



Reproductive Aspects of the Sea Urchin *Tripneustes gratilla* from the Red Sea, Egypt

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ABSTRACT

The reproductive biology of the Sea urchin *Tripneustes gratilla* from Hurgada, Red Sea, Egypt was studied in the period from January 2018 to May 2019. The specimens were collected monthly at a depth ranging from 1 to 2.5m. This study introduced data based on the monthly estimation of the gonadosomatic index and the monthly occurrence of each maturity stage relative to the total number of examined gonads. The oocyte size frequency distribution and the histological examination of the gonads of both sexes were used to describe the maturity stages. The results revealed that both males and females showed synchronization in the reproductive cycle. The highest values of gonadosomatic index (GSI) were recorded in October for both sexes and exhibited a decline in November which indicates that spawning took place in this period. *Tripneustes gratilla* population in the Red Sea showed a restricted breeding season that extended from October to February. The egg diameter varied from 4 to 60 microns throughout the gametogenic cycle.

INTRODUCTION

As the human population grows, the global demand on seafood products increases including finfish and shellfish (Sapkota *et al.*, 2008). Sea urchin gonads (roe) are considered a culinary delicacy in France and Spain, Chile, North America, Asia and especially Japan, which accounts for around 90% of the worldwide demand (Sun & Chiang, 2015). The trade of these echinoderms constitutes an extremely profitable business in some parts of the world (Junio-Meñez *et al.*, 2008). Its global production reached its peak in 1995 with an estimated 120,306 tons and quickly declined, recording only 90,257 tons in 1998 (Andrew *et al.*, 2002). While, the currently production is about 75,000 t per year (Stefánsson *et al.*, 2017). Notably, most of the production comes from

fisheries and less than 1% from the aquaculture (Carboni, 2012). As the high demand for this product worldwide remains, when a fishery collapse it moves into new fishing grounds (Keesing & Hall, 1998; Muthiga, 2005).

Tripneustes gratilla is a high-value sea urchin in tropical and subtropical waters of the Indo-Pacific region between depths of 0 to 75m (Lawrence & Agatsuma, 2007) from East Africa (Red Sea to Natal), the South Sea Islands (from the Norfolk and Kermadec Islands to the Marquesas and Hawaii), and from Australia (to Port Jackson on the east coast and Sharks Bay on the west) to southern Japan (with the Bonin Islands) (Mortensen, 1943; Lawrence & Agatsuma, 2007) being the dominant sea urchin species in the Red Sea coast of Egypt (Mahdy *et al.*, 2019). This ubiquitous echinoid inhabit a wide range of tropical habitats including coral reefs, seagrass meadows, macroalgae meadows, sandy or muddy, soft bottoms and inland salt-ponds tolerating salinities greater than 45 ppm at 15°C (Dafni, 1992; Unsworth *et al.*, 2010; Lyimo *et al.*, 2011; Lawrence & Agatsuma, 2013). *T. gratilla* is an opportunistic grazer, with a omnivorous diet and can feed on a wide variety of macroalgae, seagrass, diatoms and coral tissue scraped from rocks (Dafni, 1992; Klumpp *et al.*, 1993). It is a fast growing species in both subtropical (Dworjanyn *et al.*, 2007) and tropical environments (Lawrence & Agatsuma, 2013). This species reaches sexual maturity (Lawrence & Agatsuma, 2007) when its size gets between 60-70 mm (diameter) throughout a year. This echinoid is a round shaped- sea urchin that can reach a maximum height of 158 mm (Baker, 1968) and a greatest size of 160 mm (diameter) (Rahman *et al.*, 2014). It has a wide diversity of color patterns in the skeleton, spine and tube feet. Toha *et al.* (2015) observed 31 different colors. This color diversity is related to natural selection and habitat adaptation (Vardaro, 2010). *T. granilla* has frequently been considered as a keystone species in coral reefs for its ability to modify the benthic community structure by grazing algae (Zeina *et al.*, 2016). Moreover, it has been used in some localities as biocontrol for invasive algae and seaweeds, as for example, in Oahu Island in Hawaii (Bronstein *et al.*, 2017). However, shifts from fish to sea urchins as the dominant herbivore have potentially significant impacts on the integrity of coral reef habitat (McClanahan & Muthiga, 1988). Population outbreaks can locally produce significant declines in foliose algae and sea weeds (Eklöf *et al.*, 2008; Valentine & Edgar, 2010). *T. granilla* is considered as a pest in red algae (*Eucheuma* sp.) farms in Central Philippines, and they are constantly released manually by the farmers (Bacolod & Dy, 1986).

