

-Short Communication-

Temporal variation in reproductive pattern of windowpane oyster *Placuna placenta* (Mollusca: Placunidae) from Sonmiani, Balochistan, Pakistan

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Abstract: Windowpane oyster, *Placuna placenta* from Sonmiani on the coast of Pakistan was studied. They are pearl producing oysters well-known in south Asia. The sex-ratio in this species was found to be close to 1:1 Mendelian ratio with number of females being slightly greater than males. No sexually undifferentiated and hermaphrodite oyster was found. Five stages of gametogenesis, developing, ripe, partially spawned, spent and resorbing were identified in male and female oysters. Spawning in males and females was observed throughout the year with peak in spring and autumn and a brief resting period in winter. Gonad index in males was 1 to 5, whereas, in females ranked 1 to 4. This value was never ranked 5 in females as continuous spawning occurred throughout the year except December.

Keywords: Windowpane oyster, northern Arabian Sea, gametogenesis, spawning season

Introduction

The oysters of family Placunidae are one of the pearl producing oysters best-known in India, Malaysia, the southern South China Sea, and on the northern coasts of Borneo to the Philippines (Yonge, 1977). The windowpane oyster *Placuna placuna* in India, Bangladesh and the Philippines is fished and cultured for the shell and seed pearls for use in pharmaceutical industry (Dharmaraj and Sreenivasagam, 2002; Laxmilatha, 2015; Rahman *et al.*, 2015). They are also commonly known as Jingle shell or kapis shell in the Philippines, the name for the Jingle sound where the kapis city got its name from the delicate, translucent *Placuna* shells that are used for window glazing and shell craft (Rosell, 1979).

Biological information on the windowpane oysters is very scarce. Narasimham (1984) studied the age, growth, allometry and reproduction of *P. placenta* from Kakinada Bay, India. Young (1977) and Rosell (1979) investigated the biology and ecology of *P. placenta* from the Philippines. The window-pane shell fishery resulted in the depletion of natural stocks in the Philippines (Gallardo *et al.*, 1995) and emphasized the need of artificial propagation these oysters. Study on the broodstock conditioning, induced spawning, larval development and mariculture were initiated in the Philippines (Young, 1980; Rosell, 1984; Gallardo *et al.*, 1992 a,b). Dharmaraj *et al.* (2004) worked on larval

rearing and spat production of *P. placenta* in Tuticorin, India.

Many species of clams, cockles and oysters of commercial interest along with windowpane oyster occur in creeks, backwaters and lagoons along the coast of Pakistan in the Sindh and Balochistan region (Ahmed, 1971; Moazzam and Ahmed, 1994; Siddiqui and Ahmed, 2002, Jahangir *et al.*, 2010). Although windowpane oysters are commercially important yet they did not receive much attention of the local scientists. The windowpane oysters occur in creeks on Sindh and Balochistan coasts. These stocks are not large enough to sustain commercial utilization of this species. This study intended to examine the reproductive strategies of windowpane oyster *P. placenta* from Sonmiani, Balochistan for the future management and aquaculture of this potentially valuable and commercial resource.

Study site

Sonmiani Bay is located in Lasbela District, Balochistan. It is almost completely enclosed, bay or lagoon lying parallel to the Arabian Sea, situated at a distance of 90 km from Karachi, lies at a latitude of 25°N and longitude of 66°E. It gets drained to a great extent at low spring tides exposing the muddy-cum-sandy flats which stretch for miles. The bay is rich in

fisheries resources, has a deeper areas which is used for anchoring of fishing fleet at Dam. In Sonmiani Bay mangroves are spread over an area of about 2500 ha. The windowpane oysters buried under the soft silt sediment were randomly handpicked from the intertidal area of the sampling site (Fig.1).



Fig. 1: The location sampling sites.

Sampling

The samples of *P. placenta* were collected monthly from June 2013 to May 2014. The live oysters were transferred to the laboratory. After clearing and washing the mud from oysters, they were kept in aerated seawater for purging mud from the gut. The shell height was recorded nearest to 0.01mm with vernier calipers from umbo to the outermost growth margin. The salinity and water temperature was recorded in situ. Salinity and temperature were measured with handheld refractometer (Atago, S/Mill-E) and thermometer, respectively.

Histological preparation

The soft tissues of the oysters were dissected out by cutting the anterior adductor muscles with sharp knife to open the shell. The dorsoventrally section was obtained that provided representative of cross section of gonads. A small portion of each gonad was immediately placed in Davidson's fixative (Shaw and Battle, 1957) for 48 hours and then kept in 70% alcohol until further processing. To prepare the gonad tissues the standard procedure of dehydration, clearing and embedding in paraffin wax followed. The tissues were cut at 7µm sections, stained with hematoxylin and counter stained with eosin. The prepared slides were examined microscopically to determine sex and stages of gonadal development of the individual oysters.

