



## Gonadal development in the shortneck clam *Paphia malabarica* in relation to hydrographic parameters in Kalbadevi estuary

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### Abstract

A study on gonadal stages of the edible shortneck clam, *Paphia malabarica* was undertaken from two sites at Ratnagiri district of Maharashtra, India from February 2005 to May 2006. Histological studies showed that the clam had an extended spawning period from September to January. Maturing specimens were observed from April and the clams reached the ripe stage during May - June. Cytolysis in the gametes was noticed by the end of January. Only one spawning peak was observed followed by the regression of gonads. Minimum and maximum condition indices were observed in February and September, respectively. From February, most individuals were either in spent or in a short gonadal resting stage. Gonadal development initiated from March onwards. Active gametogenesis started from April to July. The sudden drop in salinity and temperature in September appears to act as stimuli for the initiation of spawning.

**Keywords:** *Paphia malabarica*, temperature, salinity, maturity stages

### Introduction

Of several species of venerid clams that occur along the coasts of Maharashtra, *Paphia malabarica* is important from the nutritional point of view. It contributes about 80% to the total production of clams landed annually mainly from Kalbadevi (Shirgaon creek) and Kajali (Bhatye creek) estuaries along Ratnagiri coast, Maharashtra. As large size clams are available from November to May, intensive fishing is observed during these months (Mohite, 2006).

Information on the seasonal gonadal cycle of this estuarine clam is essential to understand different physiological processes in relation to environment. Studies have shown that the gametogenic cycles in marine invertebrates are influenced by exogenous factors (Sastri, 1957; Giese, 1959) of which temperature is believed to be one of the most significant (Mann, 1979). Temperature is closely associated with geographical location and many workers have assessed the importance of geographical locations in defining and controlling gametogenesis (Holland and Chew, 1974; Meneghetti *et al.*, 2004).

The aims of the present study are to describe histologically the gonadal stages of the shortneck clam, to correlate the reproductive cycle with variations in ambient temperature and salinity and ascertain if there are differences in the reproductive cycle between the two sites.

### Material and Methods

**Study areas:** Kalbadevi estuary is situated at 17° 1' to 17° 3' N lat. and 73° 16' to 73° 18.8' E long., eight kms from Ratnagiri. River Kalbadevi flows into this estuary area, which is characterised by a bottom substrate of spit sand. Shirgaon station along this estuary is a vast stretch of marshy area and is rich in various types of flora and fauna, including green filamentous algae, gastropods and molluscs. Bhatye station (16° 58' 36" N lat. and 73° 18' 0" E long.) is about seven kms on the south of Kajali estuary. The distance between both the study sites is about eight kms. The study sites are shown in Fig. 1.

**Environmental parameters:** Ecological parameters such as water temperature and salinity of Shirgaon and Bhatye creek were recorded from February 2005 to May 2006 on weekly basis by

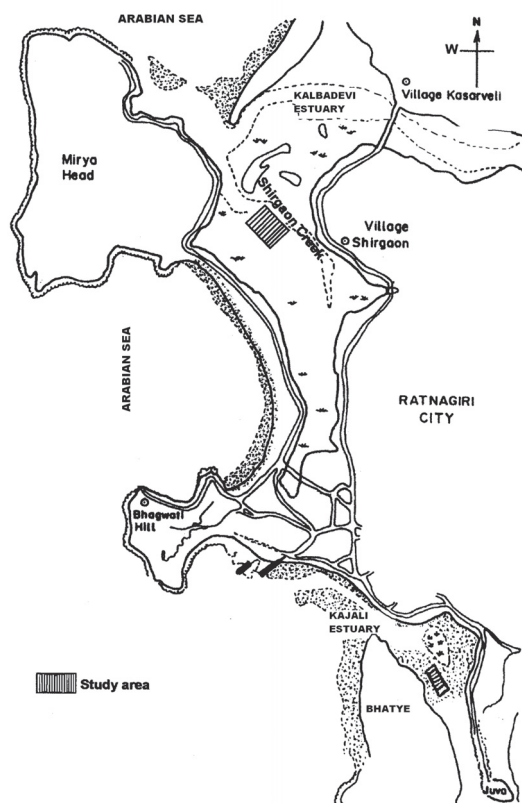


Fig.1. Map showing area of study (shown with zebra lines)