T. granilla is one of the six most commercially traded sea urchin species (Wang *et al.*, 2013). It is an economically valuable sea urchin species mainly for their gonads (roe). *T. granilla* is collected by small-scale fisheries in several areas of its distribution geographical range (e.g., Japan, Fiji, Philippines, Mozambique (Andrew *et al.*, 2002; Muthiga, 2005) and Egypt). It is gathered to supply local markets but also for export, particularly to Japan, Taiwan, Hong Kong and Korea where there is high market demand for this species (Trinidad-Roa & Pasamonte, 1988). The high fishing pressure, the

indiscriminate harvesting and lack of management for echinoid have collapsed some of their fisheries in the main producing nations (Sloan, 1985; Talaue-McManus & Kesner, 1995). This has caused a subsequent increase in *T. gratilla* aquaculture (echinoculture) practices and restocking programmes (Dworjanyn *et al.*, 2007; Pante *et al.*, 2007; Juinio-Meñez *et al.*, 2008; Wainwright *et al.*, 2018). Juinio-Meñez *et al.* (2008) noted the success of these restocking programs in northern Philippines to enhance the recovery of the *T. gratilla* fishery in northern Philippines in the 2000's. The program combined reseedling, together with an improvement in fisheries management (*e.g.*, marine sanctuary, minimum legal size). In addition, it is produced in Japan in small quantities for restocking (Shimabukuro, 1991; Andrew *et al.*, 2002). In the Mediterranean coast of Egypt, this species is gathered by hand from the coastline and by scuba diving from the coastline at a depth of 17m and sold at local markets but is not yet commercially exploited in the Red Sea, where it is more abundant. This sea urchin is also gathered to be used as a bait in Kenya (Muthiga, 2005).

T. gratilla has different reproductive patterns along its wide geographical distribution range. It frequently exhibits an annual reproductive cycle with a variable spawning season (Pearse, 1974; Radjab, 1997; Muthiga, 2005). However, Tuason and Gomez (1978) recorded that spawn occurs throughout the year in Philippines. These changes in spawning season have been correlated with several abiotic (sea water temperature, day length, moon cycle) and biotic factors (food availability and phytoplankton (Pearse, 1974; Lawrence & Agatsuma, 2013). *T. gratilla* reproductive pattern in the Red Sea was studied only by Pearse (1974). He observed an annual reproductive cycle with a spawning season in winter. This study was based on the oocyte-ovum frequency distributions between February 1967 to April 1968 in females from Hurghada, Egypt, northern the Red Sea. The current study examines the reproduction of *T. gratilla* in the Red Sea updating the results obtained by Pearse (1974) in the same place (Hurghada, Egypt, northern Red Sea). The knowledge of the reproductive cycle is essential for fishery management as for aquaculture programs. *T. gratilla* has been identified as the most commercially viable sea urchin species (Cyrus *et al.*, 2014). Thus, we have analyzed the gonado-somatic index, histological sections of gonads and the size frequency distribution of oocytes from January 2018 to May 2019. In addition, the influence of exogenous factors such as photoperiod, sea water temperature and chlorophyll (Chl a) concentration on the reproductive biology of the species was also analyzed.

MATERIALS AND METHODS

1. Study area and samples collection

Sampling site was selected in front of the branch of the National Institute of Oceanography and Fisheries (NIOF), off Hurghada City on the northern Red Sea coast.

The sampling site is located at 27° 17' 07" N latitude and 33° 46' 30" E longitude, approximately, 5 km north of Hurgahda City (**Fig. 1**). The seabed of the sampling site is mainly covered with seagrasses with some scattered coral reef patches.

