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Research Article
Effect of Temperature on Cytological Alterations in Gonads of Estuarine Bivalves at Bhatye Estuary, Ratnagiri Coast

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Abstract

Background and Objective: Histological studies give the clear and accurate forecasting on alterations of cellular framework under various stresses. Keeping this vision, present study was undertaken to highlight the cytological alterations in reproductive organs of estuarine clams like *Katleysia opima* and *Meretrix meretrix* under exposure of various temperature ranges. **Materials and Methods:** In this study, two selected estuarine clams were exposed to various temperatures ranges for 192 h such as 14, 19, 24 and 34°C, respectively. After exposure, male and female gonads (testis and ovary) were dissected out for cytological observation. The staining procedure was done by using Harris hematoxylin and alcoholic eosin stain (HE technique). **Results:** In *K. opima* clam, the male and female gonad was recorded severe alterations at high temperature (34°C) followed by 14, 19 and 24°C, respectively. However, in *M. meretrix* male gonad was noted major alteration at low temperatures (14°C) followed by 34, 19 and 24°C, respectively. Whereas, the female gonad of *M. meretrix* showed as like *K. opima* i.e., at high temperature (34°C) followed by 14, 19 and 24°C, respectively. **Conclusion:** In this attempt, the both extreme high temperature and low temperature produced adverse impact on gonadal cells and developmental process of clams. Experimental temperature shows significant and major cytological alterations in *M. meretrix* clam than *K. opima*. Present study reveals that the *M. meretrix* clam is quite more sensitive than *K. opima* to the changing temperatures ranges. These cytological alterations of estuarine clams that could be used to diagnosis the structural changes in cellular architecture under changing temperatures. This base line information would more beneficial particularly for ecologically and economically important clam species.

Key words: Temperature ranges, estuarine clams, male and female gonads, cytological alterations

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A large number of marine bivalves are inhabitant of intertidal zone, and this zone is subjected to wide range of variations in external factors like temperature, salinity, action of waves and availability of oxygen as well as food. Reproduction in bivalves was completed when gonads mature or fully developed than both male and female gonads releases their gametes i.e., spermatozoa and eggs in to the water and fertilization take place outside in water[1]. Early literatures says that, the gametogenic development in marine invertebrates is mainly depends upon the external factors[2-4]. Some authors concluded that, the reproductive behavior is very sensitive to both external as well as the internal agents[5,6]. Reproduction in clam is completed into different stages like gonadal development, spawning and fertilization and development and growth. These all stages are functionally carried out in co-ordination with the seasonal environmental parameters[7].

The reproductive behaviour in clams is a cyclic processes and it may be annul, semiannual or continuous processes. The synchronization conditions are most favorable for significant reproduction success. In external factor, temperature is one of the important factor, limiting the reproductive behavior and all metabolic activities with their distribution are regulate with temperature fluctuation[8]. These factors can effects on both gametogenesis and spawning processes of various clam species[9-13]. The temperature is also responsible to inducing the spawning behavior in certain oyster species during winter season[14]. Temperature also plays an important role in development of sex cells and spawning behavior in various clam species[15,16]. Suja and Muthiah[17] have been showed the relationship between temperature and gonad development in baby clam, *Marcia opima*. On the other hand, the temperature is regulating indirectly gonad histology and gametogenesis and all important phases of gamete development[18,19]. Osada *et al.*[20] reported quantitative analysis of pattern of gonial proliferation during sexual maturation in Japanese Scallop *Patinopecten yessoensis*. According to literatures the histological method helps in understanding the cellular and sub-cellular alterations in organ or tissue rather than external demonstration[21-23].

Present study aimed to elucidating the histological changes in gonads after exposure to low and high temperature ranges on two ecologically and economically important estuarine clams i.e., *Katleysia opima* and *Meretrix meretrix*.

MATERIALS AND METHODS

Study area: The Bhatye estuary is situated at 73°15' E and 16°51' N near Ratnagiri (Maharashtra). The Bhatye estuary is very productive with diversified mangrove habitat and aquatic resources (shellfish, finfish and other invertebrate) are abundantly found in this estuary. Estuary is formed by river Kajli, the river originates from Amba forest, which meet to west coast of Arabian Sea near Ratnagiri. The Bhatye estuary having open shore on western side with fine sandy patch and on eastern of side estuary with less sand while more muddy in Bhatye village side. Mangrove Island is found about 1 km^2 in estuary while the mangrove vegetation is spreads almost 10 km interior in to river.

Animal collection: In present study, two estuarine *Veneridea clam* species were selected for experimental studies from Bhatye estuary, the species are *Katelysia opima* (Gmelin, 1791) and *Meretrix meretrix* (Linnaeus, 1758). This study was carried out during the month of August-December, 2010).