Table 1. Criteria for identification of maturity stages in *Paphia malabarica*

Stage	Common features	Male	Female
I. Maturing	Gonad shape increasing, the digestive gland is restricted to a side, loop of alimentary canal is not always visible but seen in the area close to the body wall.	Gonad somewhat flabby, Follicles contain spermatocytes, no spermatozoa,	Gonad somewhat flabby. Follicles contain oocytes, no free oocytes.
II. Ripe	Gonads packed with sperms and eggs, shape of gonads larger, if pierced, gametes come out	Gonads full and plump. Follicles large in size, bunches of spermatozoa with tails oriented towards the lumen of the follicles. In fully ripe specimens, spermatozoa occupy the lumen.	Gonads full and plump. Large, free oocytes in the lumen with distinct nucleus, rounded to ovate, follicles closely packed without interspaces. Fully grown eggs with reduction in connective tissue. In some cases, the follicular walls found to be breaking
III. Partially spawned	Gonad size starts collapsing. Very few gametes are seen	Gonads flabby and loose, many follicles discharged, mass of spermatozoa separated from follicular walls. Majority of follicles are empty.	Gonads flabby and loose, many follicles discharged. Few mature eggs still retained.
IV. Spent	Gonads considerably shrunken in volume, Loop of alimentary canal is sometimes visible.	Gonads loose, follicles collapsed, residual sperms and phagocytes present	Gonads loose, follicles collapsed, residual eggs and phagocytes present.

adopting standard methods (APHA, 1998). The readings were averaged over a monthly basis.

**Sampling and histological studies:** Specimens of *P. malabarica* (shell length; 20-45 mm) were collected from dredge nets at weekly intervals (February 2005 to May 2006) during low tide from the Shirgaon creek area. The live clams were transported to the laboratory in thermocole box lined with wet gunny cloth. During the study period 20 clams were collected every week and sex ratio was determined. From these, 50 clams per month were randomly selected for gonad identification and histology. The specimens were examined for understanding the stages of maturity by observing gonad smears under microscope. Histological sections of the gonads were also prepared. The gonadal tissues were fixed in 10% formalin. The tissues were then processed for paraffin embedding. The tissues were sectioned (8 to 10  $\mu$ m) and were stained in Ehrlich's haematoxylin and counterstained with eosin.

Maturity stages were determined by combining the criteria followed by Nagabhushanam and Mane (1978), Narasimham (1985) and Victor and Subramoniam (1987). The stages identified were I) maturing, II) ripe, III) partially spawned and IV) spent / resting (Table 1).

**Condition index:** For determination of condition index, the clams were immersed in seawater for at least twelve hours before examination. About 25 clams were examined fortnightly. The meat was extracted from the shells and weighed precisely to a milligram. The weighed meat was dried at 60° C for 24 hours. The condition index was calculated by using the following mathematical equation (Rajapandian and Rajan, 1987).

$$\text{Condition Index} = \frac{\text{Volume of shell cavity}}{\text{Weight of dry meat}} \times 1000$$

**Statistics:** The collected data analysed using standard statistical methods. Seasonal variations in condition index were analysed by one-way ANOVA. A Chi-square test ( $\chi^2$ ) was used to analyse sex ratio.

## Results

**Environmental parameters:** The seasonal variations in water temperature and salinity of the two stations are shown in Fig. 2. At Shirgaon station, two peaks of temperature were observed, one in the premonsoon (April – May) with the highest of  $32.6 \pm 0.30^\circ \text{C}$  during April. A decrease in temperature was observed during July – August. The lowest temperature recorded in July – August was due to heavy monsoon showers. Another peak of high temperature was recorded in the postmonsoon (November – January) period with the highest value of  $33.8 \pm 0.32^\circ \text{C}$  in November. Salinity at Shirgaon ranged from  $27.0 \pm 0.45$  to  $32.0 \pm 0.49\text{‰}$ . The Shirgaon creek showed a wide fluctuation in salinity

throughout the study period of one year. A sharp decline in salinity from  $32 \pm 0.46\text{‰}$  during premonsoon (April – May) to  $27.0 \pm 0.45\text{‰}$  in September was observed. Salinity values showed a steady increase from October onwards. The salinity was positively correlated with temperature (Pearson product - moment correlation,  $r = 0.564$ ,  $p < 0.01$ ) at Shirgaon station.

