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Reproductive periodicity in the edible oyster Saccostrea cucullata from Deogad, Sindhudurg district, Maharashtra State, India.

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ABSTRACT:

The locality of Deogad (16^0 23' N ; 73^0 23' E) in Sindhudurg district of Maharashtra State, India was selected on the basis of the habitat , topography, vegetation and local market value to study the reproductive periodicity of the oyster *Saccostrea cucullata* . The maximum sizes attained by *S. cucullata* in the estuaries at Deogad was 44-45 mm shell length. However, larger sized oysters are found round the year in the estuary at Deogad.

The environmental parameters such as tidal heights ,pH, temperature , dissolved oxygen and salinity existing on the oyster beds in Deogad was recorded on every new-moon and full-moon days for a period of twelve months.

The microscopic details of the gonad tissue processed on every new moon (NM) and full moon (FM) days of each month revealed following stages ; (i) Gametogenesis ; (ii) Maturing ; (iii) Mature; (iv) Partial spawning ; (v) Complete spawning ; (vi) Recovery ;(vii) Neutral . The gonads of twenty oysters were staged for males and females separately on each NM and FM days and percentage of the males and females in these different stages were calculated.

The study on reproductive periodicity in male oysters of *S. cucullata* from Deogad showed that many oysters were in gametogenesis stage in entire June and once again on November NM. Maturing stage was seen on March NM, May FM, July NM and again on November FM and in entire December. Many oysters were in mature stage on March FM, April FM and July FM and again on January NM. Most of oysters were under spent stage on March FM, in entire August and September, and on January FM. Many oysters were under recovery stage on May NM and in entire October. Most of the samples collected in entire February and on April NM showed prominent neutral stage. The female oysters showed that the gametogenesis was dominant on March NM, in entire June and on November NM; maximum on NM of March and on November. The maturing stage was dominant in entire April and May and on July NM and December NM. Oysters under spent condition were dominant on April NM and December FM, and all the gametes were released in entire August, September and on October NM. The recovery stage was recorded in many oysters on October FM and January FM, while the neutral stage was recorded in entire February.

These different stages of the gonads have been correlated with the changes in environmental conditions over the oyster beds from the two localities. The results are discussed in the light of possible impact of the environment on reproductive events.

Key words: *S. cucullata*, Deogad, Achra, NM, FM, gametogenesis, maturing, mature, spent

INTRODUCTION :

Along the west coast of India the backwaters and estuaries are very extensive and play an important role for food production. These are widely scattered and have an area of 30.7 lakhs acres (Mitra, 1970) from which Maharashtra coast constitutes 3.0 lakhs acres combining together 2.0 lakh acres for brackish water and 1.0 lakhs acre for estuaries. These backwater and estuaries are very productive along the coast and are being used for various purposes. They are the breeding grounds of various species of marine and estuarine communities (Dwivedi, 1973). The aquaculture of bivalve molluscs has attained a considerable level of production but it is not enough to cover the demand of worldwide consumers (Susan P. *et a*l., 2010). .An extensive literature have been reviewed by Sastry(1979), Nagabhushanam and Mane (1991) and Mane (1997) on the oysters from different geographical locations. The family Ostreidae, includes the diverse species of the genera *Ostrea* , *Crassostrea* and *Saccostrea* growing naturally in suitable habitats and also cultivated for food in different parts of the world .The pink oyster *Saccostrea mordax* (=*S.commercialis,S.cucullata*) (Glude, 1974 ; Braley,1984)is a native species of oyster in most Pacific Island groups along with the giant rock oyster *C.echinata.* Local variations among Island groups are considered to be eco-morphic within the species although in some cases these variations might warrant designation as varieties (Glude, 1974).In the northern Queensaland blacklip oyster S*accostrea echinata* and the milky oyster *S.amosa* are found (Nill *et al*.,1990).*Saccostrea glomerata, S. commercialis , S. denticulate, S.echinata* and *S.mordax* from i-Indo-Pacific region identified by Gomez (1980),are either morphs or geomorphic varieties of *S.cucullata*. The range of *S.cucullata* is enormous ;from East Africa (Day 1974) to Australia (Dinamani,1974). However molecular biology studies (mitochondrial DNA 16S sequence) of *Saccostrea* populations in Australia recognized at least three different species (Lam and Morton, 2001). In Mediterranean it was recorded first in 1999 from Turkey at Erdemli and Yumurtalik, Tasucu as *Saccostrea commercialis* (Cevik *et al*., 2001).

The locality of Deogad (16^0 23' N ; 73^0 23' E) in Sindhudurg district of Maharashtra State ,India (**Fig.1**) was selected on the basis of habitat , topography ,vegetation and local market value to study the reproductive cycles of the oyster *Saccostrea cucullata*. The maximum sizes attained by *S. cucullata* (**Fig.2**) in the estuaries at Deogad was 44-45 mm shell length .

