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Studies on gonad index and fecundity of mangrove clam, *Polymesoda erosa* (Solander, 1786) of Ratnagiri coast

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Abstract
Gonadal maturation studies were carried out on one of the largest growing tropical mangrove clam species, *Polymesoda erosa* from Ratnagiri coast of Maharashtra. Gonadal maturation studies under laboratory conditions were also carried out to assess the effect of temperature and salinity on Gonad Index (GI) in two separate experiments each of 60 days duration. The optimum temperature and salinity observed for GI was 30°C and 25 ppt, respectively for both the sexes. Estimated fecundity of *P. erosa* within the range of 40 to 120 mm shell lengths was studied. It was observed that maximum numbers of matured oocytes were 1773893 whereas minimum number was 384227.

Keywords: Gonad index, fecundity, *Polymesoda erosa*

Introduction
The mangrove clams *Polymesoda erosa* (Solander, 1786) of the Corbiculidae family inhabit estuaries with sediments consisting of sand and mud in coastal areas. It is a shallow burrowing bivalve distributed widely across the Indo-Pacific mostly in the tidal flat of Southeast Asia (Bayne, 1985) [2]. The clam contributes to the artisanal fisheries in the areas of Konkn.
In India, the Konkan region of Maharashtra state, the coastal area of Zadgaon, Aare Ware and Bhandarpule of Ratnagiri block (study area) is characterized by mud flats and mangrove forests with abundant natural invertebrates and widespread local fisheries. This species of Corbiculidae family was also recorded from neighbouring states like Goa and Karnataka. Although the clam is an indigenous bivalve, it is collected for food, yet has not being cultured on a commercial scale. Though production figures of this species from natural habitat are still unknown and unreported it is popularly fished from the area. This species is thus selected to assess its reproductive biology on the basis of Gonad Index (GI) and fecundity.
Reproductive cycles of marine bivalves comprise a gametogenic phase, spawning and larval development and larval growth. These cycles may be annual, semiannual, or continuous, depending upon the species and location (Sastry, 1975) [17]. The reproductive patterns of an organism play a major role in the dynamics and the biogeography and continuity of a species. Reproductive 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.
Generally, the reproductive cycle of marine invertebrates, mainly bivalves are mostly influenced by adjacent environmental parameters and their gonads could vary from place to place over year (Lubet *et al*, 1981) [9]. Studying the reproductive cycle is essential to establish the time of spawning in any species. This, in turn, represents the starting point for recruitment, age and growth studies, which provide the basic information necessary to manage a species. Thus, the knowledge of reproductive and spawning behaviour is fundamental in understanding the recruitment and population dynamics of marine species. The reproduction of *P. erosa* has never been the subject of any comprehensive studies except for some preliminary descriptions of the spawning season (Ingole *et al*., 2002; Gimmin *et al*., 2005 [5]) [7] and its reproductive strategy (Morton, 1985) [11]. Nevertheless, no studies dealing with the reproductive cycle of this species from Indian waters are reported to date.

The underlying factors like Gonad Index (GI) that affect reproductive success in *P. erosa* and consequently the potential role of environmental factors on gonadal maturation of clams are poorly understood. In view of the fact that temperature is a determining factor of the reproductive maturity in bivalves, the objective of this study was to present the effect of temperature on Gonad Index of *P. erosa*.

Materials and methods

Sample collection

Clams were collected fortnightly over a period of 18 months from October 2016 to March 2018 from Mirya –Zadgaon (17° 00' 82''N, 73° 28' 72''E), Aare Ware (17° 07' 37''N, 73° 29' 83''E) and Waravade (17° 19' 87''N, 73° 24'84'' E) estuaries, Ratnagiri, Maharashtra, India. A total of 1358 numbers of clams were collected for fecundity analysis.

Size of the clams was not restricted for a standard size and specimens representing all the size classes were analysed. After collection, the clams were brought to the laboratory, cleaned and kept in trays. The clams were opened and sexes were determined based on the colour of gonads. The gametogenic development of *Polymesoda erosa* was investigated by microscopic observation of the gonad. The male to female ratio was determined from the colour of the gonads.

Gonad Index

Clams were deemed sexually mature if gametes were present. Histological analysis was performed to investigate differences in the stages of gonadal development of the clams as described by Ropes 1968 [15]; Morton, 1985 [11]; Peredo *et al*., 1986 [13]. Gonads from both male and female clams were placed into six qualitative categories, *viz*. 1= primordial, 2 = developing, 3 = maturing, 4 = ripe, 5 = partially spawned and 6= spent. To assign the gonads into the proper stage, the slides were first examined to scan the entire gonadal area, then under magnification (10 to 40 X) to assess random follicles. The gonadal state of each clam was described as one of the six stages based on the most dominant stage present in the clam samples analysed. For each monthly sample, a mean gonad index was determined following calculations described by Suja (2007) [21]. The number of individuals in each stage was multiplied by the numerical ranking of the stage and the sum of these products divided by the total number of the individuals in each sample.

$$\text{Gonad Index} = \frac{Number\ in\ each\ stage \times numerical\ ranking\ of\ that\ stage}{Number\ of\ animals\ in\ the\ samples}$$

About 5 of the clams from each replicate of both the experiments were sacrificed for sex for gonad index studies. Numerical ranking was given to each clam gonad as per the maturation stages. After identification of sex and determination of ranking of gonads the sample from each clam was taken for the preparation of histological slides. Each slide was examined microscopically to determine sex and stage of gonadal development. The examination of early developmental stages was needed to distinguish males and females. The stages in the gametogenic cycle were assigned based on the maturity of the follicles, gametes and a numerical value assigned as per the scale developed by Seed (1975) [18] for determining gonad index in bivalves.