At Bhatye station, the water temperature ranged from  $27.4 \pm 0.26^\circ \text{C}$  to  $33.5 \pm 0.38^\circ \text{C}$ . Two peaks of temperature were observed, one in the premonsoon (April – May) with the highest temperature of  $32.4 \pm 0.29^\circ \text{C}$  in May 2005. A gentle peak was observed in the postmonsoon (November – January) period and the highest during this period was  $33.5 \pm 0.38^\circ \text{C}$  in May 2006. Lower temperatures in August – September were due to heavy monsoon showers. Temperature increased after cessation of monsoon. Similar to Shirgaon creek, wide fluctuation in salinity was recorded in Bhatye also. A sharp decline in salinity from  $33.4 \pm 0.28\text{‰}$  during pre - monsoon (May) to  $10.3 \pm 0.39\text{‰}$  during peak monsoon season (August) was noticed. The salinity was positively correlated with temperature (Pearson product - moment correlation,  $r = 0.813$ ,  $p < 0.01$ ). There was no significant difference between temperatures at the two sites (Unpaired t - test,  $p = 0.365$ ). Bhatye showed sudden decrease in the average salinities than Shirgaon (Unpaired t-test,  $p < 0.01$ ), probably due to the proximity of Kajali river and land runoffs.

**Maturity stages and sex ratio:** The gonads of *P. malabarica* are located in the muscular foot and expand into the visceral mass as the gametogenesis progress. The frequency percentages of various maturity stages in *P. malabarica* are presented in Tables 2 and 3. Of 1380 clams examined during the study period, 635 (46.02%) were males and 745 (53.98%) were females at Shirgaon station. At Bhatye station, of 1380 clams studied, 662 (47.97%) were males and 718 (52.03%) were females. High percentage of females was observed during the study period at both stations. Both male and female clams showed more or less similar pattern in their seasonal gonadal changes.

From the histological studies of the gonads of *P. malabarica*, four stages of development were

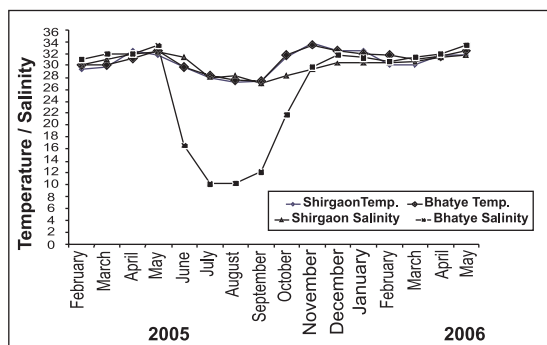


Fig. 2. Seasonal variation in water temperature ( $^{\circ}\text{C}$ ) and salinity (ppt) at Shirgaon and Bhatye from February 2005 to May 2006

Table 2. Frequency (%) of gonadal stages of *P. malabarica* at Shirgaon (Stage I - Maturing; Stage II - Ripe; Stage III - Partially spawned; Stage IV - Spent/Resting)

Months	MALE				FEMALE			
	I	II	III	IV	I	II	III	IV
February 2005	14.3	5.7	28.6	51.4	11.1	4.4	31.1	53.3
March	14.7	2.9	32.3	50.0	15.2	2.2	30.4	52.1
April	52.0	14.0	-	34.0	50.0	14.0	-	36.0
May	54.0	27.0	-	18.9	55.9	25.6	-	18.6
June	57.9	23.7	-	18.4	59.5	23.8	-	16.7
July	47.8	33.3	6.2	12.5	46.1	34.6	5.8	13.4
August	38.9	50.0	5.6	5.6	38.6	49.9	4.5	6.8
September.	33.4	58.4	8.3	-	34.1	59.0	6.8	-
October	21.0	41.9	37.3	-	22.8	40.3	36.8	-
November	12.1	33.3	54.5	-	10.7	34.1	53.3	2.1
December	10.2	32.6	38.8	18.4	9.8	33.3	41.2	15.7
January 2006	8.2	22.2	41.7	27.8	9.1	25.0	38.6	27.2
February	13.5	3.2	31.0	52.4	10.3	3.2	30.9	55.7
March	14.6	3.1	34.7	47.7	16.7	2.2	29.9	51.3
April	51.3	15.2	-	33.5	52.3	12.3	-	35.3
May	54.8	27.5	-	17.8	55.5	26.7	-	17.9

