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**TEMPERATURE INDUCED HISTOLOGICAL CHANGES IN THE FEMALE GONAD OF FRESHWATER BIVALVE, *INDONAI CAERULEUS* DURING DIFFERENT SEASONS****Jadhav M.R.\* , Gulave A.R.\*\* and Pawar S.L.\*\*\***

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[E-mail: mangesh22186@gmail.com](mailto:mangesh22186@gmail.com)**ABSTRACT**

The adult freshwater bivalve molluscs, *Indonai caeruleus* (47-51 mm in shell-length) were collected and brought to laboratory from banks of Godavari River at Paithan, during different seasons. After 24 h laboratory acclimatization, they were exposed to normal water temperature of 28.5-31.0oC (served as control) during summer, 25.5-26.0oC during monsoon and 21.5-23.0oC during winter. For experimentation, the animal was exposed to the elevation of water temperature 34.0oC, 30.0oC and 27.0oC during summer, monsoon and winter respectively for 15 days. The histological studies of the female gonads were made after 15 days. During different seasons, in control group the study revealed dominance of gametogenic phase during summer, developmental stages of oogonia during monsoon and maturation and release of oocytes during winter seasons. Due to elevation of temperature during different seasons, enlargement (growth) of oocytes as well as release of oocytes were observed at the expense of lipid globules and nutritive cells. The follicles of the female gonads expanded more and ovarian interfollicular connective tissue was observed to reduced, which was more pronounced at 34.0oC during summer compared to 30.0oC during monsoon and 27.0oC during winter season. However, in experimental group, normal developments of oocytes were observed and they were released during monsoon and winter. More release of oocytes was observed during winter at 27.0oC. The results are discussed in the light of effect of elevated temperature on the histological structure of female gonadal tissues of bivalve molluscs.

**KEYWORDS:** Elevation of temperature, female gonad, size of oocytes, bivalve molluscs, Godavari river, different seasons.**INTRODUCTION**

Many environmental factors are known to affect the physiological processes, such as respiration, excretion, reproduction in the bivalve molluscs. The phases of the reproduction i.e; gonad development; spawning and fertilization; and development and growth of zygotes are functioning continuously in co-ordination with changes in environmental components, more specifically temperature. In bivalve molluscs, synchronization of breeding periods with environmental variables especially temperature, salinity, light and food for development and growth has been extensively reported (Andrews, 1979; Sastry, 1979 and Mackie, 1984). Reproductive cycle of the bivalve species is genetically controlled in response to the environment (Sastry, 1970a). Recent studies indicate that a reproductive response is produced through an interaction of environmental factors, especially temperature, salinity, pH, light, food and endogenous factors within the organisms. After attaining a certain physiological state, an organism exposed to the required environmental pre-requisitions begins the gonad growth and gametogenesis. The temperature was considered to be an important environmental factor which affect the survival activity and metabolic processes have been reviewed periodically (Widdow, 1973).

The sequence of events related to gonad growth, gamete maturation, release of gametes and development of eggs are thermally sensitive (Kinne, 1962, 1970). Temperature also greatly influences the sexual maturity, spawning and development life stages of aquaculture species. The influence of temperature on the reproduction of marine invertebrates, including pelecypods has been reviewed by Giese, (1959a); Vernberg, (1962); Loosonoff, (1971) and Giese and Pearse, (1974). Considering the immense scope as food resource, there is a necessity of shell-fish production through manipulated reproductions in aquaculture species by altering the environmental factors, especially elevation of temperature. Hence present study was undertaken on *Indonai caeruleus*, during different seasons.