The experimental clams were collected from Bhatye estuary during low tide with the help of local fishers. The collected clams were brought to the laboratory, cleaned off to remove the adhered mud and sand particles and were kept in sea water in laboratory for 48 h at room temperature for acclimatization. The average sized (32-36 mm) clams were selected the experimental purpose.

Experiment design: The experimental work was carried out at CCMB Laboratory at Bhatye, Ratnagiri. In experimental work, two selected clams were exposed to various temperatures. Both lowered and higher temperature ranges were selected for exposure, both ranges (lower and higher) were selected on the bases of normal temperature of the month. During study period (August-December, 2010), the normal temperature was 29±1°C, therefore the experimental ranges were 14, 19 and 24°C as a below normal and 34°C as above normal were maintained. All experimental temperature i.e., lower range of temperature was maintained by ice cold water while higher range of temperature was controlled by thermostat heater.

The clams were exposed regularly to the respective temperatures for 8 days (192 h) at laboratory along with control group. During exposing period every 6 h the water of experimental sets was changed with cotton filtered sea water

and during experimentation of exposure the clams were never feed. For histological inspection, after end of exposure period i.e., 192 h the gonadal tissue samples (male and female) were selected from both experimental clams and as well as from control groups. The tissues were dissects out from clams and preserved in Bouin's fixatives to further microscopy study. Preserved tissues were removed and washed under tap water and then dehydrate in various alcoholic grades of ethyl alcohol. Dehydrated tissue were proceeded to block and slide preparation. The slides were prepared by routine micro-technique. The staining procedure was done by using Harris hematoxylin and alcoholic eosin stain (HE technique) and mounted in DPX. All the observations for microphotography (400X) were made with the help of Leica DM 2000 microscope.

RESULTS

In the present investigation, an attempt has been made to see the effect of temperature on cytological or structural alteration in gonads (male and female) of two venerid estuarine clams (*K. opima* and *M. meretrix*).

Temperature induced cytological alterations in gonads of *K. opima* and *M. meretrix*

Control: During study period, the male gonad of *K. opima* and *M. meretrix* were showed large number of developing spermatogonia/germ cells distributed along the periphery of the follicle and spermatid and spermatozoa were also observed which shows all developing phase of the gonad (Fig. 1 and 3a). Whereas, in female gonad of both clams were recorded the developing follicles with mature eggs having nucleus, nucleolus and nuclear materials. Some of the mature eggs were at the stage of autolysis or in some eggs complete autolysis was occurred (Fig. 2 and 4a).

Histological alterations in the male gonads: After exposure to various temperature ranges such as 14, 19, 24 and 34°C for 192 h, the experimental temperatures produced significant changes in the cytological structure of male gonads of both clams. At lower temperature (14 and 19°C) in *K. opima*, the outer membrane of follicle was completely disintegrated, mature spermatozoa/sperm count was meagerly noticed and few spermatogonial and spermatids were noticed in inter-tubular spaces (Fig. 1b-c). In *M. meretrix*, the complete integrity of follicle wall was collapsed which result in spreading of spermatogonial cells and spermatids in the interstitial space (Fig. 3b-c). At 24°C, there was no significant alteration recorded except some portion of follicle was impaired in both clams (Fig. 1 and 3d). At high temperature i.e., 34°C, the both clams showed the follicle membrane was ruptured from all sides. All follicular cells such as spermatogonial cells, spermatocytes and spermatids were arrested and cease their further development (Fig. 1 and 3e).

Histological alterations in the female gonads: In female gonad of *K. opima* and *M. meretrix*, at 14 and 19°C, the follicular thickness was reduced, while some follicles were damaged and shrunken follicle with follicular wall ruptured, condensation and disorientation of nucleus and nuclear materials and leaking of ooplasm in the lumen of mature oocyte were significantly recorded (Fig. 2 and 4a-c). At 24°C, the both clam shows more or less alterations in follicles and oocytes (Fig. 2 and 4d). At high temperature (34°C), the follicles of both clams were completely collapsed due to rupture of follicle membrane. In addition to this, alterations like, arrested developing oocyte, shrunken of lumen of mature oocyte and disoriented of nuclear materials were observed. Some of the follicles were observed without maturing oocytes, may be due to cytolysis (Fig. 2 and 4e). It means that at high temperature both gonads were recorded severe alterations than lower ranges such as 14, 19 and 24°C, respectively.