The effect of water temperature on Gonad Index was studied by rearing the clams at different temperatures: 26 (T_0 – control), 28 (T_1), 30 (T_2) and 32°C (T_3) with 6 replicates each for 60 days duration (December 2017 – Jan 2018) following Completely Randomized Design. Temperature of treatment T_1, T_2 and T_3 was kept at desired levels using an immersion heater system. The salinity of all the treatments was kept between, 22- 25 ppt (Gimin *et al*., 2005) [3].

Data analyses

The experimental data was analysed by using methods given by Snedecor and Cochran (1967) [20]. Data on percentage of maturity in conditioning experiment was analysed through two-way Analysis of Variance (ANOVA) using SPSS 13.0 version computer software for Windows to find out whether there was any significant difference ($p< 0.05$) between the various treatments.

Results

Sexes are separate in *Polymesoda erosa* with no incidence of hermaphroditism at three sampling stations of Ratnagiri. Mangrove clam sex can only be determined in the adult stage by the appearance and colour of the gonads. When fresh, the female gonads are black in colour and male creamy-white. The sex ratio in *P. erosa* did not differ significantly 1:1. Spawning activity was recognized as recently spawned gonads containing much water, decreases in gonad indices, and evidence of spawning and recovery from microscopic observation. The gonad is made up of interconnected gonadal alveoli and surrounds the digestive gland and the gut, infiltrating the muscular tissue of the foot.

Fecundity

Estimated fecundity of 88 individual *P. erosa* of various shell lengths is presented in Fig. 1. Within the range of 40 to 120 mm shell length, *P. erosa* contained 384227 to 1773893 matured oocytes. The larger clams appeared to contain more eggs than smaller individuals. The lack of consistency between shell length and egg count in the present study could be due to different maturity stages of the individuals.

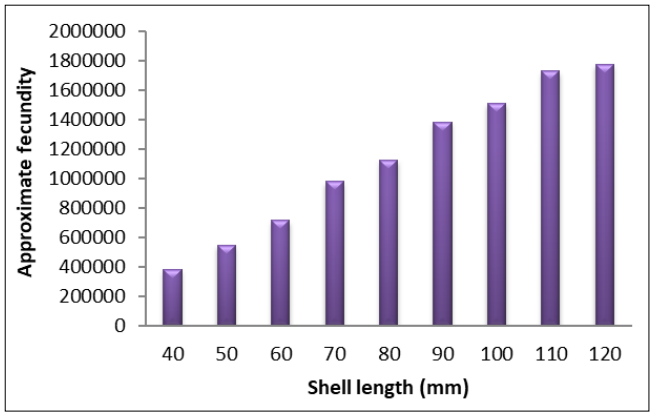

Fig 1: Approximate number of mature oocytes

Effect of temperature on gonad index of male and female of *P. erosa*

Effect of temperature on Gonad Index of male and female *P. erosa* is presented in Table 1 and Fig. 2. In case of male, the highest GI was observed in the treatment T_3 followed by T_2, T_1 and Control, respectively. One way ANOVA showed significant difference ($P< 0.05$) between treatments. Highest GI (3.31 ± 0.26) was observed in treatment T_3, whereas lowest GI was denoted in Control (1.11 ± 0.63). Tukey HSD test indicated significant difference among the treatments. Treatment T_1 differs significantly with Control, whereas, there was no significant difference observed between

treatment T_2 and T_3.

In case of female, the highest GI was observed in the treatment T_2 followed by T_3, T_1 and Control, respectively. One way ANOVA showed significant difference ($P< 0.05$). Tukey HSD test indicated significant difference between treatments. Treatment T_1 differs significantly with Control, T_1 and T_3 whereas, there was no significant difference between treatment T_2 and T_3. Highest GI (2.87 ± 0.30) was found in treatment T_2 whereas lowest GI was denoted in Control (0.67 ± 0.10).

