# REPRODUCTIVE CYCLE AND CHANGES IN ASSOCIATED PARTS OF GONAD IN *CRASSOSTREA (SACCOSTREA) GLOMERATA* (GOULD) FROM THE COAST OF KARACHI*

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## ABSTRACT

A study of the reproductive cycle of *Crassostrea* (*Saccostrea*) *glomerata* from the coast of Karachi is described for the years 1971-73. The species attains maturity of gonad in majority of specimens in April, which is continued in fluctuating manner through October. Active spawning continues from May|June to November. Comparison among the different size groups shows that spawning in youngest size group is very restricted, which may be attributed to abundance of phagocytes throughout the year. No 'inactive phase' is observed but 'indifferent phase' generally occurs in winter. Comparison with the same species of New Zealand shows that the temperature range at which reproduction is carried out is different from that of the latter locality, suggesting acclimatisation to different temperature condition of the two areas. Glycogen granules were abundant in winter which declined gradually reaching minimum value in May|June and this condition remained in summer. In September, build of glycogen starts, which attained maximum concentration in the body parts in winter (November through January). In conclusion the observations on the reproductive cycle of the species from Karachi shows a different pattern from those of temperate regions.

## INTRODUCTION

The edible oyster, *Crassostrea* (*Saccostrea*) *glomerata* (Gould) is widely distributed in the northern hemisphere — China (Si and Tze-kong 1956), Phillipines (Carreon 1969), India (Awati and Rai 1931) and Pakistan (Ahmed 1971), and it occurs also in the southern hemisphere — New Zealand (Dinamani 1974). As compared to a large number of oviparous species of oysters, the reproductive cycle of only a few has been so far studied (*C. virginica,* Kennedy and Battle 1963, Berg 1969, *C. gigas,* Imai and Sekai 1961, Berg 1969, *C. angulate,* Pascul 1970, *C. madrasensis* Rao 1956, *C. gryphoides,* Durve 1967 and *Saccostrea cucullata,* Awati and Rai 1931). Recently reproductive cycles of *C.* (*S*) *glomerata* from southern hemisphere (New Zealand) is reported by Dinamani (1974). In the present paper the results of similar studies made from Karachi coast are described, providing an important comparison of the

* Part of Ph.D. Thesis, University of Karachi.

behaviour of the species in the two hemispheres of the world. Earlier work on the reproductive cycle of this species is meagre; however, preliminary information about its spawning season is provided by Ansari and Ahmed (1972) without referring to reproductive indices, onset of gametogenesis, maturation and fattening period. The present intensive study describes these processes along with the associated changes in the glycogen content of the whole population and then intrinsic gonadal changes are correlated to some extent with the environmental factors.

## Material and methods

Dense beds of *Crassostrea* (*Saccostrea*) *glomerata* occur at Boat Club, Manora Channel, Karachi. The random samples of the oysters were collected at fortnightly intervals from the locality and these were sorted out into three size groups ranging from 0.5-1.5 cm (first) 1.6-3.0 cm (second) and 3.1-6 cm (third) in length. On the basis of the availability of these groups of oysters all the year round, 20, 10 and 5 oysters of the third, second and first size groups respectively were treated for histological study. For fixation, sectioning and staining, the procedure of Kennedy and Battle (1963) was followed. The gonadal condition of 30-40 oysters per sample of third size group was also studied on the basis of the method of Reddish (1962) and in this live-gamete study the thickness of gonad was also considered (table 1). The gonad to body ratio of the histological preparations of the oysters of third size group was computed as described by Kennedy and Battle (1963). For histochemical study of the changes of glycogen Best's carmine method (Baneroft 1967) was used. The material was fixed in alcoholic Bouin (Picric acid 1 gm, formalin 60 ml, acetic acid 15 ml, and 80% alchohol 150 ml) for 4 h and then transferred direct to 90% alchohol till yellow colour is removed after 2-3 washes in the alcohol in a day. Ultimately the material was put in absolute alchohol and blocks were prepared. For staining, sections were brought from xylene to 70%

