

## Histological study on reproductive cycle of estuarine clam *arca granosa* from Bhatye Estuary, Ratnagiri (MS) India

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### Abstract

Reproduction patterns such as production and release of gametes, fecundity and external factors controlling breeding activity have a crucial role in the continuity of populations and their adaptations to the environment. In littoral areas these mollusks subjected to unstable environments. This instability includes salinity and temperature fluctuations, variation in food availability and competitive and predatory pressure. In consequences, they are characterized by a particular demographic strategy with regards to ecological, physiological adaptations, growth, reproduction and ultimately biodiversity.

### INTRODUCTION

The term reproductive cycle is defined as the entire cycle of events from activation of the gonad, through gametogenesis to spawning (release of gametes) and subsequent recession of gonad. The clams are not reported to show hermaphrodites, they are sexually separate. In the gonad of bivalves non sexual elements such as sertoli cells and the follicle cells might be involved in hormone production. Histology has been successfully used to study concerning changes in cellular or sub cellular structure of an organ much earlier than external notification (Kumbhar, 2001)

Environment plays an important role in life cycle and physiology of the animals. Some workers emphasized that, two types of controlling factors are involved in regulation of reproduction viz. exogenous which includes environmental factors such as temperature, pH, photoperiod, food, salinity, etc. of these, the influence of temperature on the reproduction of marine invertebrates including bivalves has been discussed in reviews by many workers (Bayne, 1976; Giese, 1959 and 1976; Giese and Pearse, 1974; Loosanoff, 1971) Many investigators have worked out gametogenesis in bivalve, e.g *Mesoderma donacium* (Paredo et al, 1987). Distinct correlation between biochemical and histological data include be shown (De Jang-Brink et al, 1981). Gamete production in marine species of bivalves is known to require a great deal of energy,( Bayne, 1985) suggesting a close relationship between the reproductive cycle and energy available

for growth, a direct relationship between food availability and acceleration of the reproductive process is found in other studies also (Delgado and Camacho, 2005).

The comparative study on histopathology of gonadal neoplasms in marine bivalve molluscs and all these mixed gonadostromal neoplasms are presently diagnosed as gonadoblastomas (Peters et al, 1994). Some studied oocyte and embryo quality in *Crassostrea gigas* (Portuguese strain) during a spawning period in Algarve, South Portugal (Massupina, 1999). The studies on reproductive cycle of Indian marine bivalve molluscs have been extensively carried out by using histological techniques and based on microscopic observations. The gonad showed a number of stages in different parts of the year. In *Katelysia opima* and *Perna (Mytilus) viridis* (Nagabhushanam and Mane, 1975, a.) *Perna viridis* (Bhagade, 2000) was carried out. Nugranad carried out artificial breeding of the oriental hard clam *Meretrix meretrix* in hatchery conditions(Nugranad, 2000). The morphology of the cells during spermatogenesis and oogenesis in *Amiantis umbonella* (Mollusca: Bivalvia) was investigated by Al-Mahanna *et al.* (Al-Mahanna et al, 2001 and 2003). The reproductive strategies of an organism play a major role in the dynamics and the biogeography and continuity of a species (Ramirez– Llorda, 2002).

More recently some workers correlated seasonal biochemical variations in gonads and sexual maturation in bivalves while some showed

relationship with available food as well as the gonadal development (Camacho et al, 2003; Delgado and Camacho,2005; Osada and Mori, 2000; Stead et al., 2002). Some observed gametogenic cycle of *Ensis siliqua* in the Ria De Corcubion, Northwestern Spain and showed a clear relationship between gonadal condition index (GCI) and gametogenic stages (Dartia, 2004).

## MATERIAL AND METHODS

The clam *A. granosa* measuring 4.0– 5.0 cms was collected on monthly basis. These gonads were processed for the routine histological study of gonads. For that gonad tissues are fixed in aqueous Bouins and dehydrated passing through ascending grades of alcohol, and then proceeded for preparation of blocks using paraffin wax (58<sup>0</sup>-60<sup>0</sup>c). The sections were cut for gonads at 5 - 6µm thickness on rotatory microtome (Erma, Japan).