Table 1: Effect of temperature on gonad index of male and female of *p. erosa*

Parameters	Treatment			
	T_0 (Control)	**T_1**	**T_2**	**T_3**
GI Male	1.11±0.63 [a]	2.18±0.59 [b]	3.14±0.14 [c]	3.31±0.26 [c]
GI Female	0.67±0.10 [a]	2.01±0.22 [b]	2.87±0.30 [c]	2.65±0.27 [c]

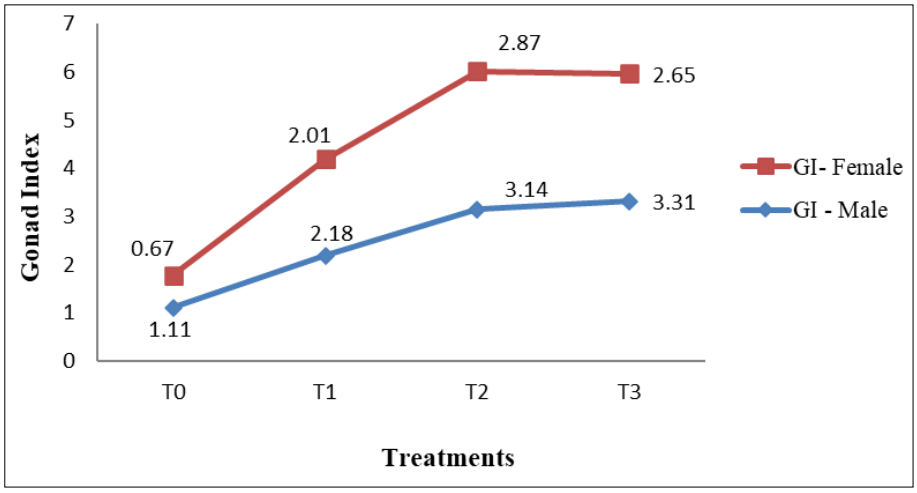

Fig 2: Effect of temperature on gonad index of *p. erosa*

Discussion

The gonads in *Polymesoda erosa* are composed almost entirely of gametogenic cells. Therefore, temporal changes in standardized gonad mass can be reliably interpreted as changes in the actual amount of gametes, thus providing an ecologically meaningful measure of reproductive output. This index increased before spawning due to the gametogenic development that produced an increase in the size of the gonad. From August to September the index decreased due to the spawning period. The sequence of events forming the reproductive cycle of invertebrates is known to be strongly affected by physical and biological environmental factors. The growth rate of the gametes thus varies with individuals, suggesting a specific response to the environmental factors. Shafee and Lucas (1980) [18] have linked the occurrence of successive individual spawning to the presence of gametes at all stages of maturity in the gonads. Various studies on the reproductive biology of bivalves have demonstrated that temperature and food are the most important exogenous factors influencing the reproductive cycle (Sastry, 1975) [17]. Gamete production in marine species of bivalves is known to require a great deal of energy (Bayne, 1985) [2], suggesting a close relationship between the reproductive cycle and energy available for growth. In fact, differences in the feeding conditions could partly explain inter annual variations (Navarro *et al*., 1989) [12] or variability between geographical points in the reproductive trend of a species. Nevertheless, there are studies that described the role of temperature as one of the most important exogenous factors for reproduction of a species (Grant and Creese, 1995) [4]. Generally, temperature needs to exceed a threshold value for vitellogenesis to proceed. Gametogenic cycles of marine invertebrates usually include a 'rest' period of reproductive quiescence following spawning. It has been demonstrated that many bivalves require a minimum threshold temperature for activation of the oocyte growth phase and although oogonia can develop below this threshold level. Sastry (1963) [16] suggested that above this threshold the rate of gamete maturation is temperature-dependent, whereas fecundity and size of the gonad is primarily determined by food availability. Experimental works have further evidenced that gonad size is strongly affected by dietary level, whereas gonad condition varies more with temperature (Heasman *et al*., 1996) [5]. Temperature also affects the transfer of nutrients needed for oocyte growth (Sastry, 1975; Rodriguez-Jaramillo *et al*., 2001) [17, 14]. In temperate areas, temperature plays a very important part in triggering bivalve gametogenesis and spawning (Shafee, 1989) [19]. In the tropics, however, temperature variations over the year are less marked; on the other hand, wide and periodic salinity variations may occur, particularly in areas affected by the monsoon (Ingole *et al*, 2002) [7]. Earlier works have demonstrated the impact of the combined action of temperature and salinity on the reproductive cycles of molluscs (Jayabal and Kalyani, 1987) [8]. At Ratnagiri, gametogenesis of *P. erosa* also peaks when temperatures are highest. Thus temperature definitely appears to affect reproductive patterns in this clam. The present study was conducted in the seaward region, hence, the effect of salinity on the onset and speed of gametogenesis in *P. erosa* therefore appears negligible, although minor salinity fluctuations after heavy rains may act as a cue for spawning. The daily temperature fluctuations to which intertidal organisms are exposed are much sharper in the warm season, when the effect of the sun is strongest, and these fluctuations of variable magnitude may well trigger spawning in bivalves. However, the fact that spawning in *P. erosa* also occurs at other times of the year shows that temperature is not the only factor affecting spawning. Changes in temperature or salinity could be distinctive inducers to synchronize spawning activity among individuals and may result in gametes being expelled into surrounding water masses favourable for larval survival (Appukuttan *et al*, 1989) [1].

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