Table 1. *Reproductive indices used in live gametes studies.*

| *Condition of gonad* | *Thickness of gonad* | *Condition of gametes in contact with sea water* |
|---|---|---|
| Fully developed | Thick | Sperm motile; Ova round |
| Preliminary gametogenesis | Thin | Sperm not motile; Ova remained elongated. |
| Spawned gonad | Thin | Sperm motile; Ova round |
| indifferent | Scarce or absent | Gametes rare or absent. |

alchohol, then direct to tap water and stained in haematoxylin. For staining in Best's carmine the sections were put in a mixture of 15 ml Best's carmine stock solution (carmine 2 gm, $k_2 CO_3$ one gm, and disalled water 60 ml), ammonia 12.5 ml and methyl alcohol 12.5 ml for 30 mintues. The sections were washed in two changes of a solution containing absulte alcohol 8 ml, methyl alcohol 4 ml and distilled water 10 ml. Later on, the sections were passed through absolute alcohol, and xylene and mounted in D.P.X. The red colour indicated glycogen. During this process a section known to contain glycogen was used as a control.

## OBSERVATIONS AND RESULTS

*Live Gametes Study*: Results of the observations in 1971 and 1972 on the basis of the live gametes study of 2,146 oysters are shown in figure 1. In January the oysters were without any gonad suggestive of complete absence of gametogenesis. By February majority of the population (73%) acquired thin gonads with gametes at their early stage. The gonad in majority of oysters became thick with fully mature and viable gametes during April (71-90%) suggestive of maturity of gonad. The gonad began to decrease in thickness, but sperms remained motile and ova fertilizable in May (63%) suggestive of spawning in the population which continued in fluctuating proportion from May to October. By November the gonad started disappearing being replaced by interfollicular tissue, and finally attained indifferent phase in winter. The trends noted in 1971 were confirmed in the year 1972.

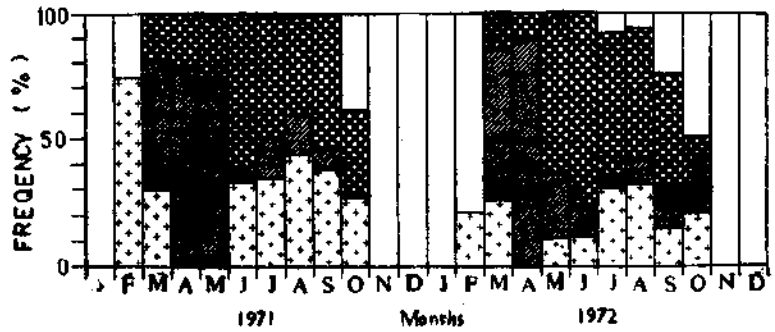


FIG. 1. Seasonal changes in gonad (third size group) of *C. (S). glomerata* on the basis of live gametes study: Plus signs indicate development; oblique lines, fully developed; chequer portion, spawning and unshaded portion, indifferent stage.

*Histological Study*: The details of changes in gametogenesis, maturation, regression of gonad and interfollicular tissue were obtained by histological study. Nine gonadal indices were differentiated (Plates I and II) as described by Tranter (1958). These indices (table 2) were grouped into: (a) early gemtogenic phase ($d_1$-$d_3$) and (b) phase of maturation of gonad ($d_4$-$d_5$), regression or spawning phase ($r_1$-$r_3$) and (c) indifferent phase (i).

