

**REPRODUCTIVE BIOLOGY OF THE GIANT CLAM TRIDACNA
MAXIMA (BIVALVIA: TRIDACNIDAE) IN THE NORTHERN
RED SEA**

By

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ABSTRACT

Giant clams are the biggest bivalves ever exist and are confined to the Indo-Pacific area. Tridacnid clams are protandrous hermaphrodite, thus the animal starts its life as male and becomes simultaneous hermaphrodite at sexual maturity age. The Gonadosomatic Index reached its maximum value in May (46) and then it dropped in June (20). The change in the GSI values indicated that spawning might have happened at that time. This was supported by histological examination to the clam gonad over one year. Ripe animals were found in early summer months and spent individuals were in summer time. Deposition of sperm and ova were synchronized and was shown by comparing the reproductive scores of both gametes. Finally, the minimum size of sexual maturity was 8 cm at which some individuals were noticed to be hermaphrodite.

INTRODUCTION

Giant clams of the Family Tridacnidae among the most conspicuous members of the benthos and are easily accessible to people who consume the entire flesh as food or their shells as ornaments which resulted in their overexploitation in many places in the world (Heslinga & Fitt, 1987). Overexploitation of giant clams reduced the stocks that led to the elimination of this important component to many coral reef environments. This situation had aroused international interest in

the conservation and mariculture of these clams (Juinio *et al.*, 1989) and made the tridacnids to be included in The IUCN Invertebrate Red Data Book as threatened species (Wells, 1983). Knowledge of the reproductive cycle of these clams is essential to an understanding of larval production and ultimately to the abundance of this ecologically important animal. Furthermore, the success of their culture relies on the ability to obtain ripe eggs and sperm when they are required (Braley, 1992).

Giant clams are functional hermaphrodites where the male phase of the gonad develops first. By the growth of the clam, female phase is encountered, and the hermaphroditic condition becomes established (Wada, 1952; La Barbara, 1975; Murakushi & Kawaguti, 1986a). In adults, the hermaphroditism in different tridacnid species was studied (Grobben, 1898; Stephenson, 1934; Wada, 1942, 1952, 1954; Rosewater, 1965; Yonge, 1975; LaBarbara, 1975; Jameson, 1976; Heslinga, 1986; Heslinga & Fitt, 1987; Crawford *et al.*, 1987; Lucas, 1988 a). The breeding seasons of the Tridacnidae were determined according to the spawning success in the laboratories. For example, Rosewater (1965) at Marshall Islands, Hardy & Hardy (1969) at Eniwetok and La Barbara (1974, 1975) in Fiji islands, induced spawning in *Tridacna squamosa* in the austral winter of 1963, 1968 and 1972. Similarly, LaBarbara (1975) and Jameson (1976) found *T. maxima* to be a winter breeder. Jameson (1976) induced spawning of *T. crocea*, *T. maxima* and *Hippopus hippopus* by macerated gonads and studied the life cycles of these species. Also, Beckvar (1981) succeeded in cultivating three tridacnid species, where spawning was induced by exposure to hydrogen peroxide.

The reproductive cycle of *Hippopus hippopus* was first studied by Braley (1984), who used regular biopsy samples from the gonads of tagged clams. Most of the information on the reproductive periodicity of the Tridacnidae has come from observations of natural spawning. The first microscopic study on gonads of giant clams was made by Stephenson (1934) on *Hippopus hippopus* on the Australian Great Barrier Reef. Although she failed to obtain artificially fertilized gametes, her study clearly showed that the clam had a breeding period peak covering the austral summer months from January till March. A detailed histological study of the reproductive biology of *Tridacna crocea* and its sexual differentiation was done by Murakushi & Kawaguti (1986 a). To investigate the seasonality of reproduction, Nash *et al.* (1988) made a monthly histological

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examination of the gonads of *T. gigas* inhabiting the northern Great Barrier Reef and were able to identify six gametogenesis stages.

Regarding The Red Sea, no studies have been made on the reproductive biology of giant clams. The present work reports on the reproductive cycle of *T. maxima* from the northern part of the Red Sea using 1 year, using histological techniques, gonado-somatic index and ova diameter measurements in different seasons. The aim of this study is: a) to define the different maturity stages in the gonad; b) to determine the spawning season and minimum size of hermaphrodite animals; and c) to compare the present results with other giant clams from different places in the Indo-Pacific.

MATERIAL AND METHODS

Between 10 and 20 clams were monthly collected over one year from the Mid Garden reef flat (Figure 1). Giant clams in this site were abundant, and the amount used in the present study formed only a small proportion of the total clam population on these reefs. In the laboratory, the shell length of each individual was measured to the nearest 0.1 mm and the soft tissues were removed, blotted, and weighed along with the wet weight of gonads (determined separately) to the nearest gram.

The gonads in the giant clams are located near the anterior edge of the shell, ventral to the excurrent muscular tissue. The gonad is whitish in color and is enclosed along with the heart and kidney within the pericardium, and completely surrounding the digestive gland. The change in the gonad color at different maturity stages was not consistent over the study period, thus it was not possible to define the different stages from the morphological appearance of the gonads.

