

A Histological Study in the Reproductive Status of the Pearl Mussel, *Lamellidens Marginalis* (Lamarck, 18 19)

P. B. Pardeshi

Department of Zoology,

M. G. V's, Arts, Science and Commerce College,

Manmad Dist. Nashik-423104

Abstract:- The reproductive status of the pearl mussel, *Lamellidens marginalis* was studied for a one year period by seasonal (monsoon, winter and summer) observation of gonadal changes through the histological study. The gonad development, spawning and fertilization which dependable on climatic factors and the richness of food supply. The gametogenic activity begins in males and females during December and January in winter also maturation phases and breeding simultaneously in both sexes were observed during summer and early monsoon seasons.

Keywords:- Male gonad, Female gonad, *Lamellidens marginalis*, gametogenesis, reproductive status.

I. INTRODUCTION

The most important factor controlling the number and spread of a specific molluscan species in any ecosystem is understood to be a reproduction. It takes place a crucial role in the taxonomic classification of distinct species, particularly in bivalve species with overlapping physical traits. The unionoidea species reproductive features and procedures are diverse and complicated, with a wide range of fecundity and brooding tendencies were reported by (Haag and Staton, 2003). Histological techniques provide data about the reproductive status of pacific oyster studied by (Lango-Reynoso et. al, 2000). The reproductive status in molluscs is a cyclic process, it may be annual, semiannual or continuous processes were observed by some workers, (Ghosh and Ghose, 1972; Nagabhushanam & Mane, 1975; Drummond et al., 2006; Siddique et.al., 2019). Growth and reproduction is grossly regulated by various environmental factors, particularly temperature and food availability reported by (Baqueiro and Aldana, 2000). Food constraints also hampered gonadal recovery during spawning events observed by (Delgado and Camacho, 2005).

The present study was undertaken, to investigate the seasonal reproductive status of *Lamellidens marginalis* in a lentic habitat of Nathsagar dam at Paithan. The histological study also aimed to examine the habitat specific seasonal changes in maturation of gametes, reproductive growth, spawning and gametogenic cycle with relation to abiotic factors.

II. MATERIAL AND METHODS

Freshwater pearl mussels, *Lamellidens marginalis* were collected from lentic habitat (Latitude 19° 29' 8.7" N, Longitude 75° 22' 12" E) (figure-1) of Nathsagar dam at Paithan during monsoon season, winter season and summer season. After brought to the laboratory, they were washed and brushed with water for removing algal biomass and mud. During each seasons, the animals were acclimatized for 24 hours in laboratory conditions. After it shells length, whole body weight, shell height, shell width were measured. After dissecting the animals, the weight of soft body tissues were measured. Five mussels were selected for the study. Gonads were removed and kept in Bouin's Hollande fixative for 24 hrs. for hardening. For further process tissues taken out from fixative and kept under tap water to remove excess fixative. Water content was removed by dehydration process, in different concentration of ethyl alcohol ranging from 30%, 50%, 70%, 90%, to 100% and xylene. After dehydration process tissues were kept in xylene wax for half an hour then blocks were made in L- shaped steel blocks. After that they were kept at room temperature for 2 hrs. and then placed inside the freezer for overnight. Ribbons were taken out from freezer and the sections were cut out at 6.0 - 7.0 μ m thickness using spencer rotary microtome. Sections taken on a slide, slides run over a flame gently to melt wax. Then sections were kept in xylene for two minutes then hydrates the slides in descending order of alcohol grade from 100% to 30% after staining, mounting was done by using DPX. Gonads were stained with haematoxylin and eosin stains by Luna, 1968). All the section was observed under the research binocular microscope and wherever necessary, measurements (particularly of oocytes and hepatic tubules) were made before microphotography. Water quality parameters, such as water temperature, dissolved oxygen, hardness and pH was measured monthly at habitat of mussels sampling sites.