Fig. 1: Map showing the coast of Maharashtra state

Fig. 2: Shells of *Saccostrea cucullata*

T eper and wider. The topography of the oyster bed on the rocks in this locality is mixed soil of mud and sand. In Deogad mangrove vegetation exits near the oyster bed. The oyster bed in the intertidal zone in Deogad get exposed to atmospheric air for a longer period. The estuary is free from water pollution and no mechanical fishing operation occurs. The water is fairly clean on the oyster beds. The environmental parameters such as tidal heights, pH, temperature, dissolved oxygen and salinity existing on the oyster bed in Deogad was recorded on every new-moon and full-moon days for a period of twelve months.

MATERIALS AND METHODS :

Environmental parameters : Temperature, salinity, dissolved oxygen and pH on the oysters habitat at Deogad was recorded on full-moon and new-moon days . The samples of sea water were drawn just before the collection of these oysters and analysed immediately. Samples were collected for determination of dissolved oxygen in 250 ml DO bottles and oxygen was fixed by adding alkali iodide for further analysis by Wrinkler's method, azide

modification. The temperature of sea water was recorded with the help of standard centigrade thermometer ^{0}C. pH was recorded with the help of standard BDH pH paper strips. Salinity was measured according to the method given by Parson *et al.* (1984). The replicates of these determinations on each fortnight were used in calculation. The height of the tide was recorded from chartdatum.

Changes in the gonads : *Saccostrea cucullata* (44-45 mm shell length) were collected from the fixed locality at Deogad in Sindhudurg district. Twenty oysters were collected on low tide of every new-moon and full-moon days for a period of twelve months. The oysters were brought to the laboratory and shucked for the flesh .The gonad was dissected and fixed in Bouin's Hollande preservatives prepared in sea water for further paraffin preparations for histological study. The paraffin blocks were cut at 7 µm thickness and stained with Mallory's triple. The observations were made under VT-20 Labo Video Scan microscope on television and whenever necessary photomicrographs were taken after measurements of the gonad contents.

RESULTS:

The environmental parameters such as tidal heights, pH, temperature, dissolved oxygen and salinity existing on the oyster bed in Deogad were recorded on every new-moon and full-moon days for a period of twelve months (**Figs. 3**).

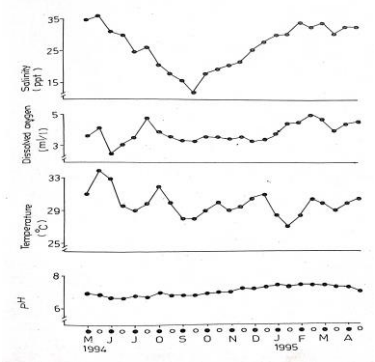


Fig. 3: Fortnight variations in the physicochemical parameters from the habitat of *S.cucullata* in the estuary of Deogad.

The tidal heights differed according to the full-moon and new-moon. Generally, the new-moon tidal heights were comparatively higher than the successive full-moon tides in both the estuaries during May to October and vice-versa in the remaining period. The pH of sea water for the oyster bed is from 6.7 to 7.5. Generally, the maximum pH values were recorded in summer season, whereas minimum pH values were recorded in monsoon. Fortnight variations in temperature sh… …num temperature was recorded in June, whereas maximum was ob… …mperature was recorded in December. The fortnight variations in the… … estuary at Deogad was ranged from 2.53 to 5.07 ml/l. The salinity sh… …t. The maximum salinity values coincided with summer season, wh… …n and influx of freshwater . The influx of freshwater in the estuary at Deogad is more.

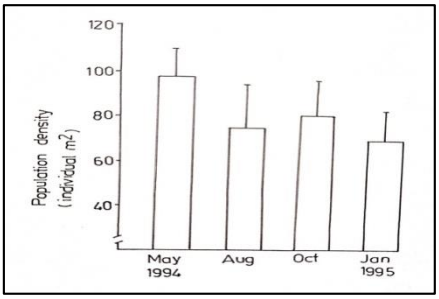


Fig. 4: Seasonal variations in the population density of *S. cucullata* from the estuary at Deogad. [Bar represents ± S.D.]

The oysters density is high in Deogad in all the season (**Figs. 4**). The maximum density was in May and minimum in January at Deogad. The oyster in Deogad showed population density 98±17.14 individuals /m^2 in May. In August it was 81±19.60 individuals/m^2.In October it was 81± 16.32 indiduals/m^2 and in January 70±13.88 individuals/m^2. The gonads of twenty oysters were staged for males and females separately on each new-moo (NM) and full-moon (FM) days and percentage of the males and females in the different stages were calculated. The results are shown in **Figs. 5** to **6**.

