as a tool for assessment of reproductive biology. Recent record provides information on basic reproductive biology, but this information false in explaining gametogenic phenological differentiation among other freshwater molluscs. Hence, there is the need of scientific practical approach to understand gametogenic mechanism in molluscs, for the better management and culture practices.

Present study had focused on phenological gametogenic phenomenon between two freshwater molluscs *L. marginalis* and *P. Corrugata*.

MATERIAL AND METHODS

Study site:

River Panchganga, originated at Prayag Sangam, Chikhli, India-16 º 44′ 4″ N 74 º 10′ 33″E was selected for study (Fig.1,a). Physicochemical parameters viz. temperature, dissolved oxygen and free carbon dioxide were analyzed during entire study period. Freshwater Uninoids species *L. marginalis* and *P. corrugata* were collected from selected spots of Panchganga river. Specimens were collected by simple hand picking technique. Shell size of collected specimens was measured with Vernier caliper. Monthly sample of *L. marginalis,* shell size (50 mm to 120 mm), and *P. corrugata*, shell size (30 mm to 80 mm) was collected during the period July, 2010 to May, 2011. Collected individuals were brought to laboratory and processed within next 24 hrs. Animals were dissected for gonadal tissue and fixed in Bovine's Fixative for 48 hrs. Targeted tissues were then subjected further for block preparation by routine microtechnique. 5-7µm cut sectioned tissues were mounted on glass slide to detect gametogenic variation among the species.

Histological analyses:

To differentiate the period of spawning and gonadal maturity, gonadal sections were stained with Haematoxylene-Eosin staining technique [18]. Stained sections were observed under light microscope. Developmental stages of male and female gametic cells were differentiated, as described by Peredo and Parada (1984)[19]. Development of ova and spermatozoa were summarized as mentioned by Haggerty *et.al* (1995) [20]. Where, ten random sections were selected for the observations of gametic cells. From male gamete, numbers of sperms per acini were calculated, whereas, in female gamete, developing and matured ova were calculated in first thirty follicles. Mean number of sperms per acini and ova pre follicle were recorded and statistically interpreted.

Statistical analysis:

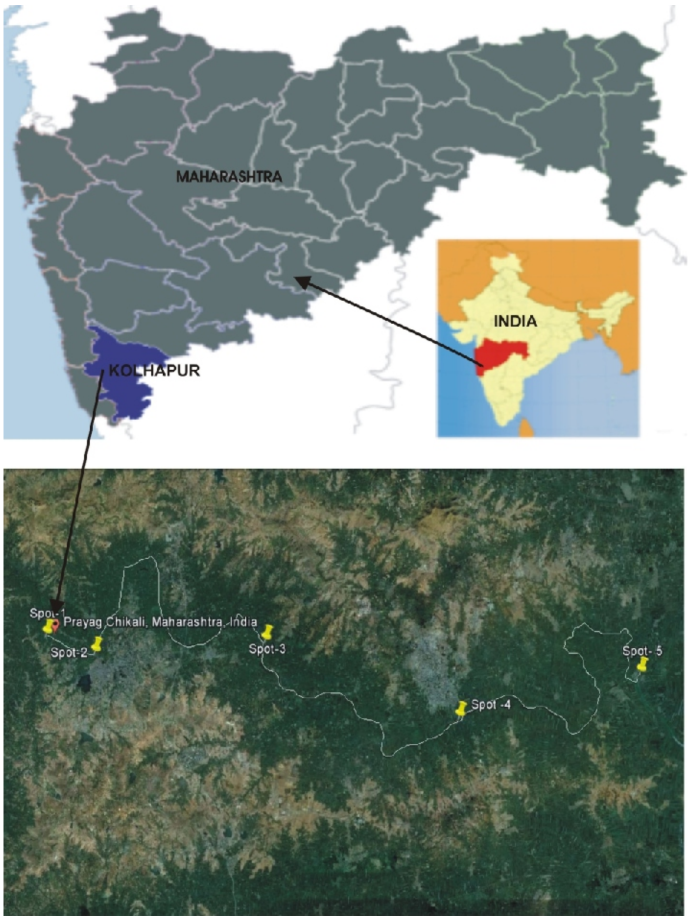
A statistical analysis of physicochemical parameters during annual period of June, 2010 to May, 2011 was conducted. Quantified data of gametogenic developmental stages were subjected to ANOVA and Tukey-Kramer multiple comparison tests. Finally, results were graphically presented for comparative analysis and interpretation of reproductive mechanism in both the species.