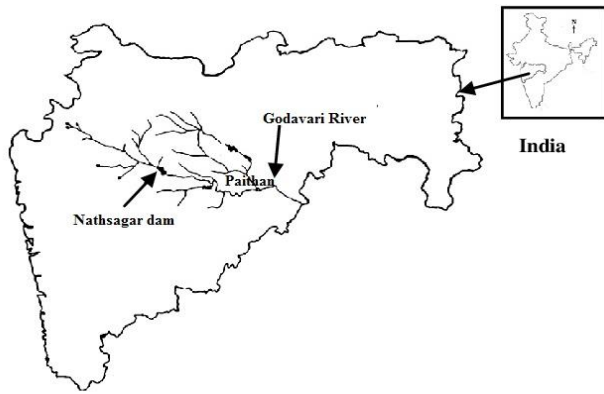


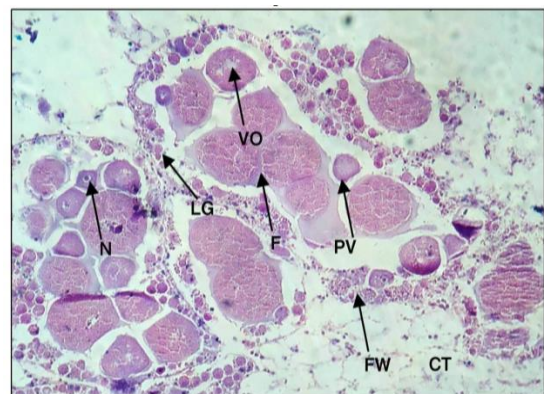
Fig. 1: Location showing study site of Nathasagar dam (Latitude 19° 29' 8.7" N, Longitude 75° 22' 12" E) at Paithan.

III. RESULT

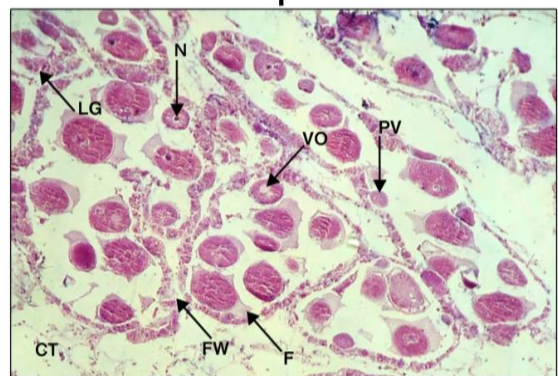
The physiochemical parameters of the water on the mussels habitat sampling sites were temperature 31.0°C-33.0°C, pH 7.5- 7.9, hardness 85.30 – 102 ppm and dissolved oxygen content 5.7428- 6.9918 ml/lit during summer; temperature 25.8°C- 28.5°C, pH 7.2- 8.0, hardness 145.35 - 164.79 ppm and dissolved oxygen content 5.6910- 7.022 ml/lit during monsoon and temperature 22.1°C- 23.5°C, pH 7.1- 7.7, hardness 107.52 - 125.02 ppm and dissolved oxygen content 6.99- 8.37 ml/lit during winter seasons.

A microscopic examination of the gonadal maturity stages in *Lamellidens marginalis* is described as follows.

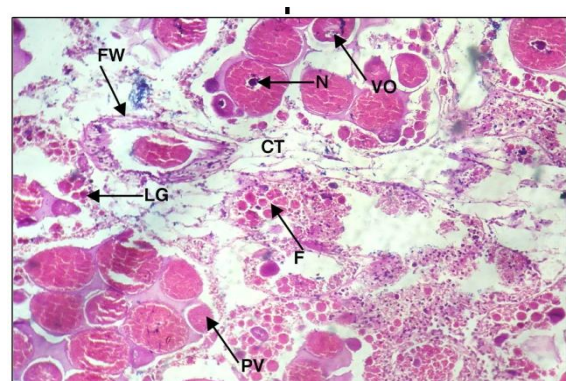
- **Gametogenesis stage:** in this stage, the female follicles were mostly packed with germ cells, nutritive cells and lipid globules. The male follicles showed sperm morulae, compact spermatids with free spermatozoa and however, the size of the interfollicular connective tissues started to be reduced.
- **Early Maturation stage:** in this stage, the very small follicles with primary or secondary oögonia and oocytes grow also vitellogenesis takes place during the vegetative phase of gametogenesis. The male gonadal tissues have reached its maximum development; Spermatids and matured spermatocytes are seen near the lumen in the follicles.
- **Late Maturation stage:** in this stage, vitellogenic oocyte enlarges in size and nucleus becomes a rounded. In males, the lumens of follicles were fulfilled with matured spermatozoa.
- **Spawning stage:** At this stage, follicles become empty due to the release of such gametes from the gonad hence follicle's size is reduced and compressed. In male follicles, sperms are released and a few yet to be released from many follicles still show spermatids and formation of sperms.
- **Cytolysis stage:** in this stage, both male and female gonads show empty follicles, very few released gametes are seen in the follicles, which subsequently undergo lysis and probably support the nourishment to the developing gametes.