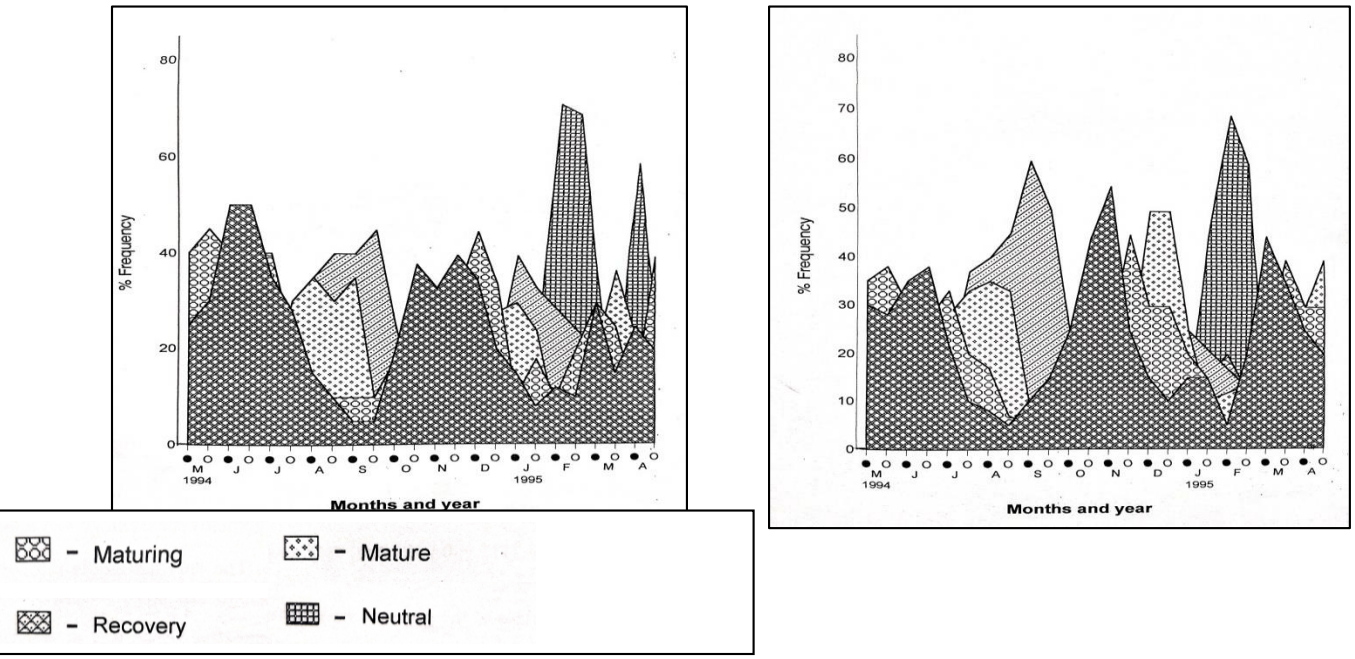
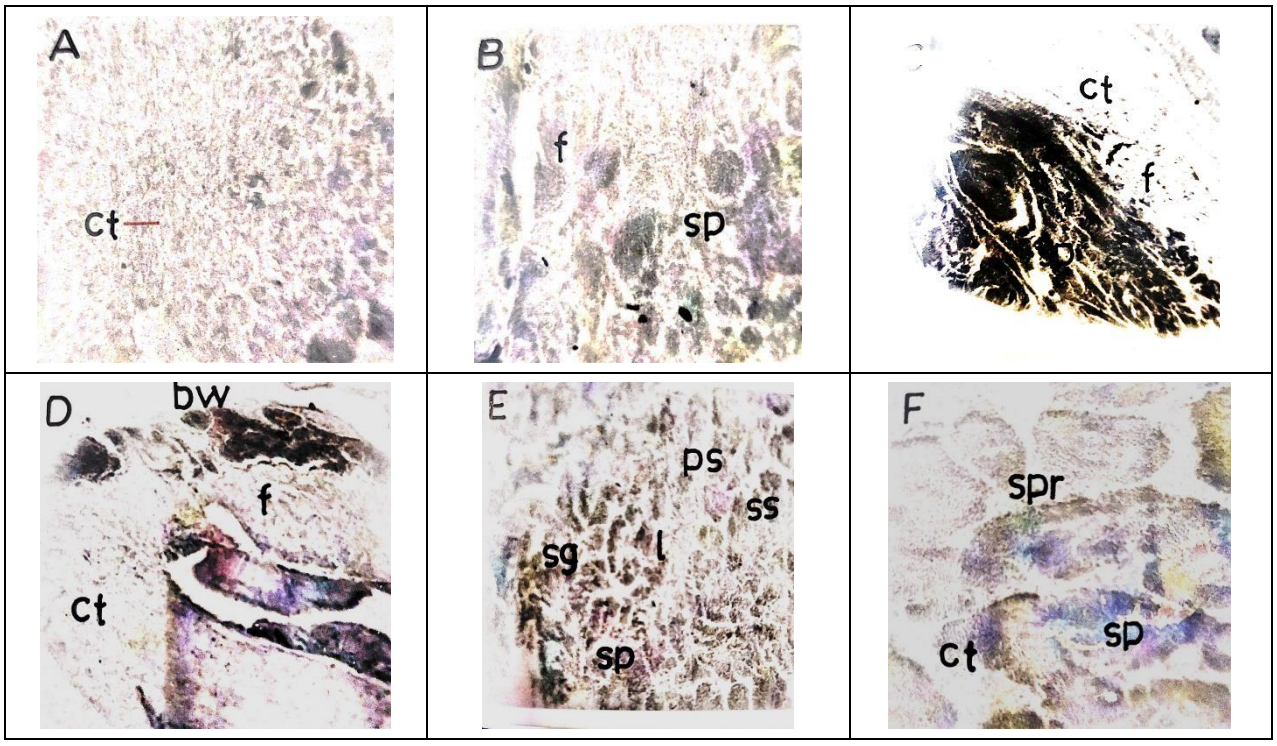


Fig. 5 and 6: Frequency polygon of male and female gonad of S. cucullata from the estuary at Deogad

The microscopic details of the gonad tissue processed on every NM and FM days of each month revealed following stages; (i) Gametogenesis; (ii) Maturing; (iii) Mature; (iv) Partial spawning; (v) Complete spawning; (vi) Recovery;(vii) Neutral (**Figs.7** and **8**).