Gonad Index (GI)

The GI was estimated by the method described by Barkati and Ahmed (1990). The gonadal condition of each oyster (N=40) was determined and grouped in different stages. The number of oysters in each stage was multiplied by the numerical ranking of the respective stage and the sum of this product was divided by the total number of individuals in the sample. A value of 1 was assigned to resorbing oysters and the increase in the gonad index indicates gonad maturation.

Physical parameters

The water temperature was 17-34°C In the sampling period and low water temperature was noted in January to April period (17-20°C). The salinity remained between 35-40 ‰ throughout the year and the highest being observed in January (Fig. 2).

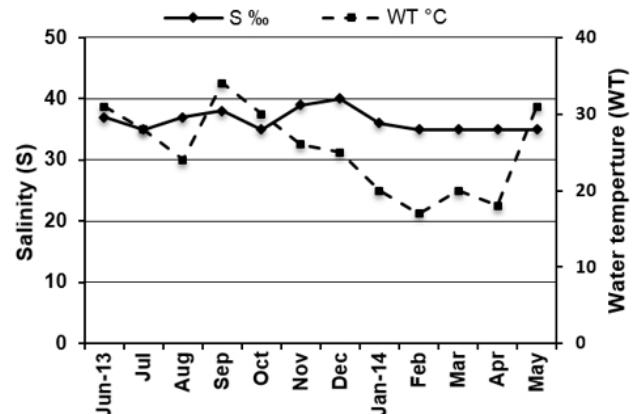


Fig. 2: Temporal variation in salinity (‰) and water temperature (°C) at Sonmiani.

Sex ratio

Chi-square test for sex determination of *P. placenta* displayed no deviation from the theoretical ratio of male and female (1:1) by representing no significant difference ($P > 0.05$) in each month (Tab. 1). The different size groups representing the 55 to 165mm oysters also showed no significant deviation from the expected 1:1 sex-ratio, though in the largest size-group females were slightly higher in number than males ($X^2 = 0.30$; $P > 0.05$). Whereas, in oysters of family Ostreidae, *Crassostrea gryphoides*, *C. madrasensis* and *C. belcheri* the sex ratios were in favor of males with undifferentiated oyster dominating in December and January (Siddiqui 1998; Siddiqui and Ahmed, 2002, 2003). Similarly Asif (1975) observed high proportion of males in *C. rivularis* but more females in *C. glomerata* and in *Saccostrea tuberculata*. Ansari and Ahmed (1972) also reported

overall sex-ratio to be in favor of females in *C. glomerata*. Joseph and Madhyastha (1984) found the male:female ratio to be 1:1 in *C. madrasensis*.

Tab. 1: Monthly Chi-square (X^2) distribution of male (M) and female (F) *P. placenta*.

Months	M	F	X^2	P.
Jun-13	24	16	1.6	0.21
Jul	17	23	0.9	0.34
Aug	27	13	4.9	0.03
Sep	15	25	2.5	0.11
Oct	21	19	0.1	0.75
Nov	14	26	3.6	0.06
Dec	17	23	0.9	0.34
Jan-14	26	14	3.6	0.06
Feb	13	27	4.9	0.03
Mar	23	17	0.9	0.34
Apr	24	16	1.6	0.21
May	11	29	8.1	0.00
Total	232	248	0.53	0.470

Stages of gametogenesis

The gonadal colour in ripe females and males were bright red and white, respectively. The five stages of gonadal development were recognized namely, developing, ripe, partially spawned, spent and resorbing. Likewise five stages (early active, late active, ripe, partially spawned and spent) of gonadal development have been reported in windowpane oyster *Placenta placenta* from the Phillipines (Rosell, 1984). In *P. placenta* from Kakinda Bay, India four stages of gametogenesis were recognized, active, and ripe, partially spawned and spent/resting (Narasimham, 1984).