(a) Monsoon Season



(b) Winter Season



(c) Summer Season

Fig. 1: Female Reproductive Status in *Lamellidens marginalis* during (a) monsoon Season (b) winter season (c) Summer season

F	=	Follicle
VO	=	Vitellogenic oocytes
N	=	Nucleus
LG	=	Lipid globule
O	=	Oögonia
FW	=	Follicular wall
PV	=	Previtellogenic oocytes
CT	=	Connective tissue

IV. DISCUSSIONS

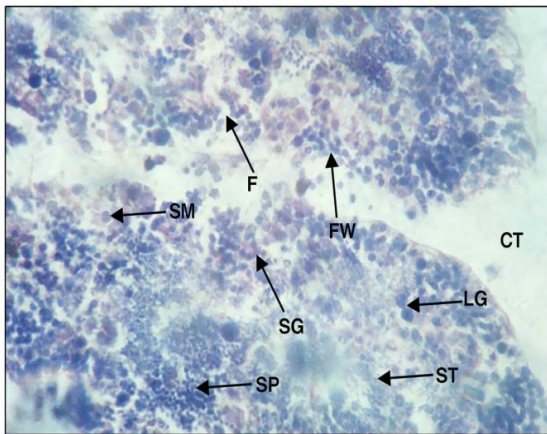
In the present study carried out on Freshwater pearl mussels, *Lamellidens marginalis* were collected from lentic habitat of Nathasagar dam at Paithan, it is revealed that, The gametogenic activity begins in males and females during December and January during winter also maturation phases and spawning simultaneously in both sexes were observed during summer and early monsoon seasons.

During study period, it was noticed that various physicochemical parameters of water and exogenous factor affects the reproductive activities and gametogenesis. Temperature is the most important exogenous factor in maturation of gametes and spawning. (Niogee et, al.2019) It has been shown that the *Lamellidens marginalis* females species spawning above 22.0 °C to 29.57 °C temperature. In contrast to the present study where the spawning were observed temperature from 25.8°C – 33.0 °C. Hence concluded that gradually rising temperature triggers the spawning whereas at rising temperature the gametogenesis process has been completely affected. (Lagade et.al.2019) had observed low and high temperature ranges show significant on follicular rupture, arresting of oogonia, autolysis in oocytes.

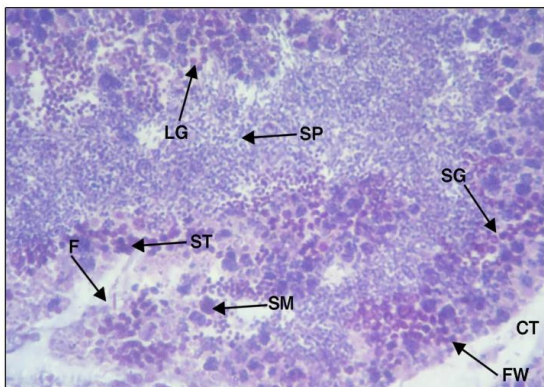
The dissolved oxygen (DO) is the most important abiotic factor influencing the biological life in an aquatic environment. In the current study, observed DO level 6.99-8.37 ml/lit during winter seasons were relatively higher than the other seasons, indicated that the values were acceptable for gametogenic phase of *Lamellidens marginalis*.(Kafi et. al.,2021) reported that low DO concentration of water retards the development of oocytes in *Lamellidens marginalis*. The pH and hardness both are important factors affecting Eco-physiological processes in the bivalves reported by (Farr and Payne, 2010). Highly acidic waters cause shell erosion and eventual death observed by (Kat, 1983). Increase in hardness in water favourable for the growth of molluscs reported by (Garg et al., 2009) In the present study habitat water of *Lamellidens marginalis* was alkaline throughout seasons with small seasonal variation. (Siddique et al., 2019) reported that the alkaline pH values were suitable for growth and reproduction of mussels.