RESULTS

For the present investigation freshwater molluscs *L. marginalis* and *P. corrugata* was selected, which showed altered phenology of reproduction, from typical reproductive cycle, which in turns had affected their rate of reproduction. Along with that, physicochemical parameters showed their greater impact on the reproduction of both species.

Physicochemical parameters:

Physicochemical parameters as temperature, dissolved oxygen, free carbon dioxide were noted playing vital role in the annual reproductive cycle. Fluctuated physicochemical parameters had drastic effects over the gametogenic cytokinesis in both male and female bivalve molluscs. During assessment period, temperature recorded varied between 25 °C to 28 °C, with



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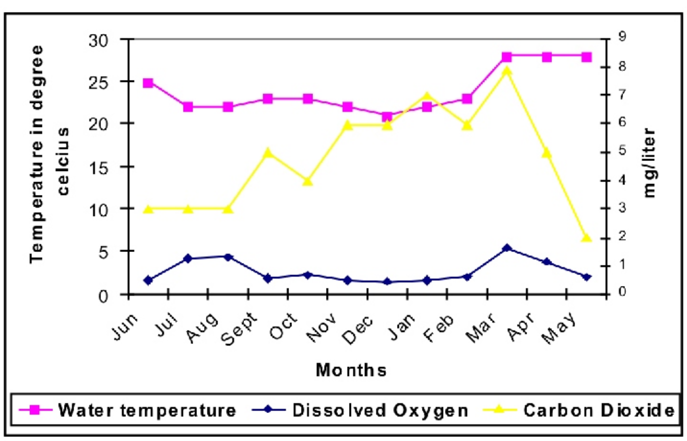

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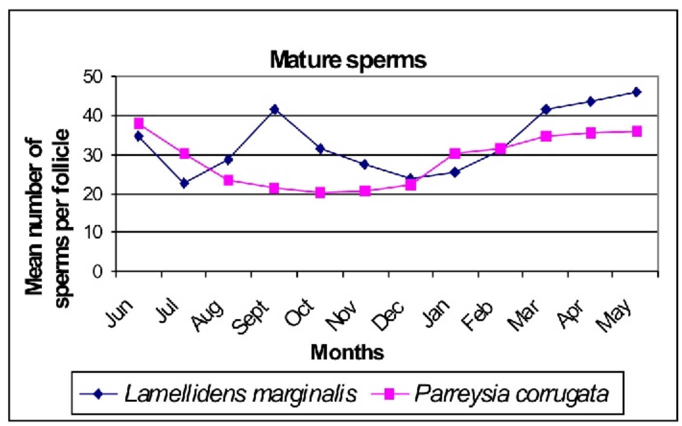
Figure 1- a) Location, b) Physicochemical parameters: - of the Panchganga River, Kolhapur, Maharashtra, India.



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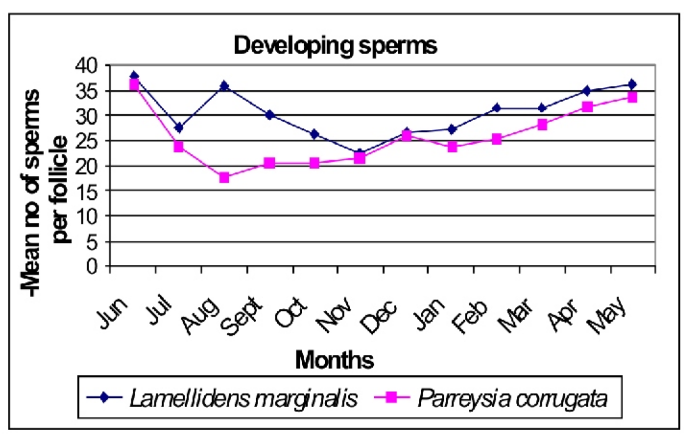

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Figure 2- a) Mean number of mature sperms, b) Mean number of developing sperms in freshwater molluscan species *Lamellidens marginalis* and *Parreysia corrugata.*