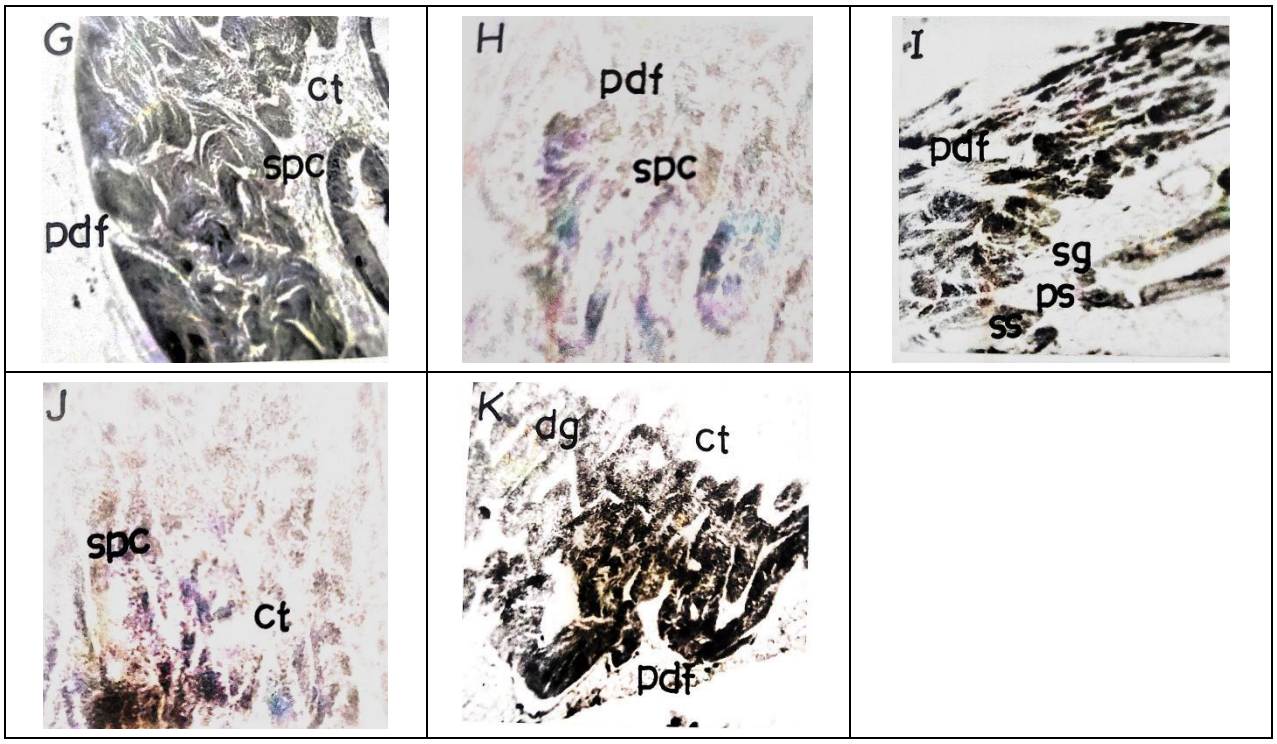
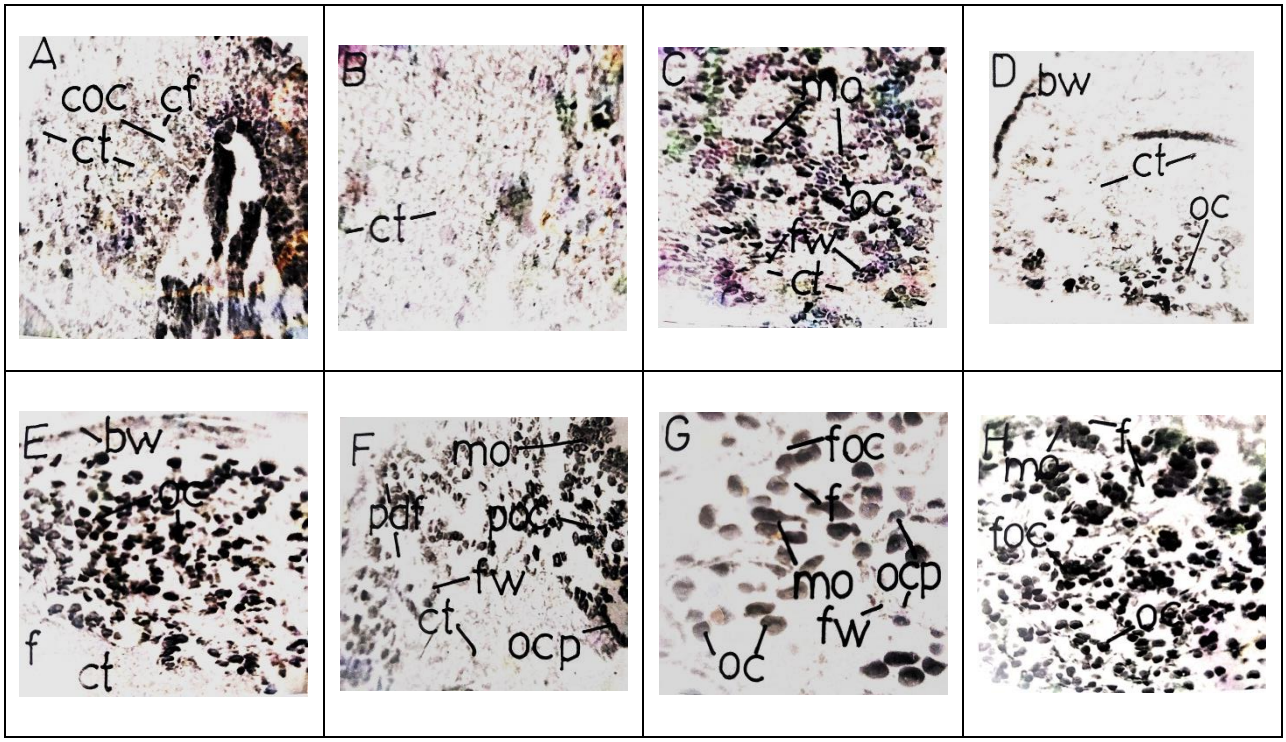