Table 3. Frequency (%) of gonadal stages of *P. malabarica* at Bhatye (Stage I - Maturing; Stage II - Ripe; Stage III - Partially spawned; Stage IV - Spent/Resting)

Months	MALE				FEMALE			
	I	II	III	IV	I	II	III	IV
February 2005	13.5	5.4	37.8	43.2	11.7	4.7	39.6	44.3
March	14.3	2.9	40.0	42.9	13.3	2.2	40.0	44.4
April	49.0	16.3	-	34.7	49.0	15.7	-	35.3
May	51.3	24.3	-	24.3	51.3	25.6	-	23.3
June	56.1	24.4	-	19.5	56.3	25.6	-	17.9
July	48.0	36.0	2.0	14.0	46.0	34.0	2.0	18.0
August	41.5	48.8	4.9	4.9	41.0	48.6	2.6	7.7
September	35.0	51.3	14.0	-	33.3	50.8	15.8	-
October	26.3	47.3	26.3	-	23.8	52.4	23.8	-
November	15.4	38.4	38.4	7.7	17.1	39.0	39.0	4.9
December	10.2	34.7	34.7	20.4	11.8	33.3	35.3	19.6
January 2006	8.3	25.0	41.7	25.0	11.4	20.4	43.1	25.0
February	12.8	6.2	38.5	42.6	10.7	5.2	38.6	45.6
March	13.9	3.9	38.7	43.6	13.8	2.8	40.0	43.5
April	46.5	19.9	-	33.7	50.6	14.5	-	34.9
May	52.9	24.8	-	22.3	52.7	27.0	-	20.3

identified. In order to check whether the observed sex ratio in each month differed significantly from the theoretical 1:1 male to female ratio; the Chi-square value for each month was calculated. The sex ratio of 1:1.17 at Shirgaon did not show significant variation from the 1:1 ratio ( $\chi^2 = 0.99$ , df =1,  $p < 0.05$ ). Similarly, the sex ratio of 1:1.08 at Bhatye did not show significant variation from the 1:1 ratio ( $\chi^2 = 0.97$ , df =1,  $p < 0.05$ ). No

hermaphrodites and undifferentiated specimens were observed during the study period.

**Gametogenesis:** Initially the gonads appeared loose and translucent. Gonads in many specimens of both the sexes were in spent condition showing few numbers of residual oocytes and spermatocytes. A few ripe clams were noticed in the samples in February 2005 at both the stations. From February to August 2005, the frequency percentage of males

in spent stage decreased from 51.4 to 5.6% at Shirgaon and from 43.2 to 4.9% at Bhatye. Females showed a decrease in spent stage from 53.3 to 6.8% at Shirgaon and at Bhatye, from 44.3 to 7.7% during the same months. The gametes, which were still present in the gonads, were in the stage of reabsorption and connective tissue was seen around them. Gametogenesis increased in the subsequent months. The frequency of males entering the maturing stage increased from 14.3 to 57.9% from February to June 2005 at Shirgaon, while at Bhatye, it increased from 13.5 to 56.1% during the same months. Frequency percentage of females entering the maturing stage at Shirgaon increased from 11.1 to 59.5% and at Bhatye, from 11.6 to 56.3% during the same period. In this phase, pronounced enlargement of follicles was noticed in both the males and females. The number of follicles in ovary and testes increased. Small oocytes as well as spermatocytes could be seen in the follicles. As a result of rapid growth of gonadal follicles during the earlier months, the gonads became full and formed major part of the visceral mass. Ripe male gonads showed spermatozoa with their tails directed towards the lumen. In the ripe female gonads, follicles filled the whole gonad with very little interspaces in between. Developing and developed oocytes were seen in the follicles.

