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# HISTOLOGICAL CHANGES IN THE MALE GONAD OF FRESHWATER BIVALVE MOLLUSC, *INDONAIA CAERULEUS*, EXPOSED TO ELEVATED TEMPERATURES DURING DIFFERENT SEASONS

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**ABSTRACT : During summer (May), monsoon (July) and winter (January), the adult freshwater bivalve molluscs, *Indonaia caeruleus (Prashad, 1918)* of 51-54 mm shell-length were collected from banks of Godavari river at Paithan and brought to the laboratory. After 24h acclimatization in laboratory conditions, they were exposed to normal water temperature (served as control), i.e. 28.5- 31.0 °C, 25.5-26.0 °C and 21.5-23.0 °C during summer, monsoon and winter respectively. For experimental groups, the animals were exposed to rise in temperature i.e. 34.0 °C during summer, 30.0 °C during monsoon and 27.0 °C during winter seasons for 15 days. (The temperature of the water was maintained by AUTOMATIC RENA thermostats). The study revealed that, in control the dominance of spermatogenic phase occurs during summer, the developmental stages of spermatogonia found during monsoon and maturation and release spermatozoa occurs during winter seasons in *Indonaia caeruleus*. Due to rise in temperature during all seasons, growth of gametes as well as their release were observed at expense of lipid globules and nutritive cells. The tubules of male gonads expanded more and connective tissue was observed to reduced, which was more pronounced at 30.0 °C and 27.0 °C during monsoon and winter seasons respectively.**
**However, in experimental groups, normal development of male gametes were observed and they were released during late monsoon and winter. More release of spermatozoa by emptying of tubules were observed at 27.0 °C during winter season. The results of the experiments are discussed in the light of histological details of male gonads of bivalve molluscs.**

***Key words :*** Elevated temperature, male gonad, bivalve molluscs, Godavari river, different seasons.

## INTRODUCTION

The reproductive physiology of molluscs is a special interest due to their importance as food for human beings. Besides this activities tends to concentrate among marine shores and freshwater systems and cause considerable toxic stress in addition to stresses caused naturally during summer, monsoon and winter due to sudden fluctuations in environment.

Many environmental factors are known to be affecting the physiology of bivalve molluscs. The phases of the reproduction i.e. gonad development, spawning and fertilization and development and growth of zygotes are functioning continuously with changes in environmental factors, (The temperature, pH, salinity, photoperiod etc.). The temperature and nutrition are the two factors that affect the physiology and composition of bivalve molluscs (Bayne *et al*, 1976; Shpigel, 1989). World wide timing of the reproductive cycle from gametogenesis to spawning is regulated by an integration of environmental and endogenous factors (Sastry, 1979). The timing and duration reproductive activity are determined by some environmental factors (Lubet and Mann, 1987).

In bivalve molluscs, Synchronization of breeding periods with environmental conditions especially temperature, salinity, light and food for development and growth of progeny has been extensively reported (Andrews, 1979; Mackie, 1984). Reproductive cycle of the bivalve molluscs is generally controlled in response to the temperature (Sastry, 1968). Recent studies indicates that a reproductive response is produced through and integration of environmental factors. After attaining a certain physiological state, when organism exposed to required environmental pre-requisitions begins the gonad growth and gametogenesis in unisexual bivalves. The temperature was considered to be and important environmental factor which affect the survival activities and metabolic processes periodically (Widdows, 1973). The sequence of events related to growth of gonad, maturation and release of gametes and development of eggs are thermally sensitive (Kinne, 1962). The temperature also greatly influences the sexual maturity, spawning and development of life stages of aquaculture species. The influence of temperature on the reproduction of marine invertebrates including pelecypod molluscs has been reviewed by Giese, (1959); Vernberg and Vernberg (1972); Loosonoff, (1971) and Giese and Pearse, (1974).

Literature shows paucity of information of stress effect of elevated temperature on reproductive cycle in male gonad of bivalve mollusc, hence present study was undertaken on freshwater bivalve, *Indonaia caeruleus*.

## MATERIALS AND METHODS

The adult bivalves, *Indonaia caeruleus*. (Prashad, 1918) of 47- 51 mm in shell-length were collected from banks of Godavari river at Paithan during summer, monsoon and winter seasons. After brought to the laboratory, they were washed and brused with water for removing algal biomass and mud. During each season, the animals were acclimatized for 24hrs. in laboratory conditions. After 24 hrs. laboratory adjustments, 20 animals were kept in each of two groups during each season. One group served as control of normal water temperature 28.5-31.0 °C during summer,25.5-26.0 °C during monsoon and 21.5-23.0 °C during winter and others were experimental i.e. exposure of animals to rise in temperature of water 34.0 °C, 30.0 °C and 27.0 °C during summer, monsoon and winter seasons respectively. The temperature of the water in experimental group were controlled by the use of thermostats (Automatic, RENA, France), fixed at the bottom of aquarium. In experimental groups, 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 appropriate temperatures from the experimental as well as control groups, were renewed at every 12-13 hrs. and experiment was conducted for 15 days during each season for better results.