DISCUSSION

In organism, reproductive behavior is the most important stage and this behaviour is chiefly control by both, the external and internal agents[6]. The external factors decide the gametogenesis processes in marine organism[5]. Amongst external factor, temperature is one of the vital agents, controlling the reproductive phases including all metabolic activities[8]. In the oyster, *Crassostrea angulata* (gigas) spawning behaviour is induced by temperature[14]. According to Ruiz *et al.*[16], the development in sex cells and spawning behavior amongst various clams are controlled by temperature.

In the present study, both gonads of clams showed severe damage in the follicle and sex cells which consequences in ceased gonad development and, proliferation of sex cells was retarded after exposure of temperature. Therefore, the normal spermatogenesis and oogenesis process in gonads were condensed. In case of male gonads, at extreme temperatures (low and high) the primary germ cell i.e., spermatogonia and spermatids were

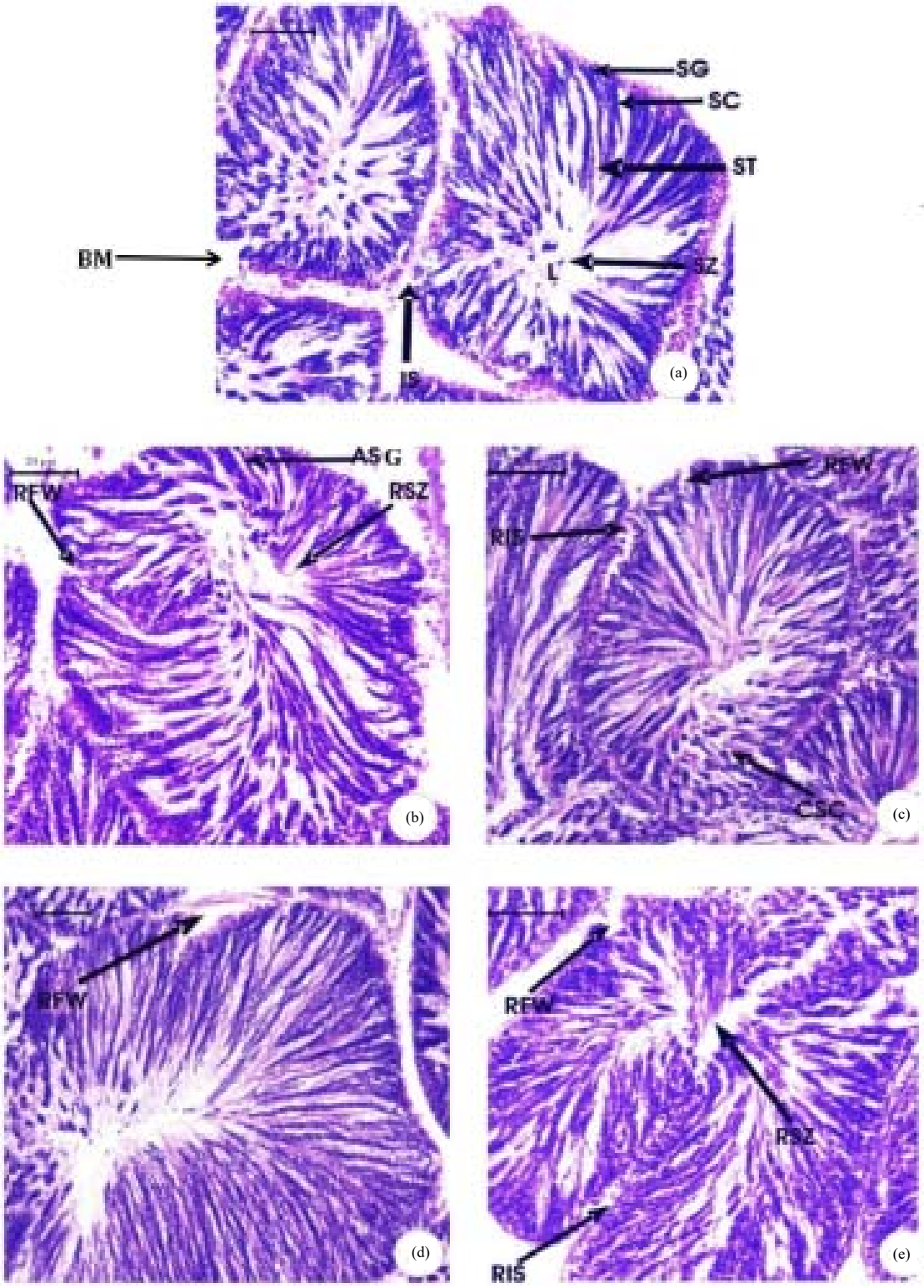

Fig. 1(a-e): Cytological alterations in male gonad of *K. opima* after exposure to temperature ranges (a) Control group (29°C±1°C), (b) 14°C, (c) 19°C, (d) 24°C and (e) 34°C