In the present investigation, it was found that the reproductive cycle of *Lamellidens marginalis* is composed of four periods; developing, maturing, spawning, and Cytolysis stages. The resting stage was not noticed during this study. The gametogenic stage was synchronous between male and female species begins during the winter season. During gametogenic stage, the male follicles showed more compact sperm morulae and the inclusions of lipid globules in the follicles. The female follicles were mostly packed with germ cells, nutritive cells and lipid globules.(Vedpathak,1989), reported that lipid globules provide the nourishment of germination of spermatogonia and oogonia.

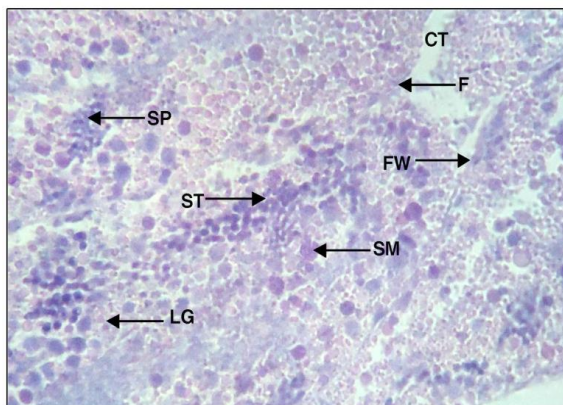
Maturation phases and spawning simultaneously in both sexes were observed during summer and early monsoon seasons. During maturing stages, vitellogenic oocyte enlarges in size and nucleus becomes a rounded. In male follicles spermatogonia, spermatocytes and spermatids are



(a) Monsoon Season



(b) Winter Season



(b) Summer Season

Fig. 2: Male Reproductive Status in *Lamellidens marginalis* during (a) monsoon Season (b) winter season (c) Summer season

- F = Follicle
- ST = Spermatids
- LG = Lipid globule
- SP = Sperms
- SG = Spermatogonia
- FW = Follicular wall
- SM = Sperm morulae
- CT = Connective tissue

distinctly seen and the lumens of follicles were fulfilled with matured spermatozoa. Similar results were obtained by (Morad et al., 2018) in freshwater mussel *Chambardia rubens*. During present study gradually rising temperature affected the gametogenesis process and triggers the spawning hence certain follicles in both sexes empty (spawning) but few follicles into maturing stages were seen. During monsoon season, cytolysis stages seen, both sexes gonadal follicles were empty except for residual gametes they probably support the nourishment to the developing gametes. In the present study, the resting phase wasn't observed, during monsoon, high pH indicated plenty of food availability; hence no resting phase was seen. Food supply can restrict gonadal recovery after spawning explained by (Delgado and Camacho, 2005).

V. CONCLUSION

This work concluded future strategies for the development of pearl culture and essential conservation of pearl mussel species of Nathsagar dam. The data generated in this study provide useful information on the reproductive status and the seasonal variation of some physicochemical parameters of water associated with the reproductive stages comprising gametogenesis, maturation, spawning and Cytolysis.

ACKNOWLEDGMENT

The authors express their sincere thanks to Molluscan Endocrinology and Physiology Laboratory, Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for carrying out this research work. Thanks are also due to Principal, M. G. V's, Arts, Science and Commerce College, Manmad for his constant encouragement and providing necessary facilities.