TABLE 2. *Reproductive indices used in Histological studies*

| *Stage* | *Fellicles size and number* | *Content of a male follicle* | *Content of a female fellicle* | *Inter follicular tissue & its glycogen content* | *Glycogen in body* | *Amoebocytes* |
|---|---|---|---|---|---|---|
| 'd1' | Very small and very few in number- (Md1) | Indifferent gonia mostly; few spermatogonia and spermatocytes (Md1) | Indifferent gonia mostly; few difinitive oocytes and oocyte 1 Fd1, Plate I-A) | Very well developed and very rich in glycogen granules (Plate I-B) | Rich (Plate 1-C) | Occasional (Plate II-1) |
| 'd2' | Bigger than d1 and few in number | Spermatocytes mostly; few spermatogonia and spermatids (Md2: Plate I-E) | Oocytes 2 mostly; few definitive oocystes and oocytes 1 (Fd2: Plate 1-D) | Developed with fair amout of glycogen (Plate 1-D) | Rich | Occasional |
| 'd3' | Big; occupy more than ½ gonad area | No. of spermatocytes roughly equal to that of spermatids and sperms. (Md3: Plate I-F.) | Ooocytes 3 mostly few oocytes 1, 2, and mature ova Fd3: Plate I-F) | Reduced with little amout of glycogen (Plate 1-G) | Rich | Occasional |
| 'd4' | Very large, occupy whole gonad area | Sperms and spermatids mostly few spermatocytes. (Md4: Plate I-I) | Oocytes 3 mostly; few mature ova (Fd3: Plate I-F) | Greatly reduced with scarce glycogen (Plate 1-1-H) | Reduced | Rare or absent |
| 'd5' | Same as above | Sperms and Spermatids (Md5: Plate II-B) | Mature ova and oocytes 3 Fd: Plate II-A) | Same as above (Plate II-A) | Reduced | Rare or absent |
| 'r1' | Same as above | Partially empty follicles with sperms and spermatids (Mr1: Plate II-D) | Partially empty follicles with mature ova and oocytes 3 (Fr1: Plate II-C) | Same as above (Plate II-D) | Reduced | Rare or absent |
| 'r2' | Reduced; less than 'r1' | Mostly empty (Mr2: Plate II-F) | Mostly empty (Fr2: Plate II-E) | Start developing with little glycogen | Little | Fairly common |
| 'r3' | Few and collapsed | Empty with fed sperms (Mr3: Plate II-H) | Empty with few mature ova (Fr3: Plate II-G) | Well developed with fair amount of glycogen | Little. | Common |
| 'i' | Very few; highly collapsed | Occasional sperms (Plate II-I) | Occasional ova | Very well developed with denses glycogen | Rich | Few |

PLATE I. T. S. of different parts of *C. (S.) glomerata*.

A: follicles at Fd stage; B: Interfollicular tissue; C: gut; D: female follicles of Fd2 stage; E: male follicle at Md2 stage; F: female follicle at Fd3 stage; G: male follicle at Md3 stage; H: female follicle at Fd4 stage; I: male follicle at Md4 stage; (all at 130 x magnification) (a - amoebocytes A; b - amoebocytes B; do - defenite oocytes; f - follicle; g - glycogen granule; i - indifferent gonium; l - cluster of glycogen; og - ongonium; o01 - oocyte1; 02 - oocyte 2; 03 - oocyte3 04 - mature ovum; S - spermatozoa; Sc - spermatocytes; Sg - spermatogonium; St - spermatid.)

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PLATE II T. S. of different parts of body of *C. (S). glomerata*. A: follicle at Fd5 stage; B. follicle at Md5 stage; C: gonad at Fr1 stage; D: follicle at Mr1 stage; E: follicle at Fr2 stage; F: follicle at Mr2 stage; G: follicle at Fr3 stage; H: follicle at Mr3 stage; I: gonad at indifferent stage (A, B, D, F and I at 130 x and the rest at 60xmagnification) (Abreviations same as in Plate I)

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*Gonad to Body Ratio:* In figure 2 the seasonal changes in gonad to body ratio is plotted for the year 1972. In January, lowest ratio was observed indicating minimal gonadial development, which is coincided with maximum development of interfollicular tissue. From mid-February onward rapid development of gonad followed reaching its peak in April (52%). By June active regression

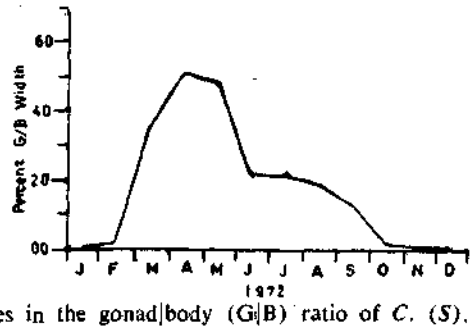


FIG. 2. Seasonal changes in the gonad|body (G|B) ratio of *C. (S). glomerata* during 1972.

of gonad contributed to the slight decline in the gonad to body (G/B) ratio and this condition continued till August. The ratio in later months declined rapidly reaching to very low level towards the end of November.