Figure7: Showing sections of gonad of male *Saccostrea cucullata* from the estuary at Deogad.
A-X 200. Neutral stage having expanded connective tissue (February new-moon).
B-X 100. Mature stage having compact follicles, some empty follicles with sperm plug in follicles lumen (March full-moon).
C-X 100. Mature stage having few follicles, full of sperms, other empty follicles collapsed in size and connective tissue expanded (April new-moon).
D-X 100. Simultaneous commencement of gametogenesis in some empty collapsed follicles, a few follicles contained many sperms and connective tissue expanded (May new-moon).
E-X 100. Compact follicles showing release of sperms and many follicles showed simultaneous growth of gametes wherein spermatogonia to sperm formation were seen (August new-moon).
G-X 100. Partial spawning and development of gametes wherein unspawned sperms undergo cytolysis (October full-moon).
H-X 200. Many follicles partially spawned, follicles collapsed in size and a few show development of spermatogonia and spermatocytes (November new-moon).
J-X 200. Magnified view of **I** with less connective tissue and unspawned sperms showed cytolysis.
K-X 100. Many follicles showing maturation og gametes with simultaneous release of gametes (December full-moon).

Abbreviations for figures 11 and 12.

ac	:	amoebocytes;	gc	:	germ cells;
A	:	phagocyte cell type A;	l	:	lumen ;
B	:	phagocyte cell type B;	mo	:	maturing oocytes;
bw	:	oyster body wall;	ngc	:	non-germ cells;
C	:	phagocyte cell type C;	oc	:	oocyte;
cdf	:	complete discharged follicles;	ocp	:	oocyte proliferation;
cf	:	collapsed follicles;	pdf	:	partially discharged follicles;
coc	:	cytolysis of oocytes;	poc	:	primary oocyte;
csp	:	clumps of spermatozoa;	ps	:	primary spermatocytes;
ct	:	connective tissue;	s	:	stem cells;
dg	:	follicles with developing gametes;	sg	:	spermatogonia;
ef	:	empty follicles;	sp	:	spermatozoa;
es	:	empty spaces of gametes released;	spc	:	cytolysis of sperms;
f	:	follicle;	spr	:	residual spermatozoa;
foc	:	free oocytes;	ss	:	secondary spermatocytes;
fs	:	follicle streaks;	st	:	spermatids;
fw	:	follicle wall;	uoc	:	unspawned oocytes.