IS: Interstitial space, SG: Spermatogonia, SC: Spermatocytes, ST: Spermatid, SZ: Spermatozoa, RIS: Reduction of interstitial space, RFW: Rupture of follicle wall, ASG: Arrested spermatogonia, CS: Clumping of spermatocytes, L: Lumen, RSZ: Reduction of spermatozoa, BM: Basement membrane

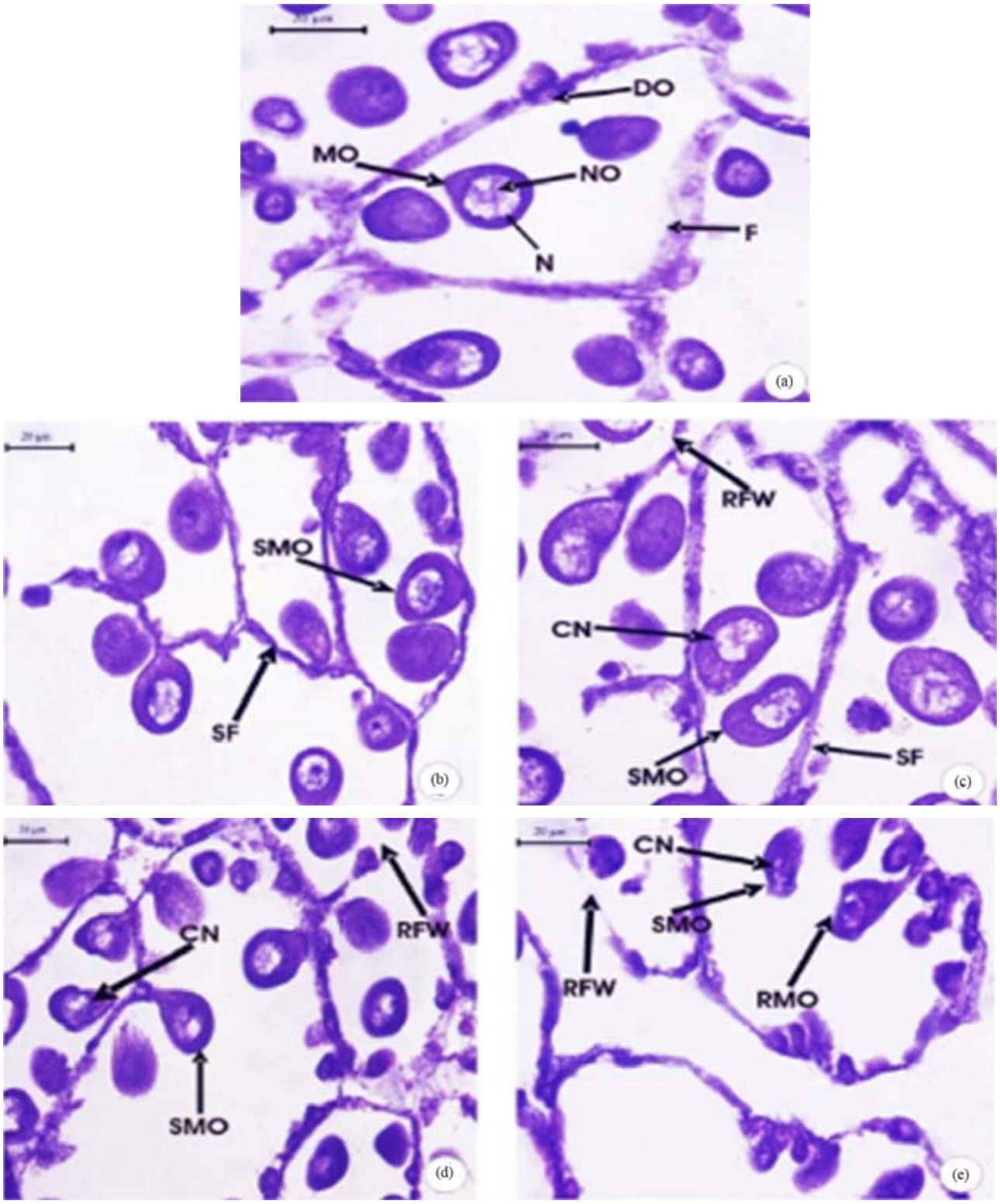

Fig. 2(a-e): Cytological alterations in female gonad of *K. opima* after exposure to temperature ranges (a) Control group (29°C±1°C), (b) 14°C, (c) 19°C, (d) 24°C and (e) 34°C