The percentage of ripe males increased from 5.7 in February to 58.4 in September at Shirgaon while at Bhatye, it showed an increase from 5.4 in February to 51.3. The percentage of ripe females with full and plump gonads increased from 4.4 (June) to 59.0 (September) at Shirgaon. At Bhatye, the frequency percentage of ripe females increased from 4.7 to 52.4 from February to September. Large, free oocytes were seen in the lumen with distinct nucleus, rounded to ovate and follicles were closely packed without interspaces. Fully grown eggs with reduction in connective tissue were seen. The rapid development of gonads was evident from the higher percentage of clams in ripe condition.

Percentage of males in partially spawned stage increased from 6.2 in July and was maximum (54.5%) in November at Shirgaon. At Bhatye, males in partially spawned stage increased from 2.0% in

July and the highest percentage of spawning was observed during January 2006 (41.7%). Females at Shirgaon showed an increase in the partially spawned stage from 5.8% (July) to 53.3% in November, while at Bhatye, the increase was from 2.00% in July to 43.1% in January. In partially spawned condition, the follicles appeared more shrunken with marked reduction in the number of gametes within the lumen. The unspawned oocytes were seen attached to the follicular walls. The gonads appear loose and shrunken with a reduced number of gametes within the lumen of follicles.

In the following months, spawning far advanced and the percentage of partially spawned clams was found to be decreasing as majority of clams were found in spent stage. Shrunken follicles with reduced number of gametes were seen in most of the spent clams. The percentage of males in spent/resting stage increased from 18.4 (December 2005) to 52.4 in February 2006 at Shirgaon. Similarly, at Bhatye the percentage of spent/resting males increased from 7.7 in June to 43.6 in March. Percentage of females in spent/resting stage increased from 2.1 in November to 55.7 in February at Shirgaon, while at Bhatye, it increased from 4.9 in November to 45.6 in February. The lumen of spent gonads contained residual oocytes and spermatozoa. Cytolysis was noticed in some follicles. The follicular walls appeared as faint lines indicating their presence. Majority of follicles were empty in the females and a few mature eggs were retained. In spent stage, the males had loose gonads, the follicles collapsed and residual sperms and phagocytes were present.

The clams entered a brief period of resting. Gonadal activity was evident from April onwards as the males and females entered the maturing stage (Plates 1 to 10). The clams showed rapid development of gonads from February to June. During May – August, clams were observed in ripe stage. Spawning was evident from September onwards as the percentage of clams in partially spawned condition increased. Spawning continued till January - February and the clams entered a brief period of resting. Consequently, the percentage of spent/resting clams decreased and again the clams entered the maturing phase.

The seasonal changes in the condition index of *P. malabarica* in Kalbadevi and Kajali estuaries are illustrated in Fig. 3. The highest value of condition

index (15.6) was recorded during September at both the stations. From November onwards, the values showed a decrease. The condition index values were

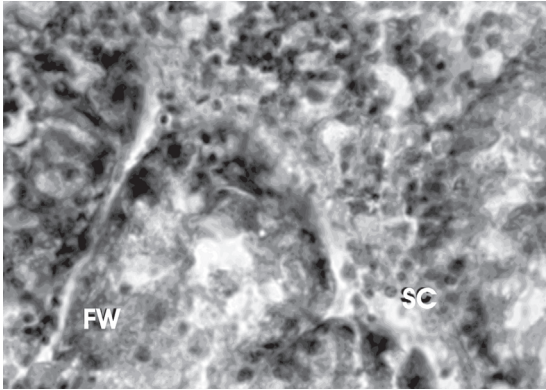


Plate 1. Gonadal stage I - Maturing (Male)  
FW, Follicular Wall; SC, Spermatocytes

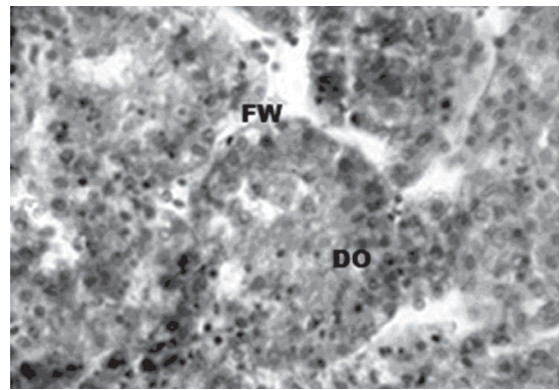


Plate 2. Gonadal stage I - Maturing (Female)  
FW, Follicular wall; DO, Developing oocytes