**MATERIALS AND METHODS**

The adult freshwater bivalve molluscs, *Indonai caeruleus* (47-51 mm in shell-length) were collected from the right banks of Godavari river at Paithan, near Aurangabad during summer, monsoon and winter seasons. Soon after brought to the laboratory, the shell-valves of the animals were brushed and washed with clean water so as to remove the fouling algal and fungal biomass and mud. During each season, the animals were acclimatized for 24 hrs. at laboratory conditions in a large aquarium containing sufficient aerated reservoir water. After 24 hrs. Laboratory

adjustments, 20 animals were kept in each of two groups, during each season. One group served as control of normal laboratory water temperature 28.5-31.0oC during summer, 25.5- 26.0oC during monsoon and 21.5-23.0oC during winter and others were experimental at different elevation of temperatures of water i.e, 34.0oC during summer, 30.0oC during monsoon and 27.0oC during winter seasons. The temperature of the experimental groups during each season was controlled by the use of thermostats (Automatic, RENA, France), fixed at the bottom of aquarium. In experimental aquarium care was taken that the bivalves were kept just away from the thermostats to avoid the direct effect of increase in temperature.

The water of the appropriate temperatures from the experimental as well as from control was renewed at every 12-13 hrs. During each season, the experiment was run for 15 days for the better results. For histological study of gonads, shell –valves of five animals from control and five from experimental were removed on 15th day. After soaking the animal bodies on the blotting paper, they were fixed in Bouins Hollande fixative for 24 hrs.. The fixative was renewed for next 24 hrs. to fascinate better fixation. During each season, the gonadal tissues of female individual from both the groups were removed and processed for preparation of paraffin blocks.

Dehydration of gonadal tissues of female individuals were done through serial grades of ethyl alcohol and tertiary butanol for better results, while xylene was replaced by toluene in the processing. The tissues were embedded in paraffin wax at 58-60oC and sections were cut at 6.0-7.0  $\mu$ m thickness using Spence- rotary- microtome. The sections were stained with haematoxyline-eosin as well as Mallory Triple Stain (MTS) and they were observed under research binocular microscope before photomicrography. Wherever necessary, the measurements of diameter of oocytes were also made and subjected for statistical analysis using student's 't' test (Dowdswell, 1957).

## RESULTS

The physico-chemical parameters of water used for experimentation were, temperature (28.5oC - 31.0oC), pH (7.68- 7.70), hardness (118-123 ppm) and oxygen contents (5.684- 6.330 ml/l) during summer; temperature (24.5-26.0oC), pH (7.79- 7.81), hardness (107-111 ppm) and oxygen contents (6.171-6.577 ml/l) during monsoon and temperature (22.0-23.5oC), pH (7.73- 7.74), hardness(97-103 ppm) and oxygen contents (6.334- 6.741 mg/l) during winter season. The results of the experiment were given in plate-1.

Histological studies carried out during different seasons revealed that in control, female follicles are diverse greatly in shape and size and they are externally lined with thin line of connective tissue and internally with simple layer of germinal epithelium. The follicles stowed different stages of oogonial development during different seasons. Each follicle contains different stages of development of oogonia and abundant nutritive cells and lipid globules particularly during summer and monsoon.