For histological study of male gonads the shell-valves of five animals from each group were removed on 15 th day. After soaking the animal bodies on blotting paper, fixed in Bouins Hollande fixative for 48 hrs. to fascilate better fixation, the fixative was renewed after 48 hrs. the gonadal tissue of male individuals from both the groups were removed and processed for preparation of paraffin blocks. Dehydration of tissues were done through serial grades of absolute alcohol and tertiary butanol. The xylene is replaced by butanol in processing.

The tissues were embedded in Rankem paraffin wax at 58-60 °C and sections were cut at 6.0-7.0 µm thickness using Weswox- Spence-rotary microtome. The sections were stained with haematoxylene-eosin and observed under research binocular microscope before photomicrography.

## OBSERVATIONS AND RESULTS

The physico-chemical parameters of water used for experiments during different seasons were, temperature (28.5-31.0 °C), pH (7.68-7.70), hardness (118-123ppm) and dissolved contents (5.684-6.330 mg/lit) during summer; temperature (24.5-26.0 °C ), pH (7.79-7.81), hardness (107-111ppm) and oxygen content (6.171-6.577 mg/lit) during monsoon and temperature (22.0-23.5 °C ), pH(7.73-7.74), hardness (97-103ppm) and dissolved oxygen content (6.334-6.741 mg/lit) during winter seasons.

The results of the histological changes in male gonad due to influence of rise in temperature were given in plate-I The histological studies carried out revealed different stages in the male gonad during three different seasons. The sexes are separate hence unisexual gametogenesis i.e. in male gonad, spermatogenesis found during summer, maturation of gametes takes place during monsoon and mature and partially spawned gametes observed during winter. During summer, in control, gametogenic stage was observed. The male follicles becomes compact and showed sperm morulae. The lipid globules found more abundant while interfollicular connective tissue reduced. During monsoon maturation of male gonad becomes active. The multiplication of sperm morulae was noticed. Lipid globules as well as interfollicular connective tissue decreased in quantity. Spermatogonia, spermatocytes and spermatids distincty seen.During winter, male follicles are placed with spermatids in the lumen. In some places follicles contain few nutritive cells and lipid globules. In this season male follicles revealed partially spawned condition. In experimental groups, due to effect of elevation temperature at 34.0 °C, most of the male follicles showed multiplication of sperm morulae and development and maturation process enhanced. Spermatogonia and spermatocytes were numerous and few follicles showed sperm morulae occupied in the lumen. Interfollicular connective tissue and lipid globules were reduced.

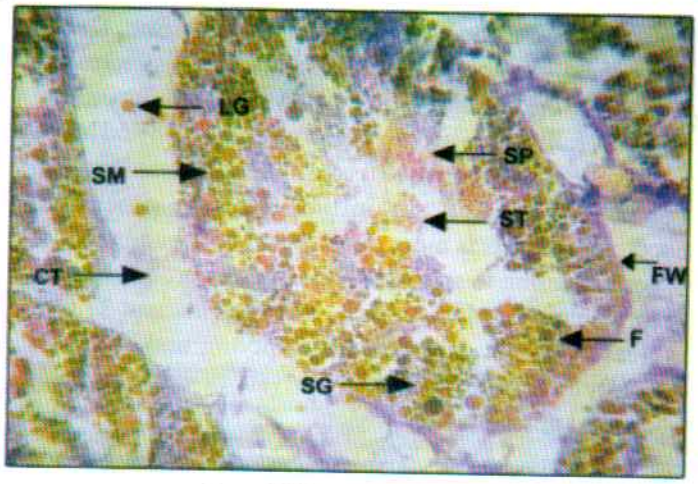
During monsoon, male follicular wall is loosely packed and interfollicular connective tissue reduced at 30.0°C, and its influence on spermatogenesis is more. The development of spermatogonia to spermatids and sperms proceeds more rapidly. During winter season at 27.0 °C, enhancement of spermatogenesis, emptying of follicles takes place. Lipid globules, nutritive cells and spermatogonia were comparatively reduced. Interfollicular connective tissue also reduced.