REFERENCES

- [1.] Baqueiro C.E., and Aldana, D. A. "A review of reproductive patterns of bivalve mollusks from Mexico", in Bulletin of Marine Science, 66(1): 13-27, 2000.
- [2.] Drummond, L., Mulcahy, M. & Culloty, S. "The reproductive Biology of the Manila clam, *Ruditapes philippinarum*, from the north-west of Ireland, in Aquaculture 254, 326–340, 2006.
- [3.] Farr, M. D., & Payne, B. S., "Environmental Habitat Conditions Associated with Freshwater *Dreissenids*", in US Army Corps of Engineers, Engineer Research and Development Center, Environmental Laboratory, ERDC/EL TR-10-24, December 2010.
- [4.] Garg, R. K., Rao, R. J. and Saksena D. N., "Correlation of molluscan diversity with physico-chemical characteristics of water of Ramsagar reservoir, in India", Internat. J. of Biodiv. and Conserva. 1(6): 202-207, 2009.
- [5.] Ghosh, C. & Ghose, K.C., "Reproduction system and gonadal activities in *Lamellidens marginalis*", The Veliger 14, 283–288, 1972.
- [6.] Haag, W. R., and Staton, J. L., "Variation in fecundity and other reproductive traits in freshwater mussels", in Freshwater Biology, 48(12): 2118-2130, 2003.
- [7.] Kat P.W., "Sexual selection and simultaneous hermaphroditism among the Unionidae (Bivalvia: Mollusca)", in J. Zool. 201:395–416, 1983.
- [8.] Lango-Reynoso, F.; Chavez-Villalba, J.; Cochard, J. and Le Pennec, M., "Oocyte size, a means to evaluate the gametogenic development of the Pacific oyster, *Crassostrea gigas* (Thunberg)", in Aquaculture, 190(1-2): 183-199, 2000.
- [9.] Luna, L.G., "Manual of histological staining methods of Armed forces Institute of Pathology. New York, McGraw-Hill. 258 pp., 1968.
- [10.] M. Abubakar Siddique, M. Asha Khatun, M. Mashur Rahman, Gias Uddin Ahmed, Mohammad Moniruzzaman & M. Jasim Uddin, "Annual gametogenic cycle of the freshwater pearl mussel, *Lamellidens marginalis* (Lamarck, 1819) collected from a perennial lentic habitat of Bangladesh", in Molluscan Research, DOI: 10.1080/13235818.2019.1682954, 2019.
- [11.] M.Morad, M.F.Fol, I.S.Gamil, M.Mansour, "Gonad variation and development of freshwater mussel *Chambardia rubens* Lamarck, 1819(Bivalvia: Mutelidae) from the river Nile in Egypt, in Journal of Bioscience and Applied Research, Vol.4, pp.495-506, 2018.
- [12.] Marina Delgado and Alejandro Pérez Camacho, "Histological study of the gonadal development of *Ruditapes decussatus* (L.) (Mollusca: Bivalvia) and its relationship with available food", in *Scientia Marina*, sci. Mar., 69 (1): 87-97, 2005.
- [13.] MD. F.H. Kafi, MD. H. Islam, MD.H.Uddin, K.A.Sumon, and H.Rashid, "Effects of dissolved oxygen concentrations on growth, survival and gonadal development of freshwater pearl mussel *Lamellidens marginalis*", in Bangladesh J. Fish. 33(1): 99-108, 2021.
- [14.] Nagabhushanam, R. and Mane, U. H., "Reproduction in the mussel, *Mytilus viridis*, at Ratnagiri", in Bull. Dep. Mar. Sci., Univ. Cochin, India, 7: 377-387, 1975.
- [15.] S.T.R.Niojee, K.F.Tonni, A.C.Barman, M.B.Tanu, S.Sku, M.J. Uddin, "Ovarian cycle of freshwater pearl mussel, *Lamellidens marginalis* (Lamarck, 1819) collected from a culture pond in Bangladesh, in Asian Fisheries Science 32:pp. 117-123, 2019.
- [16.] V.M.Lagade, S.S. Taware, S.V.Lagade, D.V.Muley, "Effect of temperature on cytological alterations in gonads of estuarine bivalves at Bhatye estuary, Ratnagiri coast, in Asian journal of Biological Sciences, 12(4) pp.860-868,2019.
- [17.] Vedpathak, A. N. "Reproductive Endocrinology of some Lamellibranch Molluscs with special reference to Environmental stress", in Ph. D. Thesis, Marathwada University, Aurangabad, (M.S.) India. pp. 1-322, 1989.