These sections were stained with Harris hematoxyline and Alcoholic Eosin and mounted in D.P.X. All the observations for the microphotography were made under the Olympus CH 20i (U.S.A.) microscope. As the samples were collected during each month, the quantitative examination of changes in the various gonadal stages is correlated with the impact of change in environment.

## RESULTS

According to the Ramirez-Llodra,, the reproductive strategies of an organism play a major role in the dynamics, the biogeography and continuity of a species. During present study, in *Arca granosa* (Red blood clam) reproductive cycle showed pronounced seasonal changes; hence the condition of gonads is divided in five stages mainly based upon the histological observations.

### i) Female reproductive cycle: (Plate – I, Fig. 1- 5)

In female clams of *A. granosa* the gonads have become full and form major part of the soft parts in the month of January. The branching nature of the germinal epithelium obliterated as a result of the fullness of the gonads which was in the ripe phase, with mature ova filled in the enlarged follicles. The follicles in females were closely packed without any interspaces, the vesicular connective tissue having been completely obliterated. After attaining maturity the ova achieves rectangular shape and this shape in *A. granosa* only, gives an indication that spawning has started (Fig.4).

In February, the follicles were shrinking in size and also there was reduction in the bulk of the gametes present in the lumen of the follicles. There

significance of contrasting feeding strategies and are some gametes in the same gonad which are yet unspent. In females, ova are less numerous and the follicular wall contains small oocytes in considerable numbers. Till the end of this month spawning has far advanced and majority of gonads were found in the spent phase. The follicles were ruptured in some cases, while in others follicular walls were present. The gonads were entered into the spent and resting phase and the sex of the individual becomes loose and translucent (Fig. 5). The follicles were completely disrupted. In few cases, formation of vesicular connective tissue has been initiated, thereby showing signs of recovery (Fig.1).

As a result of active formation of vesicular connective tissues, the translucency of the gonads was gradually lost and they became opaque. In the mid March, later signs of initiation of gametogenesis were evident in some clams. More clams have shown into the active phase. Some individuals have entered the ripe phase and a considerable number is in the resting phase. Those in indeterminable stages are slowly reduced in number. Ripe phases have also been attained by majority of the clams and a number of them showed signs of partial spawning (Fig. 4).

In the month of April, ripe clams were on the increasing owing to vigorous gametogenic activity. A considerable number of clams have entered the partially spawned phase. Spawning was vigorous and evident from the appearance of clams in the partially spawned phase. The spawning activity was continued till the end of May and later on majority of the clams were entered the spent and resting phase (Fig. 5). Formation of connective tissue is also observed in some of them by the end of the month (Fig. 1).

In the month of June, some clams were remained unspawned with gametes are still present at the beginning of July. Later, all of them enter the indeterminable stage. The gonads were in the resting phase in the month of July. A few of them contain shrunken follicles with residual ova in the lumen. In the beginning of August, the gametogenic activity becomes vigorous. The gonads can be easily distinguished from the other parts of body. In the months of September and October, few gonads were found in ripe phase. In females the follicles of gonads in the active phase become enlarged and small oocytes with rounded distal ends were found attached to the follicular wall by the peduncle (Fig.2).

The spawning activity continues till the end of the November and thereafter a majority of the clams enter the spent and resting phase.

Formation of connective tissue was also observed in some of them towards the latter part of the month. In a very few clams the gonads were in active phase in the beginning of December. Small follicles were being proliferated from the germinal epithelium.

#### **b) Male reproductive Cycle: (Plate – II, Fig. 1 - 5)**

In the month of January, rapid growth of gonadal follicles was observed due to which the gonads have become full and form major part of the body. The branching nature of the germinal epithelium obliterated as a result of the fullness of the gonads which is in the ripe phase with mature sperms. The follicles are closely packed without any interspaces, the vesicular connective tissue having been completely obliterated. After attaining maturity, the male gamete achieves rectangular shape as in the case of female (Fig. 4).