F: Follicle, DO: Developing oocyte, MO: Mature oocyte, N: Nucleus, NO: Nucleolus, CN: Condensed nucleus, SF: Shrinkage of follicle, RFW: Rupture of follicle wall, SMO: Shrinkage of mature oocyte, RMO: Rupture of mature oocyte

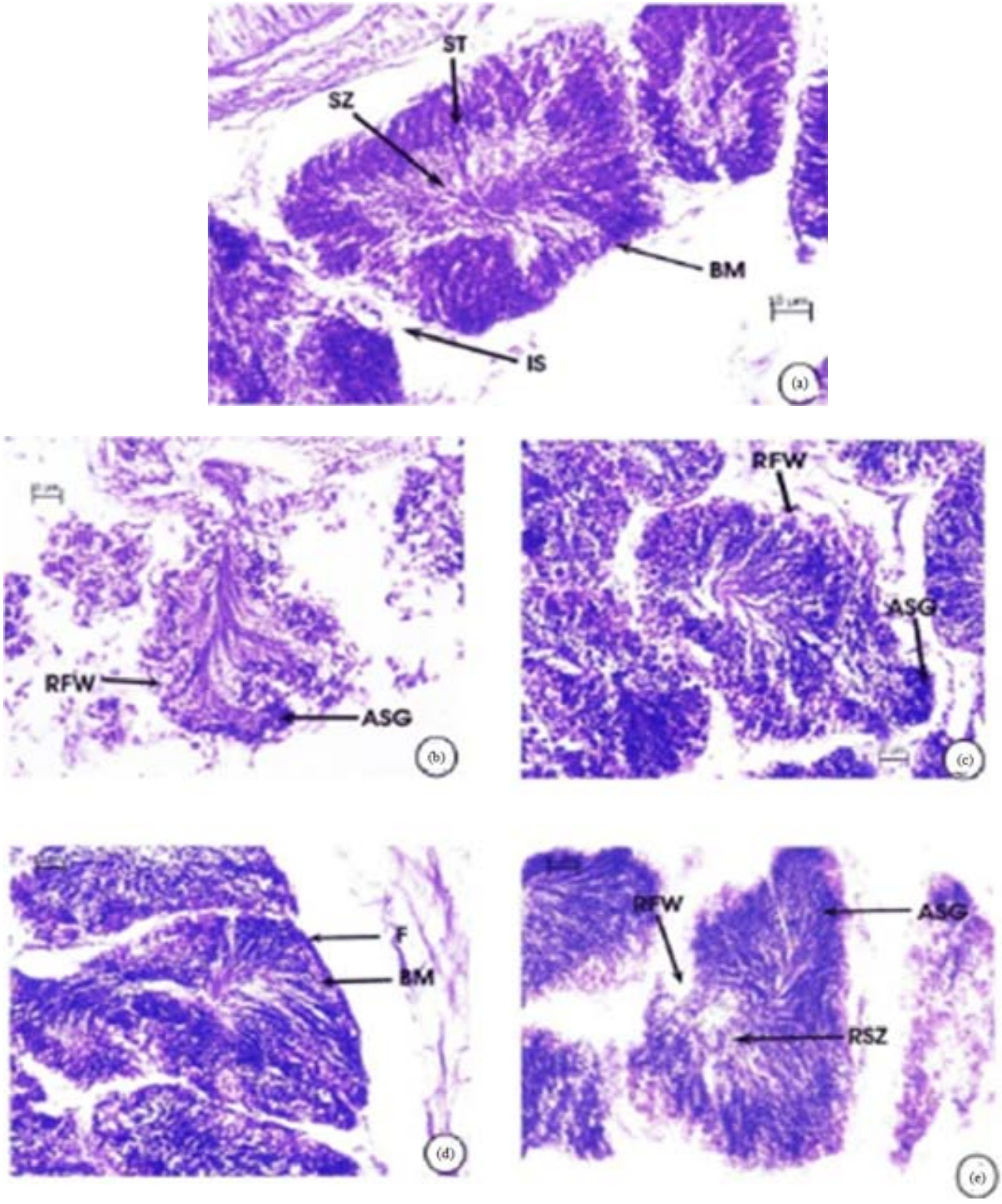

Fig. 3(a-e): Cytological alterations in male gonad of *M. meretrix* after exposure to temperature ranges (a) Control group (29°C±1°C), (b) 14°C, (c) 19°C, (d) 24°C and (e) 34°C

BM: Basement membrane, IS: Interstitial space, ST: Spermatid, RSZ: Reduction of spermatozoa, SZ: Spermatozoa, L: Lumen, RFW: Rupture of follicle wall, ASG: Arrested spermatogonia