Gonado-Somatic Index (GSI):

GSI was calculated monthly from the ratio of gonadal mass (gonads + digestive gland) to the total mass as follows:

$$GSI = \frac{W_g}{W - W_g} \times 100$$

where, W_g is the gonad wet weight in gram and W is the flesh weight in gram. It should be mentioned that the gonadal mass was used in the GSI because it was

impossible to separate the gonads from the digestive gland and any attempt to do this resulted always in damaging the gonads.

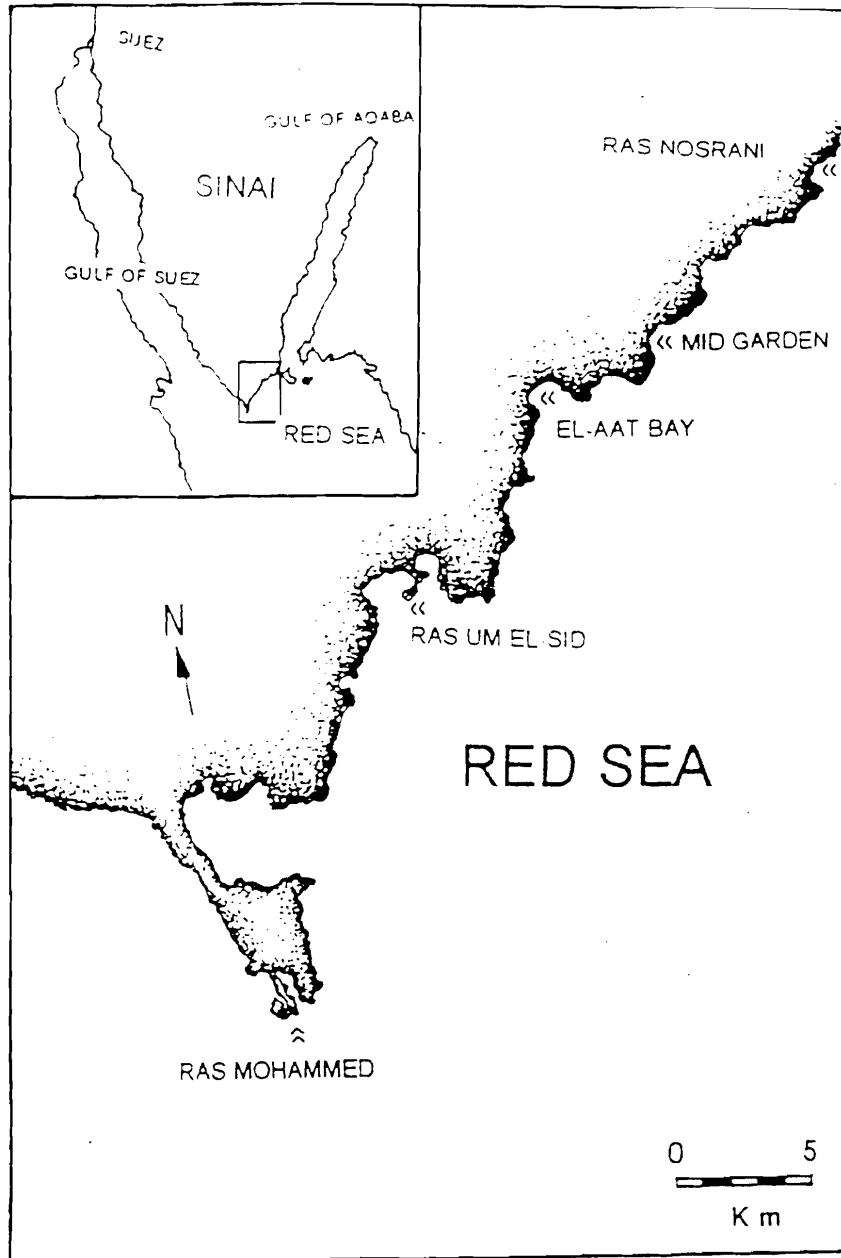


Fig. (1): Study area and sites.

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Reproductive Cycle

The gonadal mass were dissected out, fixed in Bouin's solution for 9-12 hours, dehydrated in alcohol, cleared in xylene, and embedded in paraffin by standard technique (Gabe, 1968). Gonad tissues were sectioned at 5-7 μm with a rotary microtome stained with Delafield's hematoxylin and counterstained with eosin. The sectioned material was examined microscopically and subjectively allocated to a maturity stage of development as described by Chiperfield (1953) for *Mercenaria mercenaria* and Ropes & Stickney (1965) for *Mya arenaria*. These stages were then modified by Seed (1976) for *Mytilus edulis* and used afterwards by many researchers working with the bivalves.

In the ovary portion, 4 gametogenic stages were recognised: mid, late or ripe, partial spent and spent. The cells of a particular stage were identified by the location of the ova in the gonadal follicle, by the nuclear feature (shape, size, and staining properties) and by the presence of the vitelline layer around the cytoplasm. Similarly, in the male portion, the same 4 stages were recognized. Late gametogenesis and ripe gonads were pooled as a single class (stage 3), since it was found that, in practice, many samples could not be unequivocally assigned to either category and it was not possible to differentiate between both stages in the histological sections. Similar results were recorded in *T. derasa* (Nash *et al.*, 1988). A general development stage for both testis and ovary was ascribed to each gonad sample, based on an assessment, of which gametogenesis stage was predominant.