*Gonadial Changes*: The results based on 701 oysters of the third size group are shown in figure 3-A. In January, majority of the population possessed gonad with undifferentiated sex (55%) and this indifferent phase was marked with well developed interfollicular tissue. The rest of the population was in early gametogenic stage. This phase gradually progressed and became intensive on 31st March (67%). Maturation of gametes was followed and bulk of the population (92%) reached maturity on 22nd April. This presumably contributed to first spawning observed on 15th May. Later on, the spawning phase progressed reaching its peak on 12th July (80%). The early development of gonad proceeded along with other activities and majority acquired matured gonad on 25th September which presumably contributed to second peak of spawning on 7th October (50%). In subsequent months these phases declined rapidly. The indifferent phase, associated with bulk of interfollicular tissue, appeared in August and became dominant during November through January.

The observations made in 1972 and 1973 agrees well with those of previous year with minor differences in the occurrence of different phases (fig. 3-A). In all the three years study it is observed that the early gametogenesis appeared intensively in two successive periods viz. spring (February and March) and summer (June and July) presumably contributing to maxima of matured gonads in April and September/August. Although the spawning might be started in May and terminated in November, intensive spawning occured during July to October. The indifferent phase appeared in autumn (September usually), and became predominant in winter (November through February).

*Phagocytosis*: There were two types of amoebocytes (Amoebocytes 'A' and 'B') as described by Tranter (1958). In general, the amoebocytes were common in regressed gonad and were more common in female than in male gonad, suggestive of phagocytic activities of these cells in the residual gametes after spawning. These were rare during active gametogenesis in spring and early summer and became common from August through November in the fully regressed gonad suggestive of phagocytosis of residual gametes during the period. The phagocytosis began to disappear when majroity of the population entered into indifferent phase in December.

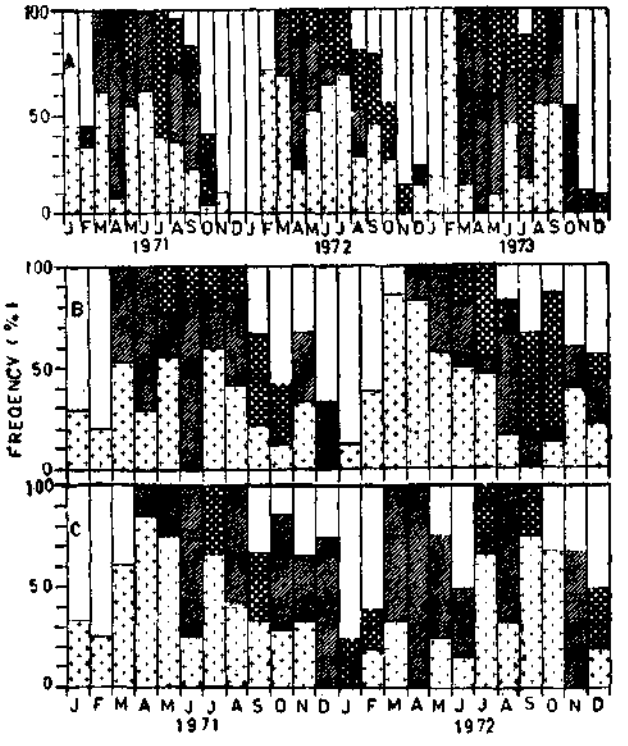


FIG. 3. Seasonal change in gonad of *C.* (*S*). *glomerata* on the basis of histological study: (A) third size group; (B) second size group; (C) first size group. (Plus signs: early gametogenesis; obliquelines: matured gonad; cheuered: regression and unshaded: indifferent phase)

*Comparison among the Different Size Groups*: The results of histological study of 166 oysters of first size group (fig. 3-C) show a sharp contrast to the higher size groups in (a) continuation of early gametogenesis all the year round with peak in April and July/September (b) maturation of gametes during April/March through the end of the year, (c) irregular and casual spawning in autumn and winter, and (d) phagocytosis was common all the year round, suggestive

of cytolosis of gametes before spawning sets in resulting in rate and casual spawning in these oyster. The observations on 386 oysters of second size group agrees very closely with that of third size group, suggestive of attainment of definite reproductive sequence in oysters at an age of four months nearly (fig. 3-B).