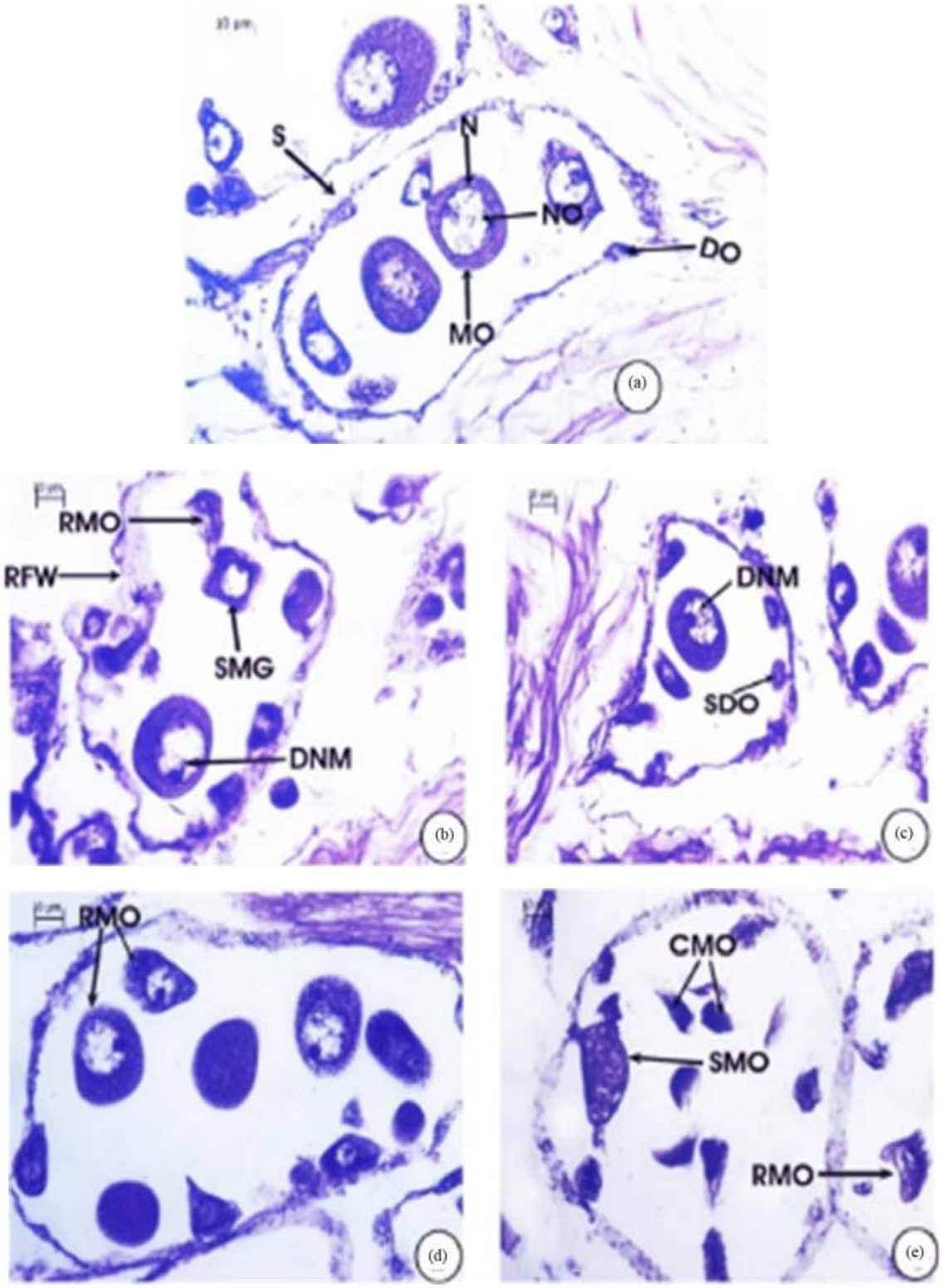

Fig. 4(a-e): Cytological alterations in female gonad of *M. meretrix* after exposure to temperature ranges (a) Control group (29°C±1°C), (b) 14°C, (c) 19°C, (d) 24°C and (e) 34°C

S: Stroma, DO: Developing oocyte, MO: Mature oocyte, N: Nucleus, NO: Nucleolus, DNM: Disorient nuclear material, RFW: Rupture of follicle wall, SMO: Shrinkage of nature oocyte, SDO: Shrinkage of developing oocyte, RMO: Rupture of mature oocyte, CMO: Cytolysis of mature oocyte

condensed, hence their further development was stopped. The follicular membrane integrity was also collapsed, so complete structural architecture of follicle in male gonad was destroyed. In earlier studies, few workers like Lannan *et al.*[15] and Ruiz *et al.*[16] has proved that the development of sex cells is temperature dependent.