The histological examination of gonads revealed that in early development follicles are small in size, few in numbers, surrounded by abundant interfollicular connective tissues. The follicles size gradually increases with the development of gonads around the digestive diverticula and the germ cells attached to the walls of follicles. In female mostly oogonia and previtellogenic oocytes protruding towards the lumen and later with the gonadal development vitellogenic oocytes are also visible. In male follicles mostly spermatogonia and spermatocytes few spermatids can be seen. In the ripe stage follicles are fully expanded and completely packed in the gonadal area, spread over the sides of digestive diverticula and the interfollicular connective tissues nearly absent. Mostly post vitellogenic or mature ova, very few pre-vitellogenic and vitellogenic oocytes present in females and a small number of spermatocytes and spermatids restricted towards the periphery of the male follicles and large number of spermatozoa filled the lumen of

the follicles. In partially spawned oysters, few unspawned ova and spermatocytes /spermatids can be seen in the lumen of the female and male follicles respectively. In spent oysters follicles are partially contracted less in number and interfollicular connective tissues reappearing. Unspawned ova/residual spermatids and spermatozoa are present. During resorption follicles collapsed and few in number, relatively empty later on with resorption and interfollicular connective tissue starts reappearing few residual mature ova or oocytes can be seen with interfollicular connective tissue in females and residual spermatozoa undergoing resorption.

Temporal variation in the gonadal stages, gonad index (GI) and spawning season in male and female oysters

Developing females were found from June to September, January to March and in May. In March peak (62%) of the females was noted and their lowest number (17%) was recorded in June. Ripe females appeared in July (46%) and their number gradually decreased to 18% in October. From March onward, they constituted 27 to 41% of the female population. The partially spawned females were found in six months with greater frequency in November (60%) and April (54%). Though spent females were also found in July, October, November but their higher frequency occurred in January (50%) and April (46%). Resorption in females was noted from July (9%) and in December it was 100% and again in February 53% females underwent resorption (Figs 3 and 4).

The male oysters with developing stage were observed from June to October (6 to 23%), January and March (29%). The ripe males were found in July to October (18 to 46%), and during March to May (27 to 41%). The males with partially spawning stage appeared in June (14.29%) and in September their percentage increased to 45%. However, from November till February no male was in partially spawned stage but in March (29%) and April (36%) their number considerably increased. The spent stage was only observed in January, September and in November all the male population was in spent condition. The maximum (100%) resorption was noted in December and February whereas in May it was 73% (Figs. 3 and 4).

In males the gonad ranking was 1 to 5 whereas in females it was 1 to 4 and they were never ranked 5 as continuous spawning capability was apparent throughout the year except for December, however,