Reproductive Scores

To illustrate the similarity between the ripeness stage of the testis and ovary at each sampling period, each gonad sample was assigned a gonad ripeness score for both tissues. Scores were assigned after the method of Dix & Ferguson (1984), as follows: early developing = 2; mid-developing = 3; late-developing/ripe = 4; partly spent = 1; and fully spent/regressing = 0. A sample index of reproductive state was then calculated by estimating the percent of clams having the same score in each month and multiplying this value by the corresponding score. This index gives a value in each month describing the reproductive status for both testes and ovaries and provides graphs of trends in condition with time. Under the scoring system, samples containing clams that were all spent would have an index of zero; whereas, samples with all ripe clams have an index of 4.

Ova Diameter

Mean diameter of 15-20 ova were measured in each specimen and this was compared to the reproductive stage of the animal during each month. Qualitative and quantitative analysis of gonad preparations was carried out using an OPTIMAS Image Analyzer, at the University of Texas at Austin, Marine Science Institute. Photomicroscopic images (10 1.25 optivar) were connected to a video signal (field area=0.639 mm²). These analog video signals were converted to a binary format using upper and lower grey-level threshold set by the operator. The image analyzer could carry out detailed area measurements and statistical analysis. Gonads were analyzed for maturity stage, reproductive scores and mean oocyte diameter in the ovaries.

In addition, the minimum size of hermaphrodite clams was determined by counting and measuring the clams having either all-male gonads or hermaphrodite ones.

Results

Total number of the clams sampled in this study were 434 and the size ranged between 4.13 cm and 22.40 cm. (mean=12.0 cm, SD=2.58).

Gonado-Somatic Index:

The mean GSI values (Figure 2) increased gradually between January (22% ± 10.9) and March (31% ± 13.6). In May the mean GSI reached its maximum (46% ± 18.6), as it ranged between 21 and 60%. This suggested that while some clams were ripe, others might have already spawned. The sharp decline in the mean GSI values in June (20% ± 11.3) showed that a spawning event might have taken place.

Reproductive Cycle:

The percentages of the various gonadal stages are shown in (Figure 3) for both testes and ovaries. None of the clams containing early gametogenic stages unless they were associated with later stages in either the ovaries or testes. Unlike the female section of the gonad, partially spent testes were found in all months except in April. In addition, late spermatogenesis stages were found in all months except in June, where clams were either partially spent (44.4%) or spent (55.6%). Mid-

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spermatogenesis stages were found in the late winter and spring months (January-April). All clams smaller than 8 cm were all-male clams (Plate 1).

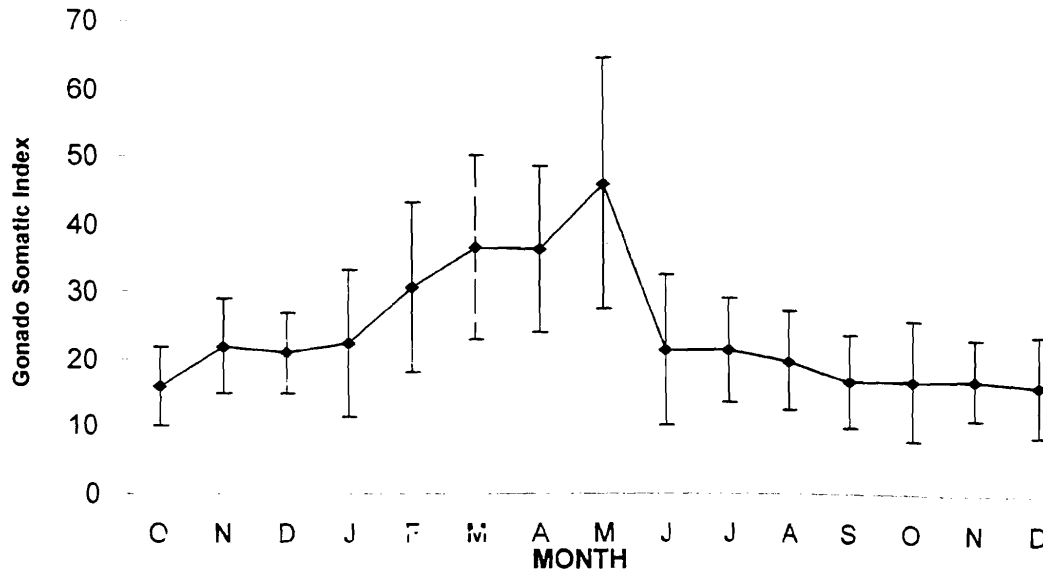


Fig. (2): Gonado-Somatic Index in each sampling period (+ S.D.)

In the ovary, clams collected in late winter and spring (January - May) were in the mid-gametogenesis (Plate 2), late-oogenesis stages or ripe, while in May, all clams were in the late oogenesis or ripe stage (Plate 3). Spent clams (Plate 4) (77.8%) were found for the first time in June along with partial spent clams (22.2%). Partial spent, spent or regressive clams, continued to exist in the following months with lesser ratios. The proportion of the spent clams were 38.5% and 8% while partial spent clams were 15.4% and 4% in July and December, respectively. Meanwhile, the percentage of late gametogenesis stage was increasing from 38.5% (July) to 88% (December). Some clams (7.7%) were in the mid-oogenesis stage in July.