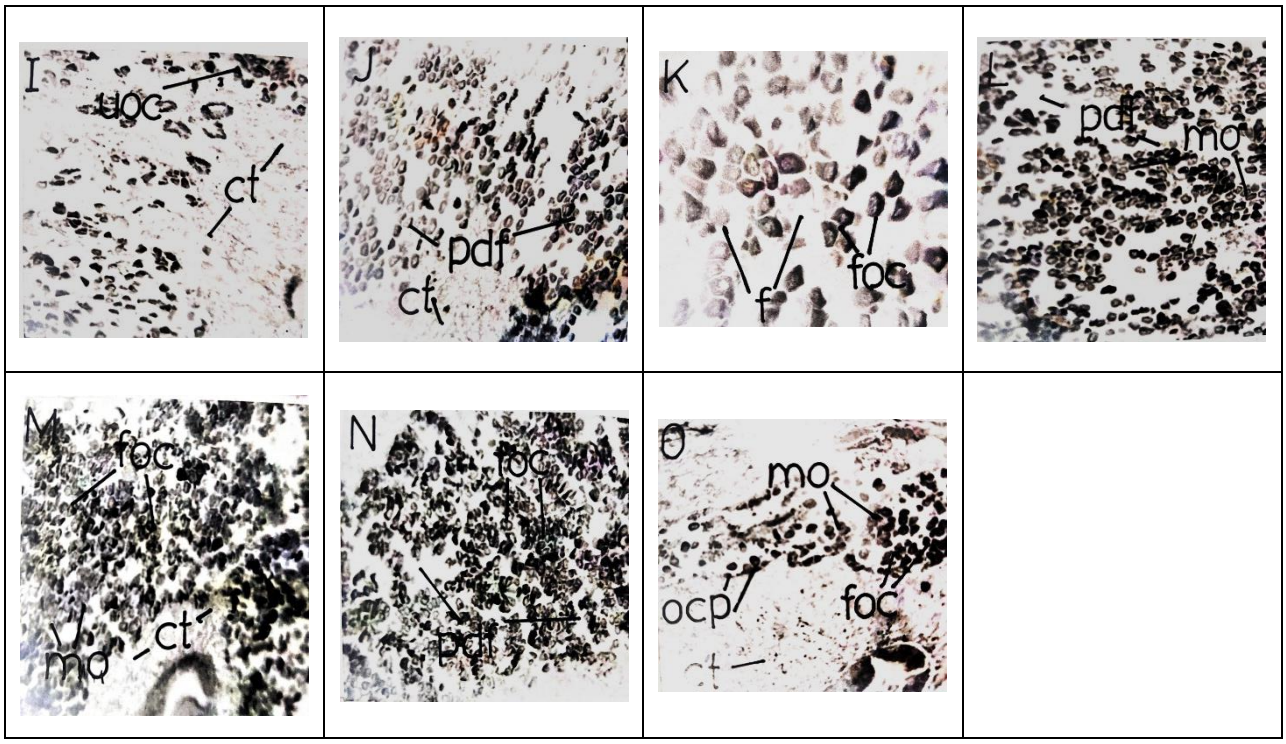


Figure 8: Showing sections of gonad of female Saccostrea cucullata from the estuary at Deogad.
A-X 100. Neutral stage having expanded connective tissue and collapsed follicles showed cytolysing oocytes (February new-moon).
B-X 100. Complete neutral stage having expanded connective tissue (February full-moon).
C-X 100. Developing oocytes in expanding follicles showing maturing oocytes and many small sized oocytes along the follicle wall (March full-moon).
D-X 100. Partial spawing wherein connective tissue was expanding and follicles showing proliferation of oocytes (April new-moon).
E-X 100. Same as **D** and new follicle streaks formed in the connective tissue which proliferated a new set of oocytes (May new-moon).
F-X 100. Partial spawning wherein few gametes undergo cytolysis, in some oocytes showed maturation and within the spent follicles new oocytes proliferated (June new-moon).
G-X 200. Same as **F** showing follicles with maturing oocytes and developing new set of oocytes (July new-moon).
H-X 100. Follicles with few matured oocytes, maturing oocytes and oocytes still attached to follicles (July new-moon).
I-X 100. Many follicles with unspawned oocytes and connective tissue expanding (August new-m00n).
J-X 100. Follicles with partial release of oocytes and no maturing oocytes or developing new set of oocytes were seen (August full-moon).
K-X 200. Follicles with many mature oocytes (September new-moon).
L-X 100. Follicles with partial release of oocytes, few maturing oocytes and few developing new set of oocytes were seen and connective tissue greatly reduced (October full-moon).
M-X 100. Follicles with few mature oocytes and many maturing oocytes and connective tissue greatly reduced (November full-moon).
N-X 100. Follicles with many mature oocytes, few follicles partially released the gametes and connective tissue greatly reduced (December full-moon).
O-X 100. Very few follicles contained mature, maturing and forming new set of oocytes were seen and connective tissue greatly expanded (January full-moon).

The study on reproductive cycle in male oysters of *S. cucullata* from Deogad showed that many oysters were in gametogenesis stage in entire June and once again on November NM. Maturing stage was seen on March NM, May FM, July NM and again on November FM and in entire December. Many oysters were in mature stage on

March FM, April FM and July FM and again on January NM. Most of oysters were under spent stage on March FM, in entire August and September, and on January FM. Many oysters were under recovery stage on May NM and in entire October. Most of the samples collected in entire February and on April NM showed prominent neutral stage. The female oysters showed that the gametogenesis was dominant on March NM, in entire June and on November NM ; maximum on NM of March and on November. The maturing stage was dominant in entire April and May and on July NM and December NM. Oysters under spent condition were dominant on April NM and December FM, and all the gametes were released in entire August, September and on October NM. The recovery stage was recorded in many oysters on October FM and January FM, while the neutral stage was recorded in entire February.