While on the other hand, female gonad showed significant alterations like decline of stroma and follicular rupture with further, arresting of oogonia, complete autolysis in oocytes and such alterations were observed particularly at low and high temperature ranges. A study by Suja and Muthiah[24] has observed significant effects of different temperatures on oocyte diameter. They have observed comparatively more decreased in oocyte diameter at 23°C range than 28°C. Honkoop and Van der Meer[25] also observed that, the temperature and immersion affects the egg size in *Cerastoderma*. However, in *Macoma* no significant change was observed, whereas the egg number was constantly affected. Paulet and Boucher[19] has stated that water temperature regulate all important phases of gamete development.

According to Laing *et al.*[26], in limiting condition clam suppressed their oxygen requirement in spite of conserving the energy and maintenance for growth. Lubet *et al.*[27] has observed that successively increasing temperatures cease gametogenesis process in the gonad of *M. galloprovincialis*. Generally, in an organism at increasing temperature, gonad become transparent, it depicted that gonad tissue was utilized for maintenance. Therefore, it affects on both developing spermatocytes and oocytes and their number was reduced.

Relatively, temperature effect was more prominent on male gonads than female gonads at extreme low temperatures i.e., 14 and 19°C and high temperature 34°C. From these changes in both the clam species, it is evident that both gonads undergo cytological alterations when exposed to temperature, whereas at extreme temperature the gametogenesis (spermatogenesis and oogenesis) process has been completely affected. In gastropod, Boyle and Yoshino[28] have reported that the gonadal development and reproductive phases are limited by external factors such as temperature, photoperiod, food availability and water quality.

CONCLUSION

This study depicts changes in cytological structure of both tissues like male and female gonad of both the estuarine clam species under different exposure of temperature ranges. The present study shows different response of both tissues at various temperatures and this information will forecast the severity of water temperature (extreme low and high) on clams and its development. In culture practices and in the view of conservative measures this information will needed in propagation of both these estuarine clam species (*K. opima* and *M. meretrix*) particularly in the context of climate change.

SIGNIFICANCE STATEMENT

This study discovered the details of cytological alterations in gonads of estuarine clams under exposure of variant temperature exposures. This study would definitely beneficial to understand the occurrence, behavior, survival, biology and physiological aspects of two estuarine clams. This attempt will help the researchers to uncover the critical areas of potential impact of short term effect of low and high temperatures on reproductive stages of two estuarine clams. The *K. opima* and *M. meretrix* clams are commercial important food species at Ratnagiri coast. So far no one researcher has focused reproductive cycle in context of changing temperature along the Ratnagiri coast. Hence, from this study we came to a critical statement that, the disturbing reproductive stages with response to temperatures it affects the maintenance and recruitment of clams.

REFERENCES

1. Jespersen, A., J. Lutzen and G. Itani, 2009. Sperm structure and sperm transfer in *Pseudopythina subsinuata* (Bivalvia; Galeommatoidea). Zool. Anzeiger-A J. Comparat. Zool., 248: 57-67.
2. Sastry, A.N., 1970. Reproductive physiological variation in latitudinally separated populations of the bay scallop, *Aequipecten irradians* Lamarck. Biol. Bull., 138: 56-65.
3. Sastry, A.N., 1979. Pelecypoda (Excluding Ostreaidae). In: Reproduction of Marine Invertebrates, Giese, A.C. and J.S. Pearse (Eds.). Vol. 5, Academic Press, New York, pp: 113-292.
4. Adiyodi, R.G. and K.G. Adiyodi, 1983. Reproductive Biology of the Invertebrates: Oogenesis, Oviposition and Oosorption. Wiley, New York, Page: 700.
5. MacDonald, B.A. and R.J. Thompson, 1985. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. I. Growth rates of shell and somatic tissue. Mar. Ecol. Prog. Ser., 25: 279-294.
6. Steele, S. and M.F. Mulcahy, 1999. Gametogenesis of the oyster *Crassostrea gigas* in Southern Ireland. J. Mar. Biol. Assoc. UK., 79: 673-686.