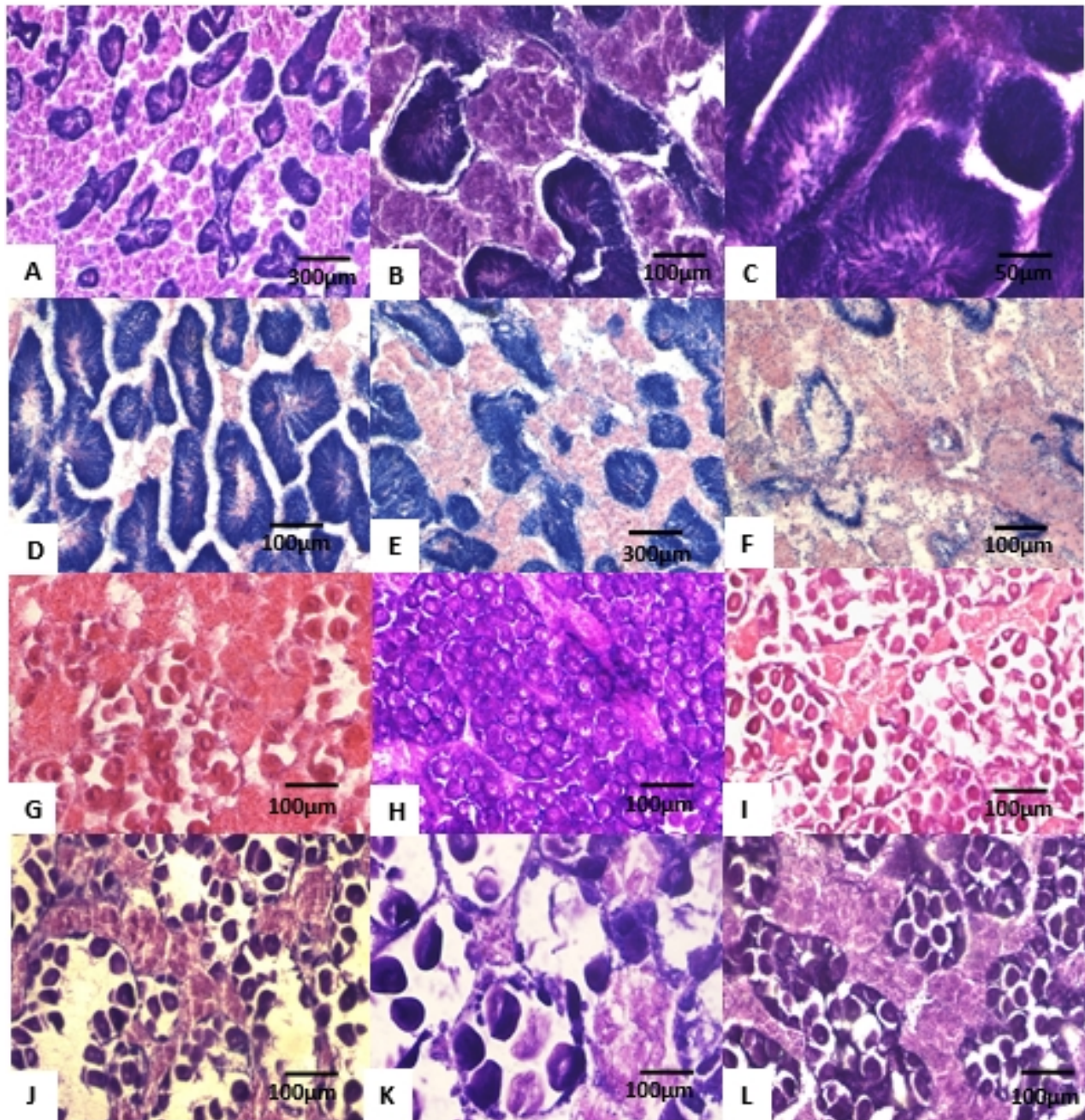


Fig. 3: Gonadal development in *P. placenta*. Male (A-F) A-B: Developing; C: Ripe; D: Partially spawned; E: Spent; F: Resorbing. Female (G-L); G: Developing; H: Ripe, I-J: Partially spawned; K- Spent; L: Resorbing.

the highest peaks (4) was observed in April. The males from June to November their GI gradually increased to 5 and ranked 1 in December and February when they were either undergoing resorption or in the early developing stage (Fig. 3). The spawning seasons were determined by combining ripe and spawning stages. The spawning in females was observed throughout the year except December and February. In the remaining months 14% to 50% females were in spawning condition while spawning in males occurred from June to October and then in March to May. In September it was 83% and

increased to 100% in April (Fig. 5).

The coast of Pakistan is situated outside the tropics experiencing very little rainfall and most part of the year temperature is $\pm 35^{\circ}\text{C}$ with a very short winter when temperature rarely falls below 20°C (Fig. 1) thus facilitating year round spawning in many marine animals (Barkati and Ahmed, 1990; Hassan, 1992; Ahmed and Hameed, 1999; Siddiqui and Ahmed, 2003; Jahangir et al., 2014). On the subtropical coast of Pakistan this strategy is adopted by nearly all bivalves such as oyster species of *Crassostrea* and *Ostrea* (Asif, 1979, 1980; Siddiqui and Ahmed, 2002,