Synchronization :

The reproductive state indices of both testis and ovary were compared throughout the year (Figure 4). This was done to illustrate the similarity between the ripeness stage of testes and ovaries at each sampling period. Both testes and ovaries exhibited very similar patterns of gametogenic development.

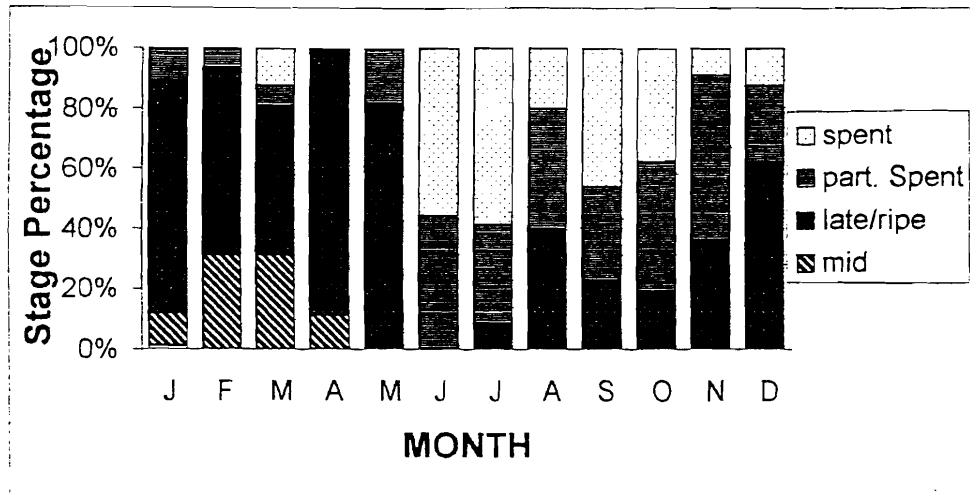


Figure 3A (♂)

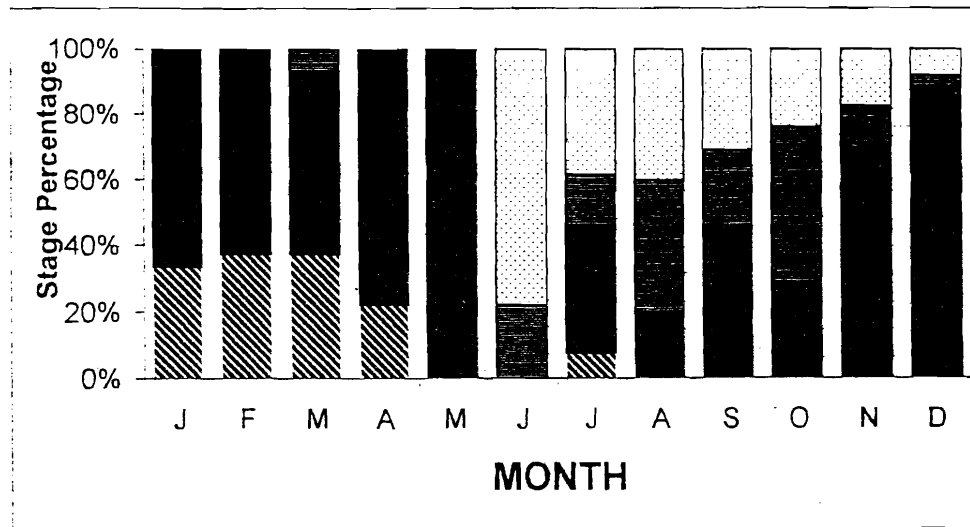


Figure 3B (♀)

Fig. (3): Different gametogenesis stages in the male and female Portions In the gonads during a year.

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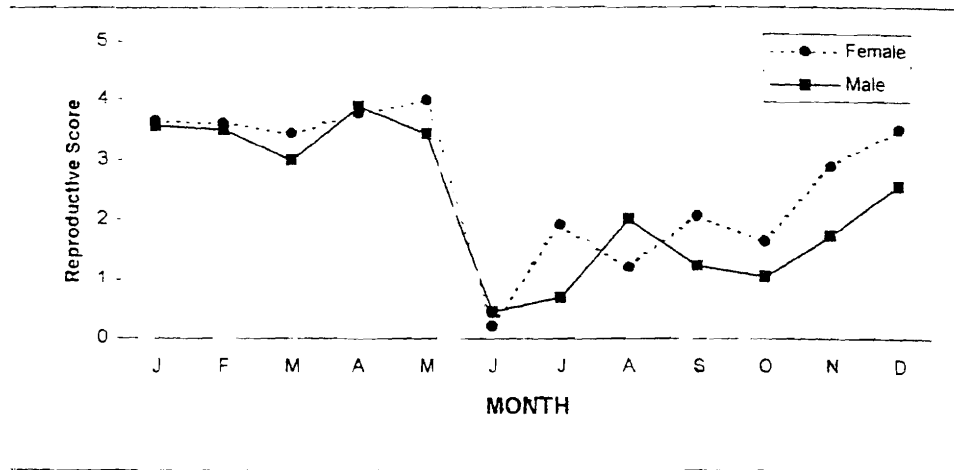


Fig. (4): Reproductive scores of male and female portions in the gonads during a year.