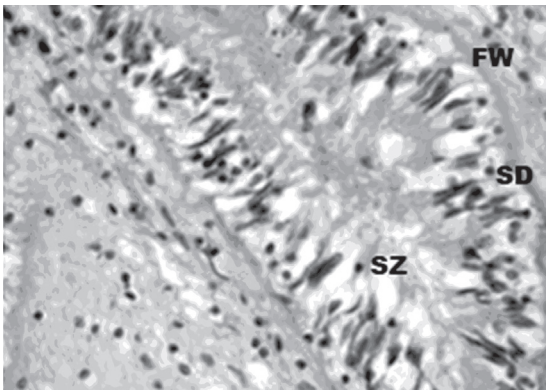


Plate 3. Gonadal development stage II-Ripe (Earlier phase) (Male)  
SD, Spermatids; SZ, Spermatozoa; FW, Follicular wall

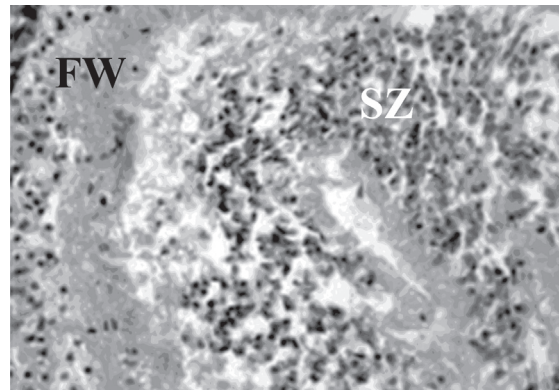


Plate 4. Gonadal development stage II-Ripe (Later phase) (Male)  
FW, Follicular wall; SZ, spermatozoa

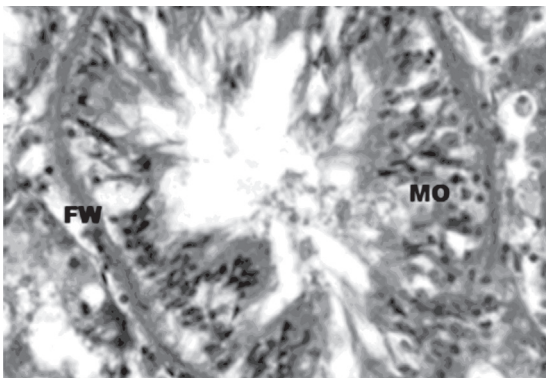


Plate 5. Gonadal development stage II-Ripe (Earlier phase) (Female)  
FW, Follicular wall; MO, Mature oocyte

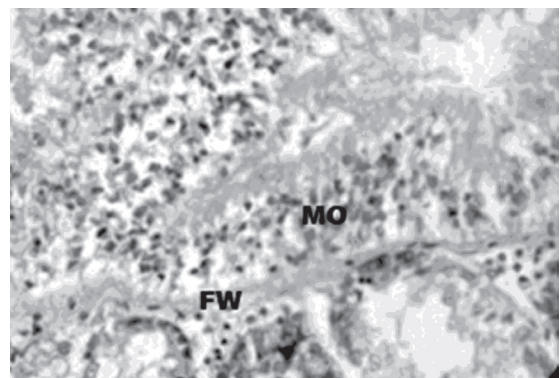


Plate 6. Gonadal development stage II-Ripe (Later phase) (Female)  
FW, Follicular wall; MO, Mature oocyte