## DISCUSSION

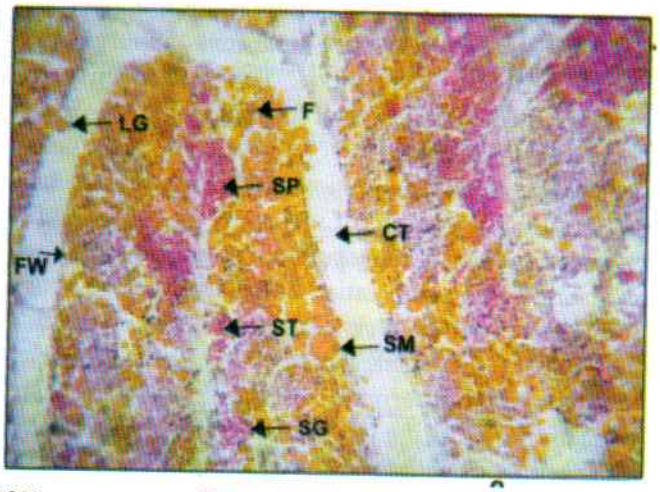
In the present study carried out on freshwater bivalve *Indonaia caeruleus* form Godavari river at Paithan, it is revealed that, in control spermatogenesis found during summer, maturation of gametes takes place during monsoon and attain maturity to spawn and partially spawned gametes observed during winter season. Since samples were collected during the period of July-August, December-January and April- May, it can't be deduced

**PLATE - 1 : Influence of elevated temperature on the histological structure of male gonad of I. caeruleus during different seasons.**