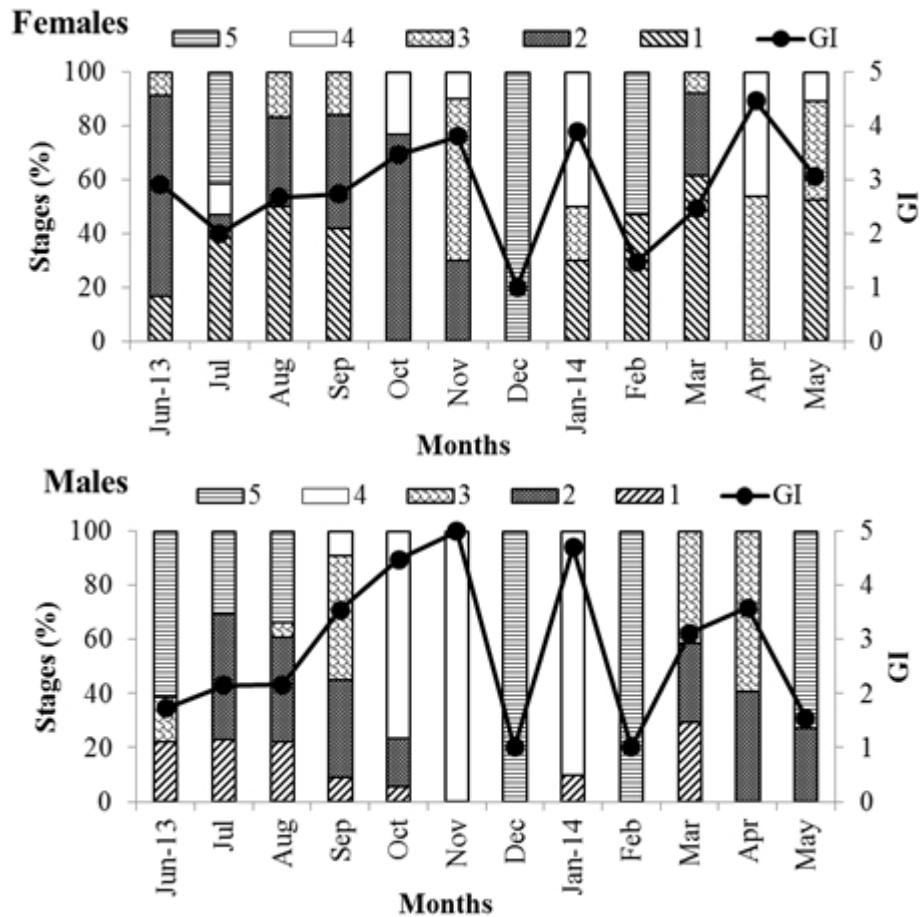


Fig. 4: Temporal variation in gametogenic pattern and gonad index (GI) of *P. placenta*.
 1- Developing. 2- Ripe. 3- Partially spawned, 4- Spent, 5- Resorbing.

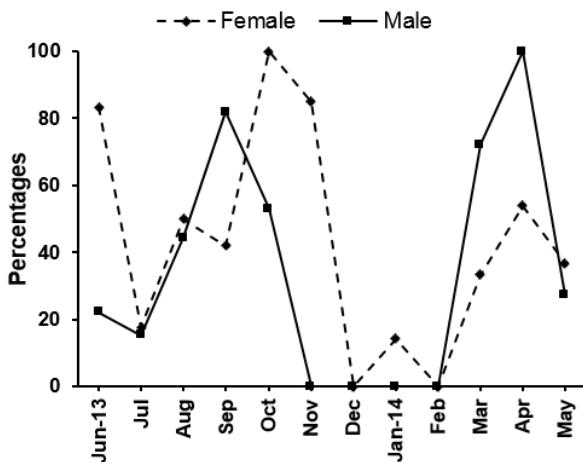


Fig. 5: Monthly variation in the spawning season of male and female *P. placenta*.

2003), clams (Khatoon, 2009; Jahangir, 2010), and blood cockle *Anadara antiquata* (Jahangir et al., 2014). This strategy is also observed in invasive mussel *Perna viridis* on the southeastern coast of United States (MacFarland et al., 2016). This may possibly be due to partial spawning strategy adopted by tropical bivalves as suggested by many authors

Walter, 1982; Rajagopal et al., 1998; Lee, 1986; Cheung, 1991; Gasper, et al., 1999; Pouvreau et al., 2000; Kripa et al., 2009).

Ahmed (1980) in his review on the breeding seasons of marine animals of Pakistan identified four groups i) monsoon spawners, ii) winter-spring spawners, iii) year round spawners and, iv) spring spawners. He further elaborated that species inhabiting high to mid-tidal zones spawn during summer for six to eight months while those live in low-tidal zones or sub-tidally spawn in winter and spring or throughout the year. *P. placenta* on our coast inhabit the low-tidal zone therefore, fall in group 3.

In Kakinda Bay India windowpane oyster *P. placenta* spawn biannually in February to May and November to December (Narasimham, 1984) similar to spawning in oyster of family Ostreidae influenced by local temperature and fluctuation in salinities particularly on its southeast coast which is subjected to heavy rainfall (Rao, 1956; Nagabhushanam and Bidarkar, 1978; Rajapandian and Rajan, 1983., Joseph and Joseph, 1986). In *P. placenta* studied from the Phillipines the spawning takes place during

March to September (Rosell, 1984).

In general, the bivalves mostly adopted three possible reproductive strategies as a result of geographical location such as high latitude, temperate and tropical regions (Yap, 1978; Berthelin 2000, Pouvreau et al. 2000, Vauhiti et al., 2016, Martin et al., 2017, Mena-Alcántar, 2017). In high latitudes or in temperate regions the summer period is brief and warm, with mild springs and autumns whereas, winters are prolonged and cold as a result, the reproductive organs in bivalves are normally inactive in winter and gametogenesis begins in early spring and spawn their gametes in spring-summer when water temperatures are high followed by resorption in the autumn. In the tropical environment multiple spawning incidences occur throughout the year, with a peak in summer months (Gosling, 2003). The spawning strategy of *P. placenta* of Pakistan is close to adopted by bivalves of the tropical environment which is supported by GI of male and female oysters.

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