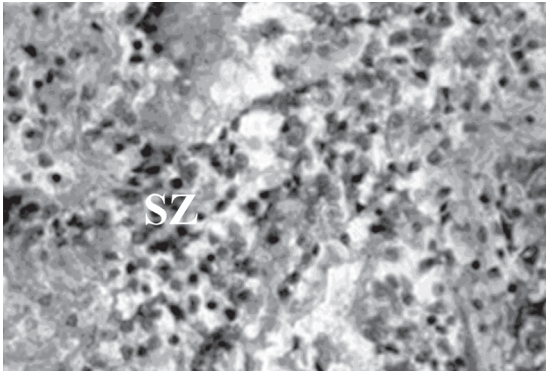


Plate 7. Gonadal stage III - Partially spawning (Male)  
SZ, Spermatozoa

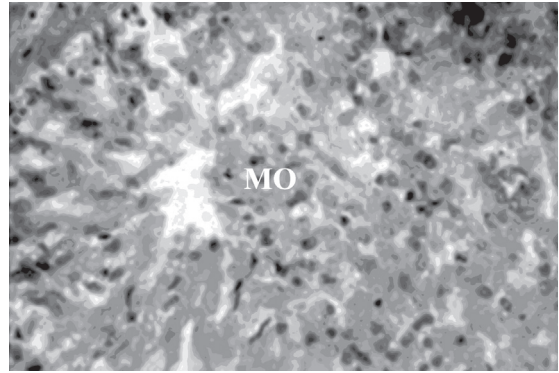


Plate 8. Gonadal stage III - Partially spawning (Female)  
MO, Mature oocytes

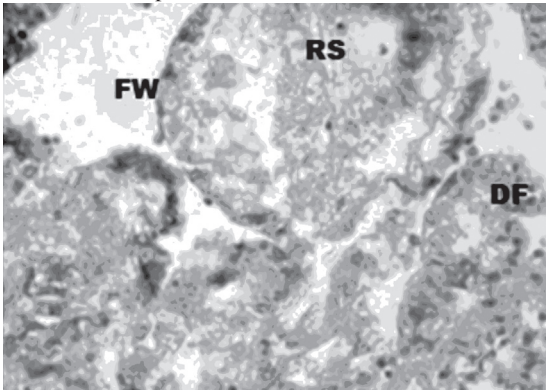


Plate 9. Gonadal development stage - IV Spent/ Resting (Male)  
FW, Follicular wall; DF, Disrupted follicle; RS, Residual spermatozoa

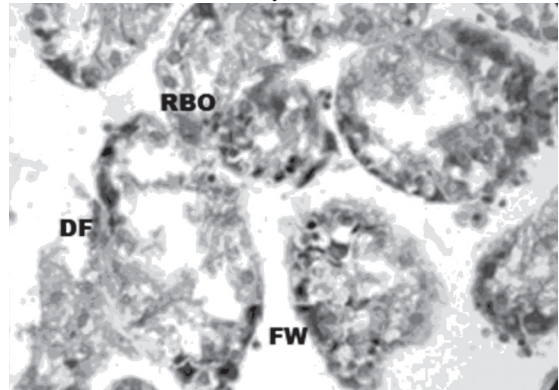


Plate 10. Gonadal development stage - IV Spent/ Resting (Female)  
FW, Follicular wall; DF, Disrupted follicle; RBO, Reabsorbing oocytes

seen to be closely related with temperature (Pearson Product - moment correlation,  $r = 0.212$ ,  $p < 0.05$ ) and salinity (Pearson Product - moment correlation,

$r = 0.398$ ,  $p < 0.05$ ) at Shirgaon. Similarly, condition index showed significant correlation with temperature (Pearson Product - moment correlation,  $r = 0.192$ ,  $p < 0.05$ ) and salinity (Pearson Product - moment correlation,  $r = 0.503$ ,  $P < 0.05$ ) at Bhatye.

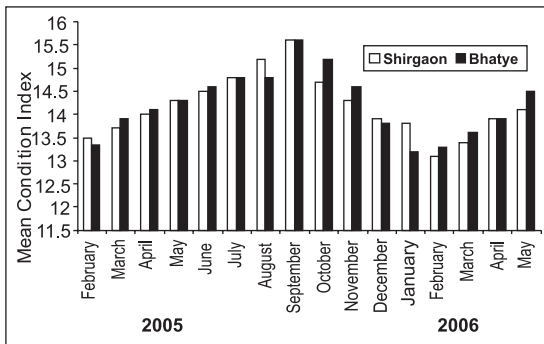


Fig. 3. Seasonal changes in condition index of *P. malabarica* at Shirgaon and Bhatye from February 2005 to May 2006

## Discussion

*P. malabarica* of Ratnagiri coast follows a definite pattern of annual reproductive cycle with a prolonged breeding period, extending from September to January/February. Gametogenesis initiated from March as clams entered the maturing stage. Ripening took place mainly in June – September. Spawning activity continued from September to January. The clams entered into spent/ resting phase from February to March. *P. malabarica* thus showed a prolonged, but single spawning season that commenced during the postmonsoon period.