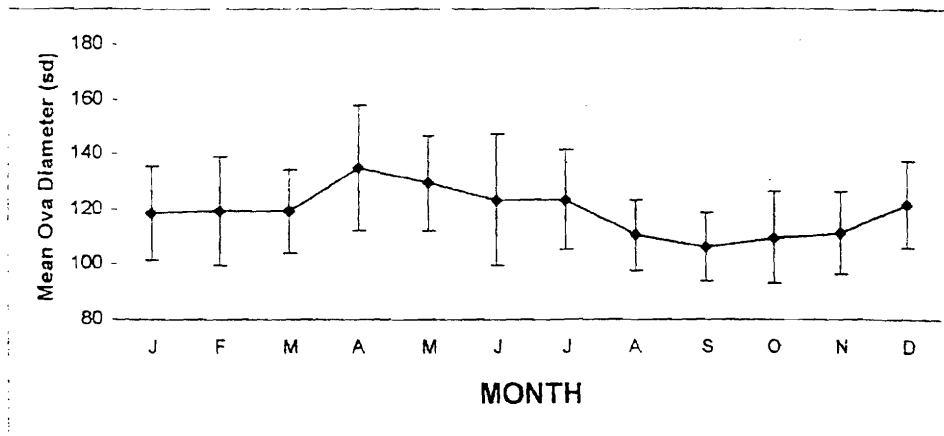


Fig. (5): Mean oocyte diameter (μm) during a year (+ S.D.)

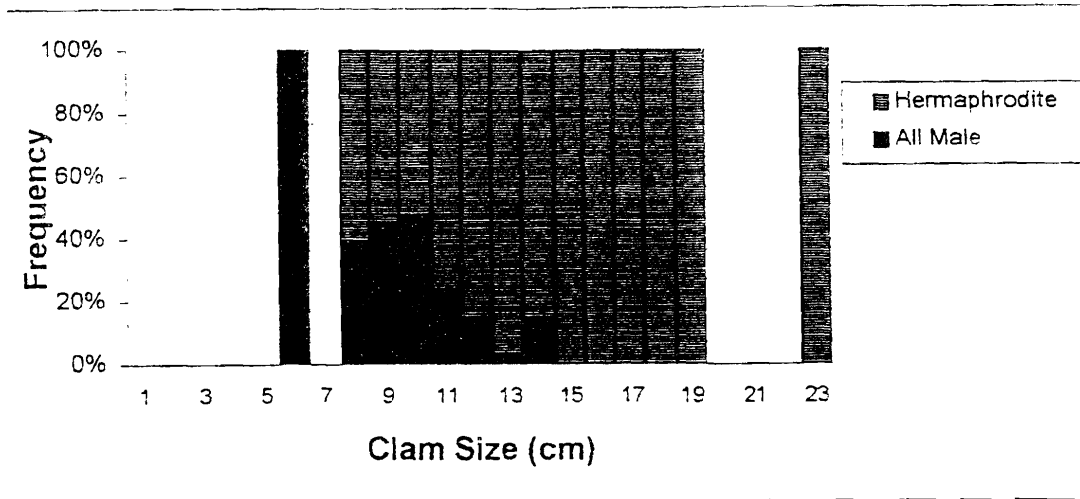


Fig. (6): Sexual condition (all-male or hermaphroditism) of the giant clam sample.

Ova Diameters:

During January and March, little gametogenesis development took place as most ovarian follicles during this time contained germ cells or oogonia. In this period, the overall mean diameter was less than 120 μm (Figure 5). The diameter increased and reached its peak in April (129 μm). Spawning commenced between April and June as evidenced by the decrease in the mean ova diameter as mature oocyte became proportionally less numerous.

Minimum Size of Hermaphroditism:

The smallest animal collected during this study was 5.5 cm in length displaying only the male gonad. All clams more than 5.5 cm were hermaphrodites where clams ranging between 8.9 and 11.3 cm in length were mostly males but associated with few ova (Figure 6). However, exceptionally one clam, 14.0 cm long was found to be exclusively male. Although no clams were found to be females only, the area occupied by the female gonad in large clams was bigger than that of the testis.