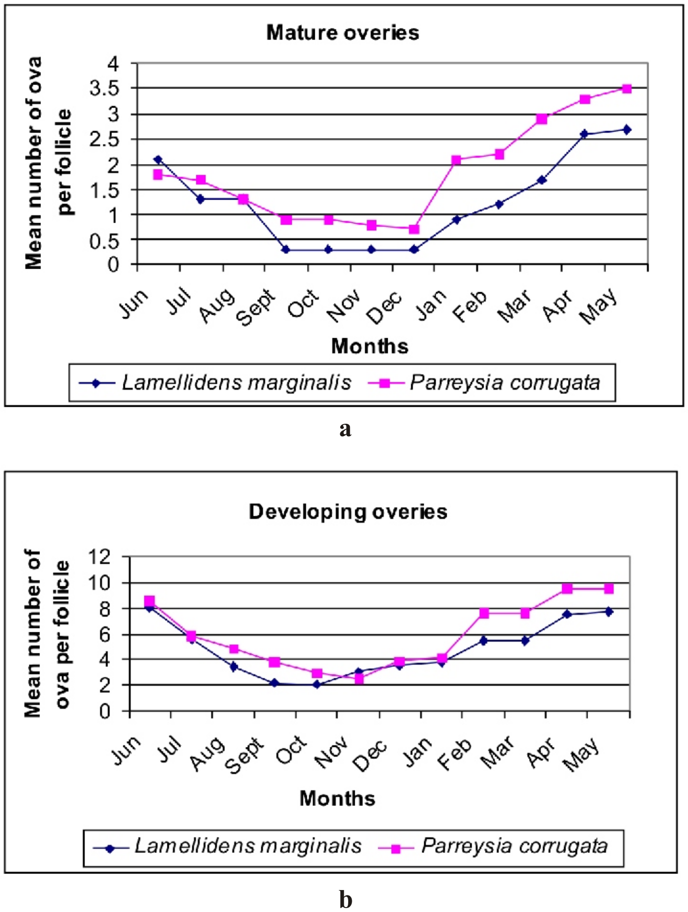

Figure 3- a) Mean number of mature ovaries, b) Mean number of developing ovaries: In freshwater molluscan species *Lamellidens marginalis* and *Parreysia corrugata.*

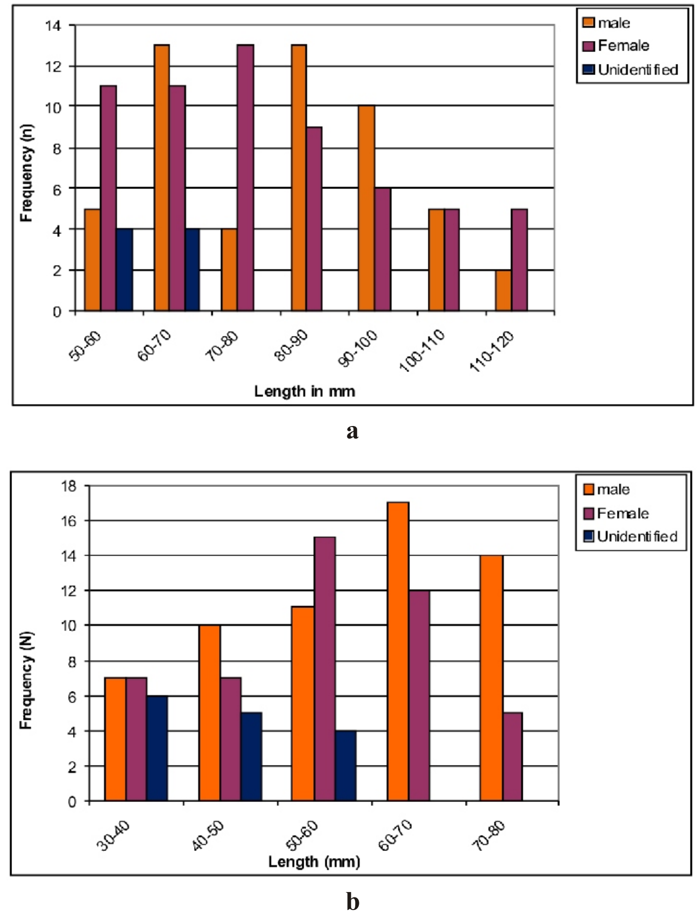

Figure 4 - a) Annual pattern of sexual differentiation in species *Lamellidens marginalis.* b) Annual pattern of sexual differentiation in species *Parreysia corrugata.*

notable seasonal variation showing highest temperature from March to May, whereas minimum recorded during months of November to January. Oxygen as one of the essential parameter had provided maximum opportunities for successful spawning; it was characterized by marked variation, with highest pick from July to August. During assessment, free carbon dioxide was noted as limiting factor for successful spawning, as increased level of CO^2 in water reflects to the minimized rate of gametogenic proliferation. Higher level of CO^2 was recorded in the months of April to May, whereas minimum level was observed during June to September (Fig.1, b).

Sexual differentiation and distribution:

Amongst collected specimens, 52 male and 60 female individuals of *L. marginalis* was noticed, while 59 male and 46 female individuals of *P. corrugata* was differentiated. Sex ratio was not observed significantly differed from 1:1 sex ratio for both the species.