The gonads were gradually turning flabby and slightly loose in consistency in the month of February. This was an indication for commencement of spawning. The follicles were shrinking in size and also there was reduction in the bulk of the gametes present in the lumen of the follicles. There were some gametes in the same gonad which were yet unspent. In some follicles of males the lumen appears empty while the spermatogonial layer continues to proliferate fresh batches of gametes (Fig. 5).

Till the end of February, spawning has far advanced and majority of gonads were found in the spent phase. The follicles were ruptured in some cases, while in others follicular walls were present. The lumen contains relict sperms which will be cytolysed by phagocytic activity. The gonads enter into the spent and resting phase and the sex of the individual becomes translucent. The gonads appeared loose and translucent. The follicles were completely disrupted. In few cases formation of vesicular connective tissue has been initiated, thereby showing signs of recovery (Fig. 1).

In the beginning of March, as a result of active formation of vesicular connective tissues, the translucency of the gonads was gradually lost and they were becoming opaque. Later signs of initiation of gametogenesis were observed in some clams (Fig. 1). Till the end of this month, more clams achieved the active phase. Some individuals have entered the ripe phase and a considerable number is in the resting phase. In mid April, ripe clams were on the increasing owing to vigorous

gametogenic activity. A considerable number of clams have entered the partially spawned phase. In the month of May, spawning was vigorous as it was evident from the appearance of clams in the partially spawned phase. Those in the ripe phase showed a decrease from the previous month as they started spawning (Fig. 3).

The spawning activity continues till the end of June and thereafter a majority of the clams entered the spent and resting phase. Formation of connective tissue was also observed in some of them towards the latter part of the month. In some clams remainants of the unspawned gametes are still present at the beginning of the month. Most of them enter the indeterminable stage in the month of July (Fig. 5).

In general, the gonads were in the resting phase till the end of July and few of them contain relict sperms to be cytolysed. In a very few clams the gonads were in active phase. The follicular wall was found to contain deeply stained uniformly sized primary spermatogonia. The connective tissue occupies the major portion of the gonad. In the beginning of August the gametogenic activity becomes vigorous. In the month of September, the follicles in male gonad obtain a definite shape, either oval or rounded. Spermatids are found near the basal membrane in the beginning and towards the centre at a latter stage. A few gonads were found in ripe phase. The masses of the active spermatozoa were arranged in radial streaks.

The gonads were gradually turning flabby and slightly loose in consistency in mid of September. This was an indication that, spawning has started. The follicles were shrinking in size and also there was reduction in the bulk of the gametes present in the lumen of the follicles. Till the end of October, spawning has far advanced and majority of gonads were found in the spent phase. The follicles were ruptured in some cases while in others follicular walls were present (Fig.5). The lumen contains relict sperms which will be cytolysed by phagocytic activity. Till the end of November gonads enters into the spent and resting phase, and the sex of the individual becomes translucent. The gonads appeared loose and translucent. The follicles were completely disrupted. In a few cases formation of vesicular connective tissue has been initiated, thereby showing signs of recovery in the beginning of December. Till the end of this month, more clams achieved the active phase. Some individuals have entered the ripe phase and a considerable number is in the resting phase (Fig. 5).

From the above observations it can be concluded that *Arca granosa* is a continuous breeder and breeds throughout the year with three peak periods in the Month of January – February, April- May and September – November.