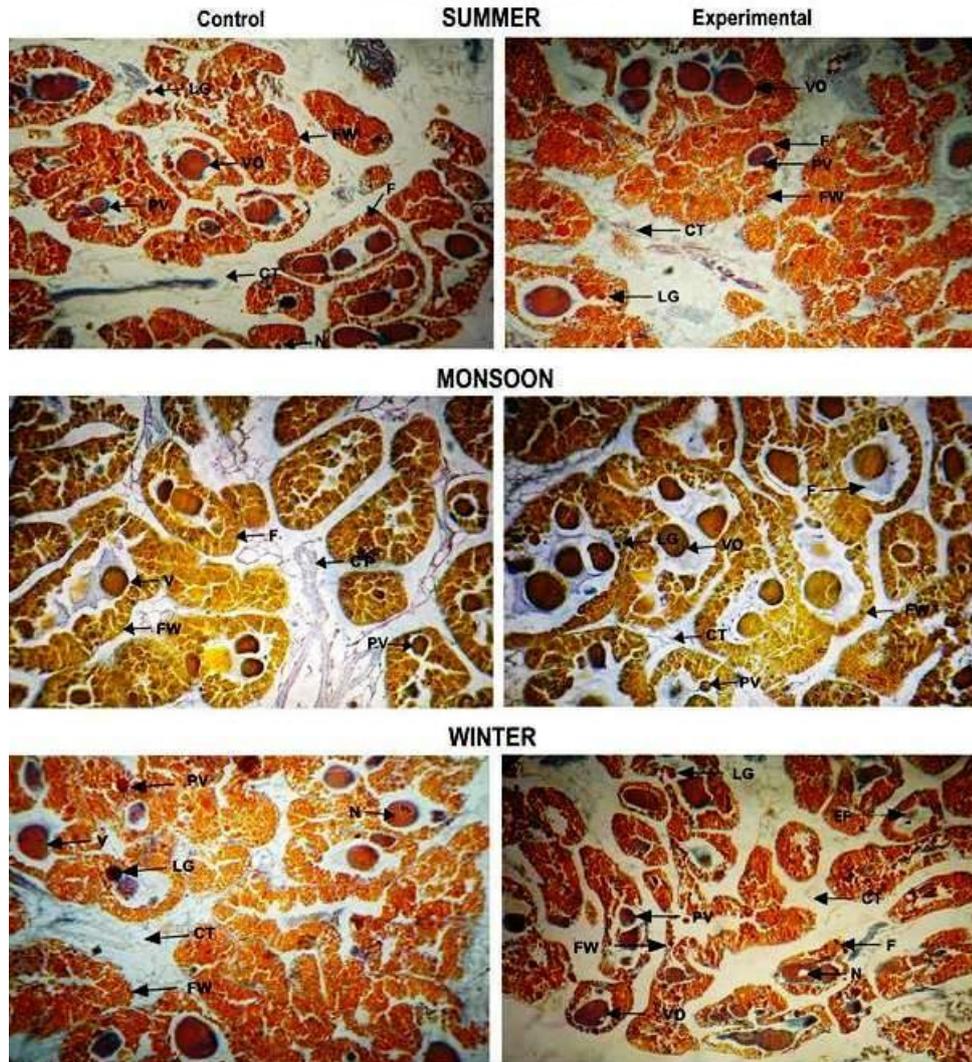
This abundance is depends upon the maturation of gametes particularly in summer. During the active phase of maturation the ovarian follicles containing ripen ova with depletion of lipid globules and nutritive cells during winter. In this season follicles showed release of ripe ova. Thus many oogonia and previtellogenic oocytes dominated during summer, whereas during monsoon and winter, vitellogenic oocytes dominated in the follicles. In female cycle, the maturation gonad takes place from summer to monsoon and fully matured gonads through monsoon to winter, and gametes are released gradually during winter. During summer, in experimental groups due to effect of elevation of temperature, follicles expanded more compared to control, which results in the reduction of interfollicular connective tissues.

Enhancement of vitellogenesis occurred in few oogonia. Quantity of lipid globules and nutritive cells decreased. Many oogonia developed to pre-vitellogenic oocytes. No release of vitellogenic oocytes from lumen of the follicles was seen but few showed lysis. This was more pronounced in many vitellogenic oocytes at temperature 34oC during summer compared to temperature 27oC during winter. During monsoon at 30oC temperature, follicles expanded more, due to this, increased vitellogenesis which resulted in dominance of vitellogenic oocytes. Few follicles showed release of vitellogenic oocytes. This release of oocytes at 30oC temperature during in monsoon was less compared at 27oC temperature during winter.

During winter due to elevation of temperature 27oC, follicles expanded more and remained oval or elongated in shape compared to control. A very few nutritive cells and lipid globules remained in the follicles. Vitellogenesis of oocytes occurred and many vitellogenic oocytes which lie free in the lumen of the follicles were also observed. Many follicles showed emptying of vitellogenic oocytes and few follicles also showed degeneration of vitellogenesis.