**SUMMER**

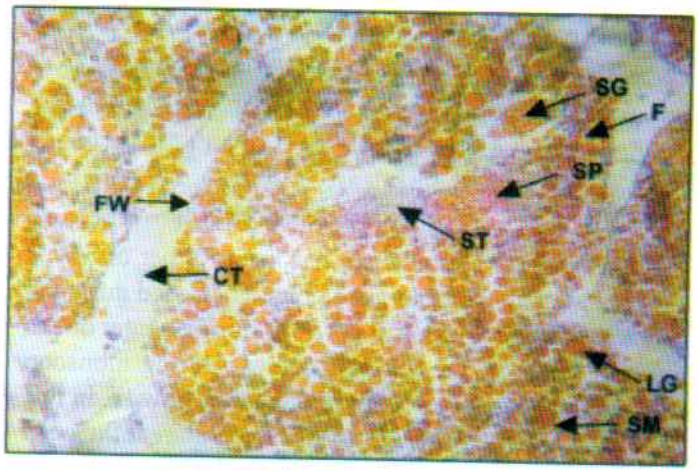



**Control (28.5-31.0°C)**

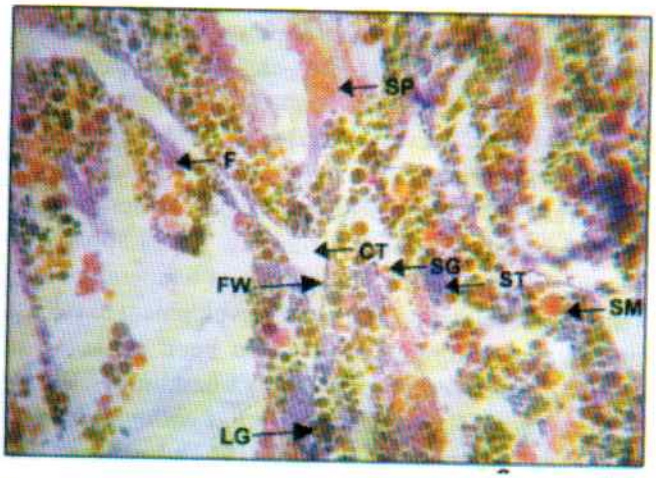



**Experimental (34°C)**

**MONSOON**

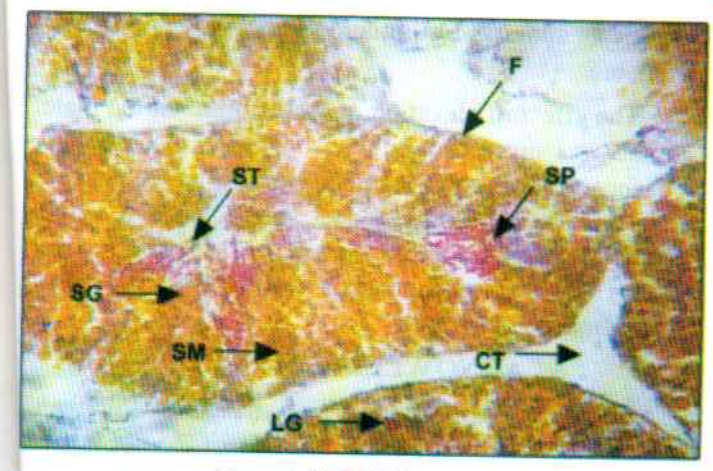



**Control (25.5-26°C)**

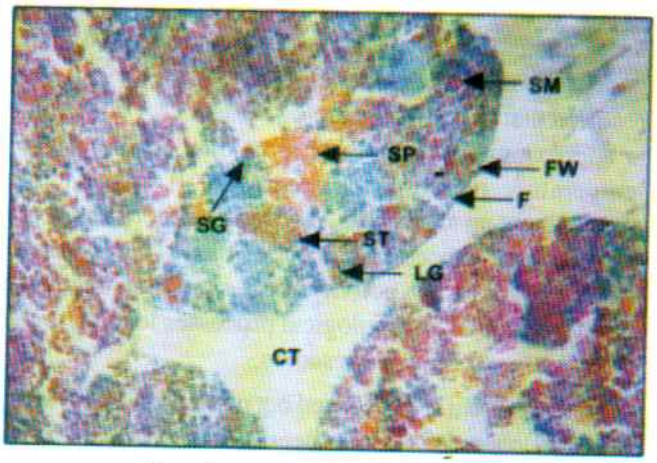



**Experimental (30°C)**

**WINTER**




**Control (21.5-23°C)**




**Experimental (27°C)**

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

the exact period of spawning. It has been shown that this species spawns during the period of September to March (Vedpathak 1990, Khatib, 1975).

During summer, histological details of male gonad revealed that, follicles becomes more compact and showed sperm morulae and the inclusions of lipid globules in the follicles were more. They were only for nourishment of germination of spermatogonia (this is also correlated with the high level of lipid contents in the gonad (Vedpathak, 1990).Maturation of male gonad becomes active during the period of monsoon, due to favorable climatic condition of rainy period with and plentiful food availability. The mobilization of nutrients for the gonad during rainy season appears to accelerate the maturation and multiplication of male gametes. Hence, in male follicles spermatogonia, spermatocytes and spermatids are distinctly seen. During winter, male follicles are packed with spermatids with few nutritive cells. In this season, male follicles revealed partially spawned condition. In many marine bivalves , gametogenesis can be induced outside the normal reproductive period and development of male and female gametes can be accelerated by exposing them to suitable temperatures (Sastry, 1979). Loosonoff and Davis (1963) collected *Mersenaria mersenaria* during winter and exposed them to elevated temperature, which induced gametogenesis outside their normal breeding period. Generally, males apparently requires shorter period of exposure of temperature than females. Acceleration of gamete development to release at elevated temperature has also been achieved in *Patinopecten yessoensis,* by (Yamammoto, 1951); *Mytilus edulis*, (Lubet, 1956); *Mytilus galloprovincialis* (Lubet and Bourcurt1963, Sastry 1963), suggested that gamete development upto maturity in *Aequipecten 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 directly proportional to the rate of temperature increase. Acceleration of male gamete development up to spawning by elevation of temperature appears to be successful only after the animals passed their post-spawning activity. post-spawning recovery involves complex physiological processes leading to the accumulation of nutrient reserves.

In the present study carried out on freshwater bivalve, *Indonaia caeruleus* it has been observed that during summer the male gametes are retained in the spermatogenic phase, enhanced the rate of spermatogenesis at temperature 34.0°C. even though the mortality was observed. At temperature 34.0°C, follicles expanded more compared to winter. Due to effect of elevation of temperature most of the male follicles showed multiplication of sperm morulae and development and maturation process enhanced. The number of spermatogonia and spermatocytes are increased. Due to this quantity of lipid globules and nutritive cells decreased. However during monsoon, at elevation of temperature 30.0°C, follicular wall becomes loosely packed and hence interfollicular connective tissue and lipid globules again reduced. At 30.0°C, its influence on the process of spermatogenesis is more, the process of development of spermatogonia to spermatid and sperms proceeds more rapidly compared to summer. During winter, at 27.0°C, release of male gametes and emptying male follicles occurred. It is to note that no lysis of male gametes occurred during winter season.

From the study, it can be suggested that during winter season elevation of temperature at 27.0°C and during monsoon at 30.0°C can be induce the growth and release of male gametes.

However, comparing the elevation of temperature at 34.0°C during summer, it can be suggested that the temperature requirement for the growth of male gonad and gametes and their release in *Indonaia caeruleus* is 27.0°C, 30.0 °C as per experimental results.

In *Indonaia caeruleus* it has been further observed that lipid contents from male gonad increased during at 30.0°C during monsoon and 27.0°C during winter due to enhancement of growth of gametes. During monsoon though many gametes showed enhanced growth. In this study on *Indonaia caeruleus,* the temperature influenced on the initiation of spermatogenesis during all season. Sastry (1968) has suggest that temperature act as a triggering stimulus for initiation of the growth phase of gametes.

The effect of elevation of temperature on the male gonad was more pronounced during monsoon. The enhancement of growth of male gametes may be due to nutrient reserves to the gonad, since monsoon is the favorable season for the development of the reproductive organs because during monsoon the food materials in the habitat media is plentiful.

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