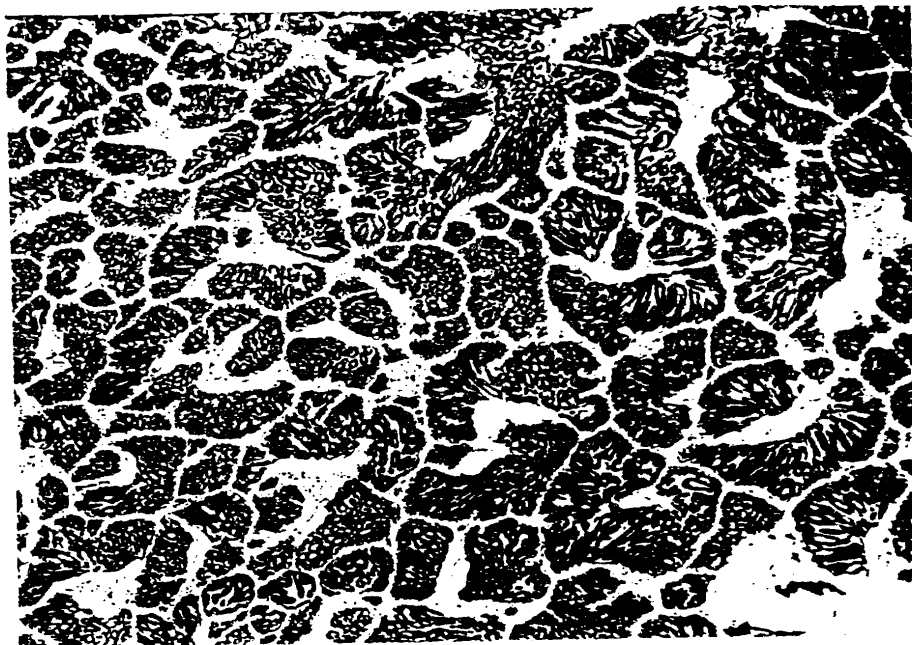


Plate (1): Plate 1. All-male clam (6 cm) with all the developing spermatogenesis

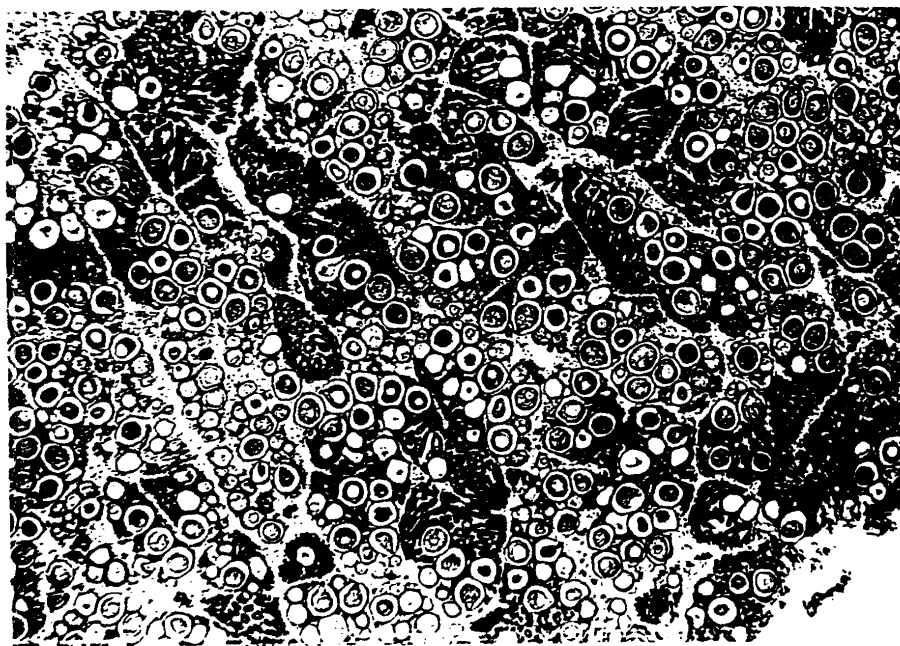


Plate 2. Section in *T. maxima* collected in fall, to show mid gametogenesis stages.
The vitelline layer is absent from some ova. The ova are more round. (400 X)



Plate 3. Section in *T. maxima* collected in early summer, to show clams at late gametogenesis or ripe stage. The ova started to be crowded.

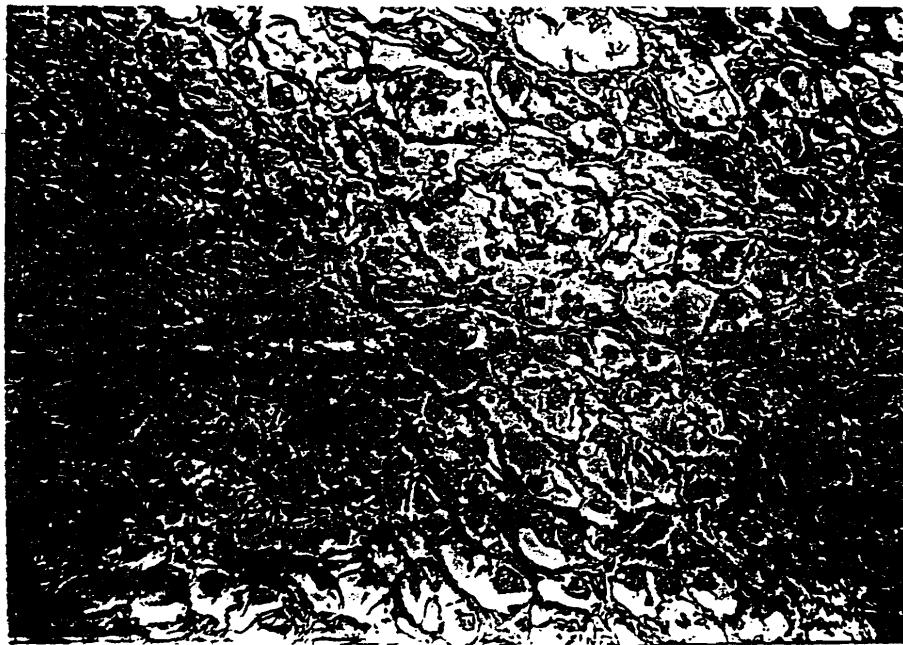


Plate 4. Section in *T. maxima* collected in late summer, to show spent clams. The ovarian follicles are almost empty of ova.