DISCUSSION:

In the present study it was observed that the gametogenesis in *S.cucullata* from Deogad was at peak in both the sexes with lowering of salinity to 30.75 ppt and temperature to 33^0C in June NM before commencement of monsoon and for the second time in October FM at the time of rise in salinity to 19.25 ppt and temperature to 30^0C and for the third time in March NM at the time of rise in salinity to 34.63 ppt and temperature of 30^0C. Many females on October FM and March NM and many males on June NM showed dominant gametogenic stage compared to those females occurred in samples during pre-monsoon and males during post-monsoon.

The maturaing stage in *S.cucullata* from Deogad was at peak in females with lowering of salinity to 24.37 ppt and temperature to 29^0C in July NM, while in male in October FM with rise in salinity to 17.97 ppt and temperature to 29^0C and again for the second time in November FM in female oysters with rise in salinity to 21.81ppt and temperature to 29.5^0C, while in male oysters in December NM with rise in salinity to 25.66 ppt and temperature to 30.5^0C, for the third time in female oysters in March FM with lowering of salinity to 32.06 ppt and temperature to 29^0C, while in male oysters in March NM with a rise in salinity to 34.63 ppt and lowering of temperature to 30^0C, and for the fourth time in male oysters in April FM with rise in salinity to 34.63 ppt and temperature to 30^0C. Many females on November FM and March and many males on December NM and April FM showed dominant maturing stage compared to those females occurred in peak monsoon and males during post -monsoon and early summer

The mature stage in female *S.cucullata* from Deogad was at peak in June NM with lowering of salinity to 30.75 ppt and temperature 33^0C, while in male oysters the peak was in July FM with the lowering in salinity to 25.06 ppt and temperature to 29.9^0C, for the second time in female oysters the peak in this stage was in August NM which coincides with lowering in salinity to 20.53 ppt and rise in temperature to 32.6^0C, while in males in November FM with rise in salinity 21.84 ppt and rise in temperature to 29.5^0C, for the third time many female oysters in this stage December NM with the rise in salinity to 25.66 ppt and temperature to 30.5^0C. In male third peak observed in March FM with the slight lowering in salinity to 32.06 ppt and temperature 29^0C, and in male oysters fourth peak in mature gonad was observed in May NM with rise in salinity to 34.63 ppt and temperature to 31^0C . Many females on December NM and many males on March FM and May NM showed dominant mature stage to those females occurred in samples in peak monsoon and summer those males found in peak monsoon and early winter.

In the present study on *S.cucullata* from Deogad on the coast of Sindhudurg district it was observed that the spawning period as determined on the basis of occurrence of spent gonads of oysters was extended and differed during post-monsoon. There was a difference in spawning of females and males in this estuary. The spent stage on both the sexes of oysters from Deogad commenced from June FM and the percentage of oysters in this stage increased till September FM and later decreased. Once again a part of the population in this stage occurred on January NM and March FM. Extended period in the male oysters in this stage was seen than in females. Many males spawned than females in winter season and many females spawned in monsoon and beginning of post-monsoon than males. A small population of female oysters spawned in May but no male appeared in this stage during this period. The spawning period in both the sexes from July NM to October NM coincided with low salinity range 16.69 to 25.61 ppt and lowering of temperature to 28.5-29.5 ^{0}C. It was found that at the estuary of Deogad, *S.cucullata* spawned due to the drop in salinity ,in the monsoon season. During July to October the salinity of the estuary at Deogad was 22.66 ppt but lowered to 19.25 ppt during the peak of spawning. At Deogad for the second time peak of spawning was observed during December and January during which the salinity increased from 28.22 to 30.75 ppt. The temperature at Deogad on oyster bed varied before 27 to 32^0C.

The recovery stage in female oysters from Deogad occurred from September NM to March FM , while in

males this stage was seen in June NM , on August NM from September FM to November NM , on January NM , on March FM and April NM . The recovery stage was extended in females than males, wherein it was interrupted.

The neutral stages in female oysters from Deogad was observed August FM to November NM, December FM to March NM and in entire April, the peak was observed in entire February. This stage was observed in male from August FM to November NM , from January FM to February FM , and from March FM to April FM. Many oysters from both the localities were in this stage in February and males in April also .

The intrafollicular tissue in resorption of residual gametes was noticed in present study on *S. cucullata*. Large number of amoebocytes were observed around the follicles in certain periods of reproductive cycle, especially during post spawning periods. Extensive studies were carried out by Takatsuki (1934) on O. edulis and Tranter (1958a) on the amoebocytes in the former report and the phagocytes in the latter. Various types of amoebocytes and their role in gonad verginion in *S.cucullata* have been described by Yennawar (1997). In the present study, it was found that generally the amoebocytes of type A(as shown by Yennwar,1997) were in the size range 0.223 to 0.39 μm in their largest area in oysters from Deogad, Much variation in the size range was observed in the gonads of oysters from Deogad. The largest sized amoebocytes in oysters from Deogad were 0.974 μm. The largest sized amoebocytes were found in the gonads of oysters form Deogad on February NM and FM at the time many neutral oysters appeared in the samples and some female oysters showed recovering of the gonad. Appearance of large sized amoebocytes was again observed on October NM during which time the spawning in females and males was terminated and few oysters were in the recovery and neutral stages. On the other hand, the amoebocytes occurred frequently in the gonad at various stages of spawning, recovery and neutral during different seasons. The maximum sized amoebocytes occurred in July FM during which time many female oysters were spent and few were under recovery, while a few males were spent. From April FM to September NM and from January NM to February NM generally large sized amoebocytes appeared when compared to those found during rest of the period. During April to September there were frequent appearance of gonads of males and females, either under recovery or neutral and spent gonads occurred throughout this period. Again for the second time these large sized amoebocytes appeared when the gonads of many oysters were neutral and both spent and recovering gonads of oysters also occurred. However, it is probable that because of this redevelopment of gametes the number of oysters in gametogenic and maturing stages occurred round the year. Fully mature gametes were produced by large number of individuals only during certain period of the seasons, probably revealing that oysters could have spawned almost round the year with peaks differing in the estuaries at Deogad. Many oysters after partial release of gametes showed redevelopment of fresh gametes and only a small population showed recovering of gonad, the unspawned gametes were lysed.