7. Kamble, S. and D. Muley, 2015. Histological study of reproductive cycle of estuarine clam, *Katelysia opima*. Int. J. Adv. Res. Biol. Sci., 2: 39-43.
8. Mann, R., 1979. The effect of temperature on growth, physiology and gametogenesis in the Manila clam *Tapes philippinarum* (Adams and Reeve, 1850). J. Exp. Mar. Biol. Ecol., 38: 121-133.
9. Calabrese, A., 1970. Reproductive cycle of the coot clam, *Mulinia lateralis* (Say), in Long Island sound. Veliger, 12: 265-269.
10. Keck, R.T., D. Maurer and H. Lind, 1975. A comparative study of the hard clam gonad developmental cycle. Biol. Bull., 148: 243-258.
11. Hesselman, D.M., B.J. Barber and N.J. Blake, 1989. The reproductive cycle of adult hard clams, *Mercenaria* spp. in the Indian river Lagoon, Florida. J. Shellfish Res., 8: 43-49.
12. Kanti, A., P.B. Heffernan and R.L. Walker, 1993. Gametogenic cycle of the Southern surfclam, *Spisula solidissima similis* (Say, 1822), from St. catherines Sound, Georgia. J. Shellfish Res., 12: 255-261.
13. Lubet, P.E., 1994. Reproduction in Molluscs. In: Aquaculture: Biology and Ecology of Cultured Species, Barnabe, G. (Ed.)., Ellis Horwood Limited, London, pp: 138-173.
14. Patel, B. and D.J. Crisp, 1960. The influence of temperature on the breeding and the moulting activities of some warm-water species of *Operculate barnacles*. J. Mar. Biol. Assoc. UK., 39: 667-680.
15. Lannan, J.E., A. Robinson and W.P. Breese, 1980. Broodstock management of *Crassostrea gigas*: II. Broodstock conditioning to maximize larval survival. Aquaculture, 21: 337-345.
16. Ruiz, C., D. Martinez, G. Mosquera, M. Abad and J.L. Sanchez, 1992. Seasonal variations in condition, reproductive activity and biochemical composition of the flat oyster, *Ostrea edulis*, from San Cibran (Galicia, Spain). Mar. Biol., 112: 67-74.
17. Suja, N. and P. Muthiah, 2009. Effect of starvation and temperature on gonad development of baby clam, *Marcia opima* (Gmelin). J. Mar. Biol. Assoc. India, 51: 21-25.
18. Newell, R.I., T.J. Hilbish, R.K. Koehn and C.J. Newell, 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* L. (Bivalvia, Mytilidae) from localities on the east coast of the United States. Biol. Bull., 162: 299-310.
19. Paulet, Y.M. and J. Boucher, 1991. Is reproduction mainly regulated by temperature or photoperiod in *Pecten maximus*? Invertebrate Reprod. Dev., 19: 61-70.
20. Osada, M., S. Nakamura and A. Kijima, 2007. Quantitative analysis of pattern of gonial proliferation during sexual maturation in Japanese scallop *Patinopecten yessoensis*. Fish. Sci., Vol. 73. 10.1111/j.1444-2906.2007.01470.x.
21. Auffret, M., 1988. Histopathological changes related to chemical. Mar. Ecology-Prog. Ser., 46: 101-107.
22. Livingstone, D.R. and R.K. Pipe, 1992. Mussels and Environmental Contaminants: Molecular and Cellular Aspects. In: Development in Aquaculture and Fishery Science, Gossling, E. (Ed.)., Elsevier Pub. Co., Amsterdam, pp: 425-456.
23. Au, D.W.T., 2004. The application of histo-cytopathological biomarkers in marine pollution monitoring: A review. Mar. Pollut. Bull., 48: 817-834.
24. Suja, N. and P. Muthiah, 2007. The reproductive biology of the baby clam, *Marcia opima*, from two geographically separated areas of India. Aquaculture, 273: 700-710.
25. Honkoop, P.J.C. and J. Van der Meer, 1998. Experimentally induced effects of water temperature and immersion time on reproductive output of bivalves in the Wadden sea. J. Exp. Mar. Biol. Ecol., 220: 227-246.
26. Laing, I., S.D. Utting and R.W.S. Kilada, 1987. Interactive effect of diet and temperature on the growth of juvenile clams. J. Exp. Mar. Biol. Ecol., 113: 23-38.
27. Lubet, P., J.P. Gimazane and G. Prunus, 1981. Etude ducycle reproduction de *M. galloprovincialis* (Lmk.) Mollusque Lamellibranche a la limitemeridionale de 1981 son aire de repartition. Comparision Avec les Autres de Cteurs de Cette Aire, Haliotis, 11: 157-170.
28. Boyle, J.P. and T.P. Yoshino, 2000. The effect of water quality on oviposition in *Biomphalaria glabrata* (Say, 1818) (Planorbidae) and a description of the stages of the egg-laying process. J. Molluscan Studies, 66: 83-93.