DISCUSSION

Marine invertebrates have a cyclical pattern of reproduction which can be divided into three phases: (1) gametogenesis and vitellogenesis, (2) spawning and fertilization and (3) larval development and growth. Each species has a variety of genetic adaptations, which coordinate these reproductive events with the environment to maximize the reproductive success (Newell *et al.*, 1982). It has been documented that the duration of each stage of the cycle may be variable among species (Sastry, 1979). The gametogenesis cycle may either be annual, semiannual, or continuous depending upon the species and environment.

Gonado-Somatic Index (GSI)

Beside the histological study, the gonado-somatic index was calculated to study the change in the gonad weight in relation to the flesh weight over the period of study. It was difficult to separate the gonad tissue from the digestive gland tissue which was formerly encountered in the oyster *Ostrea puelchana*, where the digestive gland-gonad complex was analyzed as one unit (Castro & de Mattio, 1987).

The maximum value of GSI in the present study was found in May in which the gonad represented about 45.9% of the flesh weight. The index declines afterwards till it reaches 16% in October and this decline may be a result of a weight loss of the gonad during spawning. Similar results were observed on *Hippopus hippopus* and *Tridacna crocea* by Shelley & Southgate (1988) who found that the gonad can reach 40% of the body mass just before spawning and decreased to 10% at spawning time. The GSI results of the present study may indicate that the spawning event took place during May and extended over the summer months.

Reproductive Cycle:

The gametogenesis stages in the male portion did not have a consistent trend over a year. This was not the same in the ovary, where the stages had a clear trend over the period of the study. Accordingly, the reproductive cycle of the ovary was used in the discussion.

In the present study, the gametogenesis of *T. maxima* proceeded throughout the year and may have started in January where 33.33% and 66.67% of the clams

were in the mid and late gametogenesis, respectively. While in May, 100% of the clams were in late gametogenesis or ripe stages, and in June, 77.78% of the clams were in the spent stage as they had already spawned. This proportion decreased to 38.46% in July and continued to decline in the following months till it reached 8% in December. These results show that *T. maxima* in the Red Sea has a well defined spawning season in June and stays till late summer.

In other parts of the Indo-Pacific region, different tridacnid species showed various trends in their reproductive behavior. *Hippopus hippopus*, for example, has a breeding peak in austral summer (Stephenson, 1934; Rosewater, 1965; Jameson, 1976; Yamaguchi, 1977), whereas *Tridacna squamosa* may spawn in the early austral winter (Rosewater, 1965; Hardy & Hardy, 1969; LaBarbara, 1974, 1975). The difference in time of spawning may result from population differences, either induced or genetic, between the giant clams in the Great Barrier Reef of Australia and those of the more easterly Indo-Pacific sites (LaBarbara, 1975). *T. maxima* was found to be a winter breeder in Fiji and Guam islands (LaBarbara, 1975; Jameson, 1976).

Reports of lack of well defined reproductive cycles in invertebrates from sites close to the equator contrast those away from the equator (Pearse, 1968). The effect of latitude on the spawning season of many bivalves was studied by Barber & Blake (1983) who found that different population of the bay scallop, *Argopecten irradians*, have different spawning seasons at different latitudes. A probable reason for this observation could be temperature variation. However, another explanation is that reproduction is an energetically expensive process that is dependent on an adequate food supply which may vary at different temperatures and time of the year at different latitudes. Local and regional differences have been reported for populations of *Mytilus edulis* (Newell *et al.*, 1982), *Placopecten magillanicus* (MacDonald & Thompson, 1986), and *Mercenaria mercenaria* (Hesselman *et al.*, 1989). In the present study, the temperature varied between 17 °C and 29 °C in March and July, respectively. This temperature difference between the winter and summer might be the reason of the well defined spawning season in the northern Red Sea. Another explanation for the seasonality of the spawning in the giant clams is the phytoplankton densities necessary for the survival of larva and settled clams (Braley, 1988). Himmelman (1975, 1980) has shown that natural phytoplankton induced spawning of temperate invertebrates.

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The interaction between exogenous (e.g., food availability, temperature, and salinity) and endogenous (e.g., nutrient reserve, hormonal cycle and genotype) variables may determine the initiation and duration of the various phases of the cycle and thus ensure synchrony of gamete development within a population (Gabbot *et al.* 1976). This is of prime importance for dioecious species having external fertilization, which requires synchronized liberation of gametes. In all clams in the present study, there was a notable absence of early oogenesis stages. Also, there was no ovary with both early and mid gametogenesis stages at the same time. However, within the same sampling period, there was a range of gametogenesis stages between individual clams, where individuals range from mid stage and ripe to spent. On the contrary, in testis, it was common to find a wide range of gametogenesis stages within the same individual. Along with the spermatocytes and the spermatids, the ripe spermatozoa may be found in the same gonad. The same observations were noticed by Nash *et al.*, (1988), with *T. gigas*, where the testis maturity process did not have the same trend of the ovary. Testis development was found to be a continuous process in which both early and late stages were found simultaneously. This difference is consistent with, and may account for, the observed differences in frequency of spawning of eggs and sperms. During the study period, partial spent testes were found at different months. There were partially spent testis in every month except in April. This may indicate the possibility of sperm discharge at any time of the year. This was confirmed by the trials of the artificial spawning during winter time where sperms were released whenever the clams were stimulated by serotonin (neurotransmitter). This was not the case with the ova which were not laid at all in winter. Gwyther & Munro (1981) compared the spawning of giant clams and showed that *H. hippopus* could be stimulated to release sperm throughout the year.