In the present study it has been noted that *S.cucullata* in the estuary at Deogad gets exposed to atmospheric air during each low tide due to its intertidal habitat. This makes the oysters to feed only during the high tides. It is most possible that these oysters don't get more chance to feed. In this habitat it is likely to create inadequate nutrient supply for the gonad development and maturation. It was further noticed that mangrove vegetation persists near the oyster bed in the estuary at Deogad. It is likely that this vegetation can create impact on the nutrients richness in seawater at this estuary, nutrient rich seawater at Deogad. Since oysters at Deogad are exposed to each low tide, there appears to be energy demand for maintenance , metabolism and during the period of gonad growth it is possible that less energy is supplied for the gametes development and therefore, there is an extended period of post spawning to recommencement of gametogenesis. In oysters from Deogad this period was led down in males during entire October and again in February April NM and May NM, while in females during October FM and from January FM to February FM. This indicated that oysters from Deogad passed through a comparatively longer period of recovery and neutral condition before commencement of gametogenesis. Though the quantitative data on gametes produced by individual oyster was not obtained, the observations on histological preparations and scanning as television markedly showed less numbers of gametes being produced in oysters from Deogad. The afore mentioned longer preparatory period for oysters from Deogad probably show the buildup of body reserves and metabolic status before commencement of new gametogenic cycle. It is possible that the oysters from Deogad are more opportunists to allocate energy for reproductive efforts during a short period of favorable conditions received during high tide and exhibited a direct dependence of nutrient availability with the change in the environment.

In Kelwa back waters, Bombay (Mumbai) the south- west monsoon diluted the salinity from 28.59 to 13.51 ppt in July and stimulated spawning in *C. gryphoides* (Durve, 1965).This spawning continued till September. The spawning in a number of oysters has been reported as a protracted phenomenon. One or two

peaks within a breeding seasons lead to an extended breeding season (Mane, 1997).

The time required to reach spawning condition has been shown as a function of the daily rate of production of gametic tissue . Reports indicate that the controlling mechanisms that might become responsive to environmental influence at the beginning of the cytoplasmic growth phase or gametogenesis in response to endogenously regulating system through neurosecretory cycle (Bidarkar, 1975; Nagabhushanam and Mane, 1976; Desai and Nimavat, 1983). A population responding to environmental influence and modulating endogenously would initiate gonad development at the same time and progress towards maturity synchronously. The rapid expansion of the Pacific oyster in the Dutch and German Wadden Sea (Diederich et al. 2005, Dankers *et al*. 2004) and the observed patterns in growth and reproduction suggest that this species has not reached its eco-physiological limits yet. In fact, the northern distributional limit of C. gigas already extends as far as Denmark (Bos *et al*. 2006, Dankers *et al*. 2006).

In the present study sorting of the oyster gonad tissue into different reproductive stages revealed that *S. cucullata* from both the locality showed simultaneous maturation of gametes as the gametogenesis was advanced. Oysters under gametogenesis and maturation occurred round the year, however, dominance of each reproductive stage differed from one season to another. Once the matured gametes are produced spawning commenced and advanced with the response to stimuli received exogenously and endogenously from monsoon to post-monsoon period (Salunkhe, 1999). The period of spawning in males from both the locality was longer as compared to females. It was further observed that not all the mature gametes were released by the oysters from the locality and unspawned gametes were cytolysed in the recovery state which was longer in females than males in the oysters from Deogad. However, successive events in the reproductive cycles in oysters may be affected directly by various factors, and a detailed analysis requires a separate evaluation of each phase. Our knowledge of the environmental interactions and mechanism controlling the pattern of reproductive activity in oyster population is still fragmentary. With the control of reproductive activity and stimulation of gametogenesis in oysters maintained under controlled conditions, it should be possible to determine the effect of various environmental factors singly and in combinations, and to elucidate the mechanisms coordinating the reproductive response at the whole organism level. More data on the influence of various exogenous and endogenous factors controlling the beginning of gametes growth and mechanism, coordinating maturation and spawning in oysters are essential to effectively analyse the reproductive strategy in oyster population from the given locality.

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