*Changes in Glycogen Content*: Glycogen content were not measured quantiatively but density of glycogen granules in the body tissue were considered, which provides some estimate of the seasonal abundance in these tissue during 1973. In January, maximum concentration of the glycogen granules in the interfollicular tissue, mantle, gut and digestive diverticulae were observed (fig. 4) suggestive of fattening period. The concentration of the granules in the interfollicular tissue declined along with the active gametogenesis during February but such a declination in the granules occurred in other body parts. By April the glycogen granules in the body parts started thinning out and became virtually absent in the interfollicular tissue and this was associated with intense maturation of gametes. Appearance of the granules in the interfollicular tissue and the mantle occurred during September and thick concentration was noted in the later months. By November the entire body parts started rebuilding of glycogen coinciding with main events of fattening in the winter.

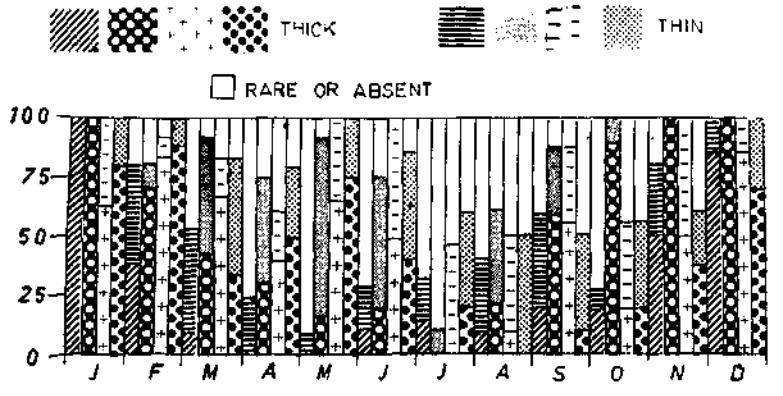


FIG. 4. Seasonal changes in the concentration of glycogen granules in interfolicular tissue, mantle, gut and digestive diverticulae in *C. (S.) glomerata* during 1973.

*Effect of Temperature and Salinity on Reproductive Cycle*: In figure 5-A the surface temperature of the locality of the bed of *C. (S.) glomerata* during the year 1971-72 is plotted, which shows winter minimum of 20.5°C in January and summer maximum of 31°C in July. The annual range of temperature of 11.5°C is high enough to influence the reproduction in the oyster. In January and February temperature remained 20.5-22°C which was sufficient to promote early gametogenesis (27-35%) in the population. In March, the temperature rose to 24-26.5°C, which coincided with maturation of gametes and became predominant at 27-28°C in April (75.5%). In May, the temperature increased

and spawning occured. The temperature remained high (27-31°C) from July through October contributing to intensive spawning during the period. It was noted that glycogen content remained at its lowest range during the period, presumably due to utilization of the reserves of the glycogen for the maturation