The relative growth rates of gonadal and somatic tissues are such that somatic growth far exceeds gonadal growth, prior to sexual maturity. There appears to be competition between gonadic and somatic development in bivalves (Menesguen & Dreves, 1987), which may be used to explain why there was a poor correlation between shell and tissue growth ($r=0.341$) in the present study. Such a poor correlation can be exaggerated by spawning events (Seeds, 1980). Shell growth can nearly stop during reproduction periods, which can result in shell and somatic growth being out of synchronization (Bricelj *et al.*, 1987). From a commercial point of view, it was found that the condition of the amount of flesh relative to the

shell size of bivalves typically follows a cycle closely related to that of reproduction. Bivalves also are suitable for harvest only if their condition and gonad appearance meet acceptable commercial standards.

Ova Diameter:

The results of the mean ova diameters, reinforced the information obtained from the histological study and the gonado-somatic index. The ova diameter was maximum in April followed by a decline till September indicating the start of spawning. Between January and March, oogenesis proceeded as most ovarian follicles during this time contained oogonia, with an overall mean diameter of 119 μm . By April the oogonia developed into oocytes the mean cell diameter increased to 126 μm . Spawning started in May-June as evidenced by a decrease in the ova diameter, as mature ova became less numerous. It will be noticed that the minimum ova diameter of *T. maxima* in the present study was less than that obtained by LaBarbara (1975) and Shelley (1989). Such a difference may be attributed to a genetic change brought about by ecological differences.

Synchronization:

To illustrate the similarity between the ripeness stage of the ovary and testis at each sampling period, the reproduction score was calculated for both ovary and testis. The mean score for both sexes exhibit a very similar pattern of gametogenesis, but self fertilization was never recorded which may be due to the spawning behavior. Thus while in functional hermaphrodite gonads, the eggs and sperm mature simultaneously, simultaneous spawning does not occur (Wada, 1954). Sperm are released first and after some time (between 30 and 180 minutes), the eggs are released (LaBarbara, 1975; Jameson, 1976; Gwyther & Munro, 1981; Crawford *et al.*, 1987; Heslinga & Fitt, 1987; Lucas, 1988 a; Nash *et al.*, 1988; Braley, 1988).

As mentioned before, the giant clams are protandrous hermaphrodites, in which the male phase mature first followed by the female. In certain functional forms of hermaphrodite bivalve as in *Pecten irradians*, the ovarian and spermatid tissues occupy different portions of the gonads, whereas, in the tridacnid clams, both male and female acini are found side by side throughout the whole extent of the gonad. La Barbara (1975) found that *T. maxima* is hermaphrodite when it is between 15 and 20 cm in size. She made this observation with clam that grew and spawned artificially in the laboratory. The presence of hermaphrodite individuals

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in histological sections does not relate to their ability of spawning and other tests should be done to ensure that.

There was clear overlap in the size of clams having only a male gonad and these with a hermaphrodite character. The size range of all male animals was 5-14 cm. Similar overlap was noticed by Shelley (1989) in *Hippopus hippopus*.

Conclusions:

- 1- The histological study showed that *T. maxima* is a protandrous hermaphrodite, in which the animal possess a male gonad first, followed by the female phase. The animal is then a simultaneous hermaphrodite.
- 2- In *T. maxima*, it was difficult to separate the gonad tissue from the digestive glands. As a result, the gonado-somatic index (GSI) was estimated using the gonad-digestive gland complex. GSI showed that the gonad comprises 16 % of the flesh weight in October 1989. This value increased to about 45 % in May and it dropped to 20 % in June. The increase in the values showed that the gonads were building reproductive material during early winter. In May, when the GSI decreased abruptly, spawning might had taken place. The spawning event extended during summer and early fall.
- 3- Four different maturity stages were determined in the gonads. In the female portion, the mid and late stages were found in winter months, while partial and complete spawning were in the summer and the first completely spawned clams were found in June. This agrees with the GSI measurements. Contrary to the female, male portions did not have this trend. Spawned male follicles were found most of the time in the study period. This leads to the conclusion that *T. maxima* can release sperm at any time in the year.
- 4- There was a synchronization in the deposition of both sperm and ova. This was shown by the similar trends of the reproductive scores of both gametes. They reached their peak in May, after which the score dropped to their minimum values in June.
- 5- Ova diameter was another sign of the spawning timing. The mean ova diameter reached 135 μm in April. The decrease in the diameter was gradual where the ova reached 106 μm in September. This may indicate that not all the clams in

populations may undergo spawning at the same time. This variability was supported by the big standard deviations around each mean in some months.

- 6- The minimum size *T. maxima* attaining the hermaphroditic state in the studied area was 8 cm, although exclusively male clams were encountered until a length of 14 cm. There was a clear overlap between both conditions. It is recommended to extend the reproductive biology study to the rest of the Red Sea areas, to complete the work on *T. maxima* in this zone of the Indo-Pacific region.

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