Similar developments were reported by Nagabhushanam and Mane (1978) on the reproduction and breeding of *Katalysia opima* from Kalbadevi estuary at Ratnagiri. Gametogenesis in *K. opima* started in June and by October the clams reached a fully ripe stage. A second gametogenic activity was reported in January and by March, majority of the clams reached the ripe stage. Their studies showed that the clam spawn twice in a year, while *P. malabarica* from the same estuary showed a single spawning season. Nagabhushanam and Dhamne (1977) observed that *P. laterisulca* from Kalbadevi estuary had a prolonged spawning period extending from mid-September to the end of March with two peaks. Mane and Nagabhushanam (1979) have observed the reproduction in *P. laterisulca*, *K. opima* and *Meretrix meretrix* along Ratnagiri coast. *P. laterisulca* was reported to have a prolonged spawning season extending from mid September to the end of March with two peaks, one in October – November and the other in February – March. *K. opima* started to spawn from October representing partial shedding of gametes and in November, spawning is more or less complete, as majority of the clams have shed the gametes. *M. meretrix* spawn immediately in September and majority of the clams complete the spawning in October and November. Kalbadevi estuary inhabits four species of venerid clams. It was seen that the spawning seasons of all the species coincide. But in *P. malabarica* the spawning chases in January – February, while in others, it may continue till March. The present study showed that the gametogenesis in *P. malabarica* starts immediately from March onwards.

Appukuttan (1996) reported that *P. malabarica* from Ashtamudi estuary was mature during October - January indicating the peak spawning period. In Ashtamudi estuary, spawning commenced in the postmonsoon period and only single prolonged spawning was observed for this clam.

Gametogenesis cycles of clams are closely related to the time and duration of spawning. Timing, duration and number of gametogenesis cycles may vary within the same population of a species. In the present study, gametogenesis was seen to be initiated,

with a short gap, in February - March at both the stations. There was a rapid development of gonads from March - April onwards, which coincided with higher salinity and lower temperature values. The gonads increased in size and gonad development was evident from the histological observations. *P. malabarica* reached the ripe stage during May - August and spawning was observed from September, when a decrease in salinity and a marginal increase in temperature was observed. It was observed that all the clams were ripe by July - August and maturity was maintained until September. Salinity reduced to its lowest due to heavy monsoon during July - September and a sudden rise in the salinity was observed in October, reaching peak in December - January. This indicates that a sudden fall and rise in salinity in September - October may have triggered spawning in the mature population. Similarly, lower temperature during August - September coincided with the initiation of spawning in September. Seasonal variations in *P. malabarica* with respect to temperature and salinity can be associated with the spawning cycle in *P. malabarica*. Similar spawning behaviour was reported in *Branchidonotes variabilis* (Wilson and Hodgkin, 1967) and *P. laterisulca* (Nagabhushanam and Dhamne, 1977). Gametogenesis is reported to be controlled by temperature in *Venus mercenaria* (Loosanoff and Davis, 1950a). In temperate conditions an optimum temperature is required for spawning ((Loosanoff, 1937a, b; Loosanoff and Davis, 1950a, b). But in tropical conditions, the water temperature is higher throughout the year. In the present study, higher gametogenic activity in *P. malabarica* was observed during premonsoon at higher temperature and spawning started in September when the temperature decreased. This suggests that extreme high and low temperature initiates gametogenic activities in *P. malabarica*.

Sex change is often reported in Pelecypoda as a result of age, rapid rate of growth or change in the environmental conditions. However, no evidence of such sex reversal was observed in the present study. Further, no significant difference in the number of males and females was observed, even though the percentage of females was found to be slightly higher throughout the year.

In summary, the shortneck clam along the Ratnagiri coast undergoes gametogenesis from February to June when the temperature and salinity show higher values, followed by vigorous spawning from September to January when the temperature and the salinity decrease due to monsoon and suddenly increase from October onwards. After a prolonged spawning period from September to January, reabsorption of the gonads takes place. *P. malabarica* showed the same pattern of gametogenic cycles in the two study locations.

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