For *L. marginalis*, shell size was represented by unique pattern of distribution for male and female, with an average count from 50 to 80 mm size (Fig.4, a). In case of *P. corrugata* in each class averagely male dominates overall pattern of distribution while female count was relatively lowered (Fig.4, b).

Histological differentiation of gametogenic cells in both species:

Histology of male and female gametic cells was carried out by normal histological assessment, where gonadal transect of both the species was subjected to HE technique. The observed gametogenic differentiation summarized was as follows.

Male gametogenic cycle:

Histological assessment in both the species *L. marginalis* and *P. corrugata* showed spermatogenesis throughout the study period. Sectional view described the developmental phases, such as spermatogonia, spermatocytes that were recorded as developing sperms whereas spermatozoa along with spermatids as mature sperms. Highly significant level of differences was noted for mature and developing sperms ($p< 0.001$, Tukey-Kramer multiple comparison test). In comparison amongst both, *L. marginalis* showed highest count of mature sperms per acinus, in the period of May to June and September to October, while in *P. corrugata* mature sperms per acinus was noted during May to June which had implied its critical gametogenic differentiation (Fig.2,a).

During the investigation, distribution of developing sperms was remarked by highest pick from June to August and thereafter resides down up to December. From January, onwards it increased drastically up to May, in case of both the species (Fig.2, b).

Female Gametogenic Cycle:

Histological assessment of female gonadal transects denoted, oogenesis throughout the year. The phases of development recorded from sectional view as developing oocytes and matured oocyte. Significant level of differences ($p< 0.001$, Tukey-Kramer multiple comparison test) was observed for mean number of ova per follicle in developing and matured stages. In the annual period during assessment, matured oocyte number was slowed gradually from July to December and thereafter increases drastically from January to May. Pick in oogenesis was recorded during April to May and Dec to January for mature oocyte (Fig.3, a).

Developing oocyte was represented by pick from April to May; and thereafter recorded by little alterations, for both the Uninoids (Fig.3, b).

DISCUSSION

Reproductive mechanism was considered as synchronously graded phenological process by which population density kept under check or maintained in the ecosystem. Reproductive physiology of these animals reveled that, various physicochemical parameters had great influence over the phenological phenomenon of reproduction. During study period, it was noticed that temperature of water affects the reproductive behaviour and gametogenesis. Average water temperature between 20 to 25 °C was marked as most suitable for spawning activity and noted during rainy season from June to September. Maximum amount of dissolved oxygen was analyzed in the water from June to September and considered suitable for the gametogenic events. Carbon dioxide content of water was observed very less during the same period in flooded river water. During the study, it was analyzed that physicochemical conditions provided greater opportunities for spawning and hence found suitable to increase rate of fertilization to sustain against peril of freshwater instability.

Quantitative analysis eliminated all lacunas and semantic problems associated with qualitative study and put forth meaningful ecological reports as described by Barber and Blake (1991) [21]. Freshwater Uninoidae molluscs *L. marginalis* and *P. corrugata* both were bradytichtic in breeding behavior and showed annually active reproductive cycle. Gametogenesis was occurred throughout the year with differentiations in developmental stages. Both species showed similar pattern of gametogenic differentiation and development as previously documented for some other molluscan species [22-23].

Quantified data of mature sperms and ova showed gametogenic differentiation in both sexes during similar breeding time, resulted by increased quantum of reproduction. During the experimentation, we concluded that for *L. marginalis,* gamete maturation was triggered during December to May and thereafter rate of gamete regeneration and maturation was resides down from June to October, representing their breeding period. In *P. corrugata* breeding period was restricted up to period of June to August when gametic proliferation and maturation was assessed. Maximum pick was noticed during onset of breeding season in case of both the species.

In case of *L. marginalis* male to female sex ratio was recorded not significantly altered from 1:1 sex ratio and continues with unique pattern of distribution in each class. Whereas, for *P. corrugata* significant differences was noted in sex distribution amongst each class. Breeding period was also restrained for particular duration viz. June to August. During study, those were the limiting factors observed, which had depleted rate of fertilization affecting phenomenon and successful spawning and numerical distribution of the species in the selected study area. *L. marginalis* showed adapted breeding period from that of typical breeding period of *P. corrugata*. All those mentioned condition made spawning phemenon fully efficient with maximum outcome of reproduction. Which in turns resulted to dominant quantum of *L. marginalis* in Panchganga river.

CONCLUSION

Present investigation enlightens the mechanism of reproduction with implicational analyzing methodology in the advanced cultural practices, which will proved immensely important to overcome the threat of extinction of commercially important species in the natural ecosystem for maintaining the ecological balance.

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