**PLATE - 1**

**Histological changes in the female gonad of *I. caeruleus* exposed to elevated temperature during different seasons.**



- |                             |                          |
|-----------------------------|--------------------------|
| F - follicle                | VO - vitellogenic oocyte |
| CT - connective tissue      | LG - lipid globule       |
| FW - follicular wall        | N - nucleus              |
| EF - emptying of follicle   | V - vacuole              |
| PV - previtellogenic oocyte |                          |

**DISCUSSION**

The present study on *Indonaia caeruleus* from Godavari river at Paithan revealed that, gametogenesis and maturation of gametes takes place during summer and monsoon and attain full maturity to spawn during winter. Since samples were collected during July-August, December- January and April- May, it cannot be deduced the exact period of spawning. It has been shown that this species spawns during the period of September to March (Khatib,1975).During period of summer the histological details of female gonad revealed that inclusions of lipid globules in the follides were only for the nourishment of germinating gametes,(this is also co-related with the high level of lipid content in the gonad).The gametogenesis becomes active during the period of monsoon with plenty of food availability. High temperature and less food availability appears to affect gametogenesis during summer. The nutrient reserves from different bodypart are also lowered down in this season. Thus, food availability and mobilization of nutrients for the gonad during monsoon appears to accelerate the maturation of gametes. During winter, female gonads became ripe and the lipid content increased. In some bivalves, gametogenesis can be induced

outside the normal reproductive period and gamete development can be accelerated by exposing them to suitable temperatures, (Sastry,1979). Loosanoff and Davis (1963), collected *Mersenaria mersenaria* during winter and exposed them to elevated temperature, which induced gametogenesis outside the normal breeding period. Generally females apparently requires a longer period of exposure to temperature than males. Acceleration of gamete development to spawning at elevated temperatures has also been achieved in *Patinopecten yessoensis* (Yamamoto, 1951); *Mytilus edulis* (Lubet,1956), *Mytilus galloprovincialis* (Lubet and Bourcart,1963). Sastry (1963), suggested that gamete development upto maturity in *Argopecten irradians* can be accelerated after gametogenesis has been initiated and the rate of development upto maturation is depends upon temperature. Chipperfield (1953), has reported that the rate of gametogenesis in *Mytilus edulis* is approximately proportional to the rate of temperature increase. Acceleration of gamete development upto spawning by elevation of temperature appears to be successful only after the animals have passed their post-spawning activity. Post -spawning recovery involves complex physiological processes leading to the accumulation of nutrient reserves.

In the present study on *Indonaia caeruleus*, it has been observed that during summer when the female gametes are retained in gametogenic phase, enhanced gametogenesis at temperature 34oC, even though the mortality was observed. At temperature 34oC, follicles expanded more compared to winter and vitellogenesis also accelerated. Due to this, quantity of lipid globules and nutritive cells decreased. The gametes with enhanced growth undergo lysis. However, during monsoon elevation of temperature to 30oC caused more pronounced effect, and during this season release of mature oocytes were less than elevation of temperature 27oC during winter, however very few female gametes showed lysis.

During winter alike to summer and monsoon female follicles expanded more and hence interfollicular connective tissue reduced and enhancement of growth of female gametes occurs. At the elevation of temperature 27oC release of gametes occurred. It is important to note that no lysis of gametes occurred in this season. From the given study it can be suggest that during winter elevation of temperature to 27oC can be induce normal female gametes growth and their release.

However, comparing the elevation of temperature at 30oC during monsoon and at 27oC temperature during winter, it can be suggested that temperature requirement for growth of female gonad and oogenesis probably lies between temperature 26oC - 27oC as per the experimental results. It has been further observed that in *Indonaia caeruleus* lipid contents from female gonad increased during winter at 27oC and also during summer at 34oC, due to enhancement oocytes growth. In monsoon at 30oC though many oocytes showed enhanced growth. Sastry (1968,1970a) has suggested that temperature act as a triggering stimulus for initiation of the oocyte growth phase.

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