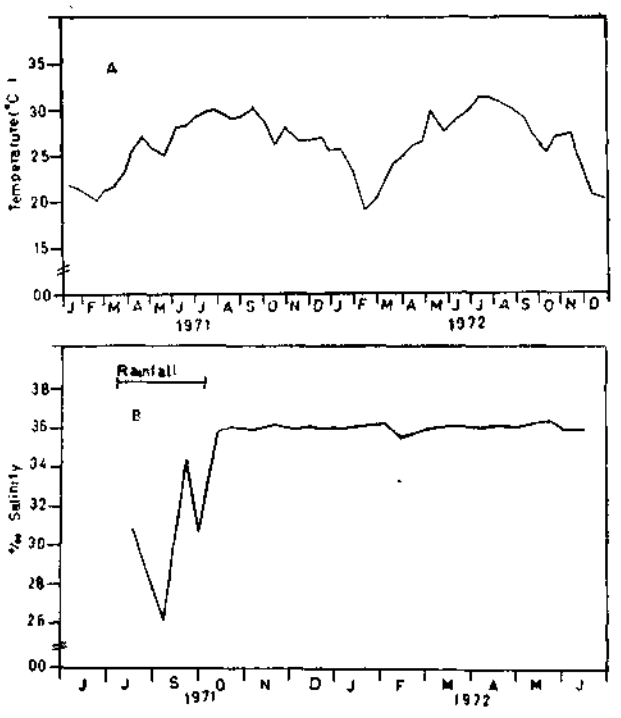


FIG. 5. Seasonal changes in: A - sea-surface temperature and B - surface salinity of Manora Channel.

of gametes. In winter months with fall of temperature to 21°C majority of the individuals having accomplished prolonged reproductive activity had little body reserves so they entered into fattening phase with loss of sexuality. During the period, glycogen is stored in the well developed interfollicular tissue and other body parts.

In Manora Channel the salinity remained 36% most part of the year, however it declined during short timed and irregular rains (average preciptation of the locality is 7" per year) and a steep fall in salinity was observed from August to October, which coincides with intensive spawning period of the species.

## DISCUSSION

The present observations suggest that gametogenesis starts in late January, progressing in later months to full maturity of gametes in April. The

gametogenesis along with maturation of gametes continues in subsequent months through September. The trends of gonadial development is well established by gonad to body ratio. The prolongation of gonadial development in this species is neither reported from this coast by Ansari and Ahmed (1972) nor from New Zealand by Dinamani (1974). The prolonged occurrance of the ripe gonads may be attributed to two reasons: (1) The high temperature condition of the coast during the period (22°-31°C) as compared to New Zealand where the annual range of temperature is 14-25°C (Dinamani 1974), and (2) The latitudinal difference in between the coast of Karachi (24°50' N) and New Zealand (41° 16' S). Such a difference in the presence of ripe gonad is also reported in *C. virginica* which is restricted to spring development in temperate region, but prolonged in tropical southern states of America (Menzil and Hopkins 1952, Lee et al 1960).

As a consequence of prolonged gonadial development, the spawning in the species continues from May through November with intensive spawning from July to October. This trend in the spawning of the species is not reported by Ansari and Ahmed (1972) who report that spawning is confined to five months (from May through August and October) while in New Zealand form it is reported during December, and January the summer months of the region (Dinamani 1974). The lengthening in the period of the spawning is well shown in warm water form of *C. virginica* (southern states of America, Manzil and Hopkins 1952; Lee et al, (1960) as compared to restricted spawning in its cold form.

In winter months majority of the oysters loses their sexuality and accumulate glycogen to be utilized in intensive gonadial development. This indifferent and fattening phase of reproduction in the species from the coast of Karachi is different from the inactive phase of cold water form where full gametogenesis is arrested in its early stage and this condition continues in winter (Berg 1949). The absence of inactive phase is also reported in the same species from New Zealand (Dinamani 1974). In this species ambisexuality do occur which is not confined to a particular season (Asif 1979).

The reproductive behaviour of the youngest group of *C. (S) glomerata* distinctly differs from the third size group. In the first size group, who were less than three months old, very irregular and casual spawning occurred in mostly in male individuals presumably due to phagocytosis of gametes. Dinamani (1974) reported absence of spawning in spat of the same species from the coast of New Zealand. The minor difference may be attributed to high temperature condition which helps in rapid maturation of gonad resulting in spawning.

## Acknowledgements

I wish to express gratitude to Prof. S. M. Haq, Director, Institute of Marine Biology, Univeristy of Karachi, for his guidance, encouragement and

useful criticism during this work. I am highly indebted to Dr. M. Ahmed, Associate Professor, Institute of Marine Biology, for supervision of this work at the initial stage until his departure for Venezuela on an assignment in 1972.

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