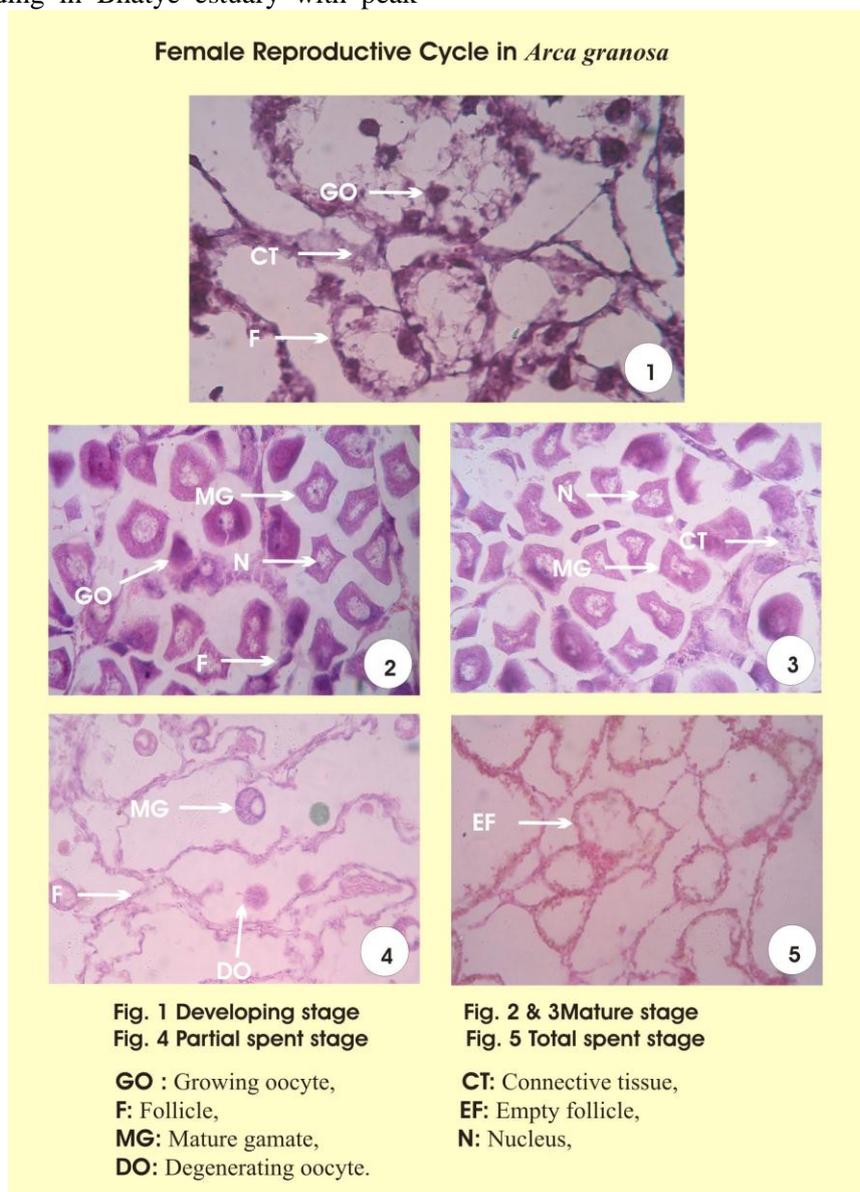
**Discussion**

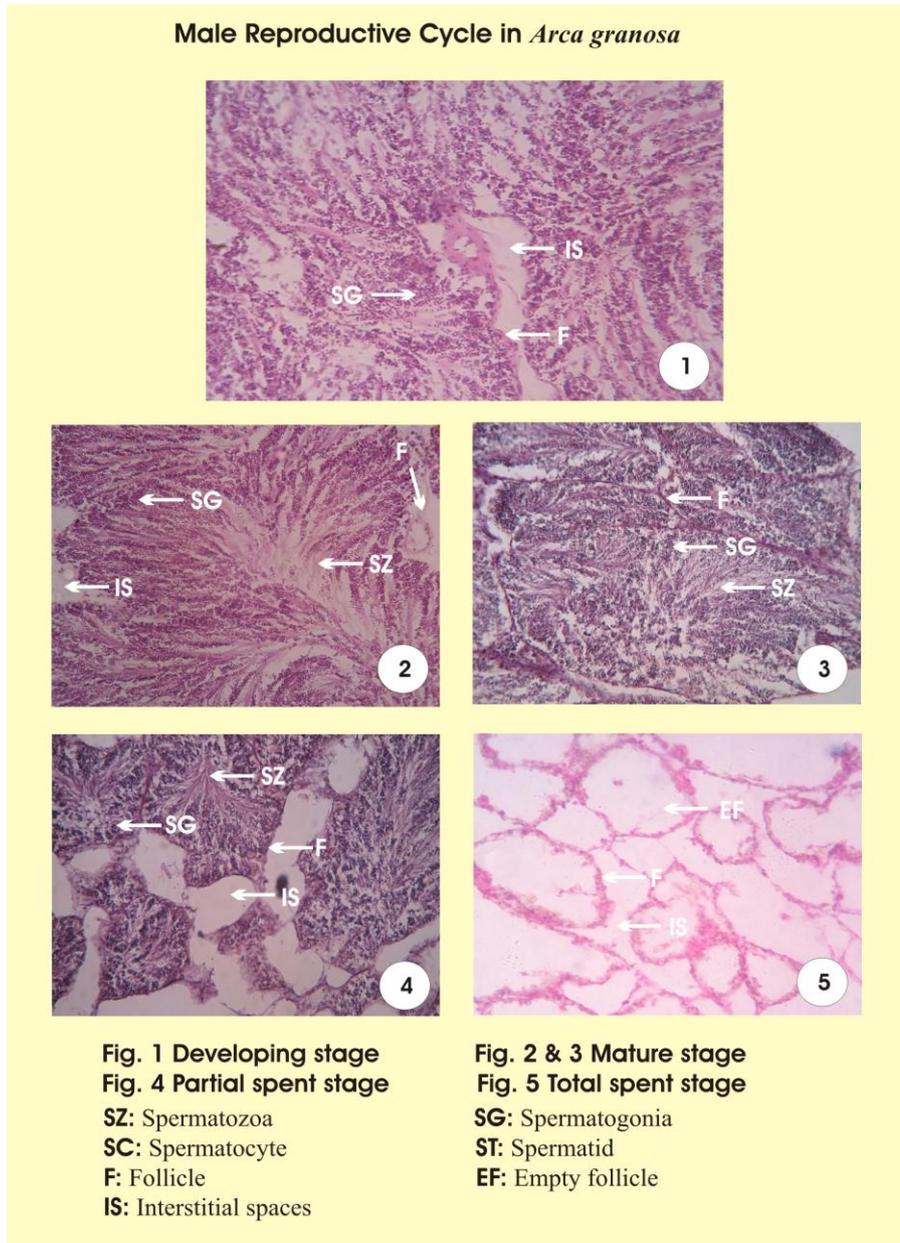
*Arca granosa* is primarily marine and is found burrowing in sand and mud in quite shallow waters, but secondarily it invades backwaters and estuaries in which its distribution is confined to regions in the closest vicinity of the mangrove. The gonadal growth and gametogenesis in marine bivalves were affected or regulated according to the environmental factors like salinity, temperature, light and availability of food. From the present investigation it was clear that, *Arca granosa* is continuous breeder.

In present study, *Arca granosa* showed continuous breeding in Bhatye estuary with peak

periods in April to May, September to October and from December to January. The period from June to September was resting period in this clam. *Martesia striata* in the estuarine waters near Cochin, breeds for 8 months in a year, when salinity is high and this activity is interrupted with a decrease in salinity during the period of monsoonal rains (Balasubramaniam, 1960).

The reproductive strategies of an organism play a major role in the dynamics, the biogeography and continuity of a species.<sup>22</sup> Reproduction patterns such as production and release of gametes fecundity and external factors controlling breeding activity have a crucial role in the continuity of populations and their adaptations to the environment. Jernegren et. al., found similar reproductive cycles and life history traits in congeneric bivalves with different modes of nutrition (Jernegren et al, 2007).





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