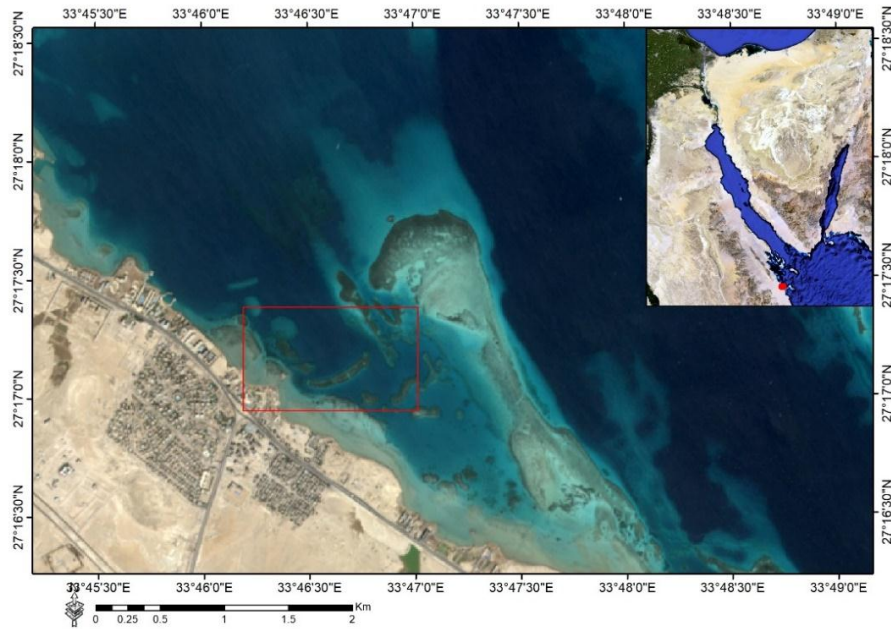


Fig. 1. Sampling location off the NIOF in Hurgahda, Red Sea

The study was carried out in the period from January 2018 to May 2019. The individuals of *T. gratilla* were randomly collected at depths that ranged from 1 to 2.5 meter. A total of 339 specimens were collected throughout the course of the study. The sampling was monthly carried out on the seagrass beds adjacent to coral patches or in the crevices where they were hidden. At the laboratory, each specimen was gently rinsed with filtered sea water (FSW) to be ready for the subsequent measurements, dissection and extraction of gonads (**Fig. 2**).

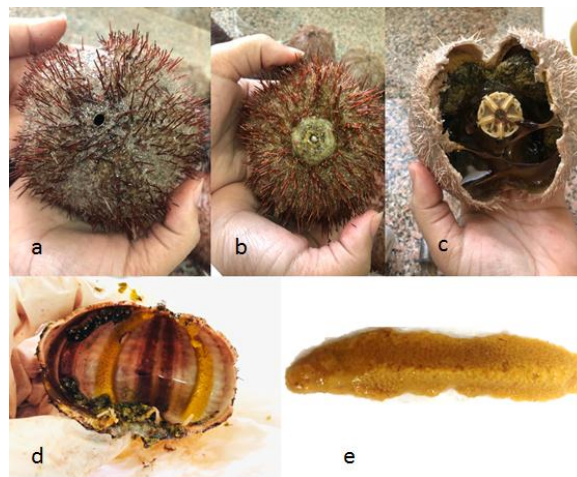


Fig. 2. Dissected specimens of *T. gratilla* showing **a**; Aboral side, **b**; Oral side, **c**; Aristotle's lantern, **d**; Two lobes of gonad, **e**; Gonad lobe.

2. Estimation of gonadosomatic index (GSI)

On a monthly basis, the mean gonadosomatic index (GSI) values were estimated for males and females *T. gratilla*. The total wet weight and the gonad weight of each specimen were measured using a digital balance (to the nearest 0.01 g). The percentage of gonad weight to total weight was adopted to calculate the gonado-somatic index (GSI) according the following equation (Allain, 1975):

$$(\text{GSI}) = \text{gonad weight (g)} / \text{total weight (g)} \times 100$$

3. Maturity Stages

The maturity cycles of both sexes of *T. gratilla* were studied using three main tools, including: general appearance of the gonads and its microscopic examination (gonad smear), size frequency distribution of oocytes in females and histological examination. Four main stages were determined according to the classification of Fuji (1960) with some modifications. The stages of oocytes maturity could be recognized, namely: pre-vitellogenic, vitellogenic, post-vitellogenic and partially spent. The Four main stages of maturity could be recognized in males; namely, pre-mature, maturing, mature and partially spent.

4. Sex ratio

The sex ratio was determined in the whole sample according to the occurrence of each sex within each size interval (1 cm intervals) and in the total seasonal samples. Significance of the difference in sex ratio from 1:1 was tested using the Chi square test.

5. Size frequency distribution of oocytes

To study maturation based on the oocyte size frequency distribution, between 7 to 30 females were used to monthly determine the oocyte diameters. The oocyte diameters were measured by using eye piece micrometer for at least 10 oocytes per examined female.

6. Histological studies

A small section from the gonad of each specimen was cut and preserved in 10% formalin solution for fixation for 24 hours. Processing of samples was performed using standard histological techniques serial. Sections were cut at 6-7 μm thickness with an automatic microtome (LKB 2016). Tissue sections were stained by Eirilch's hematoxylin (EH) and counterstained with eosin Y (E) (Bancroft & Stevens, 1996). The sections were assigned to four developmental stages according to the scale of Fuji (1960), with some modifications based on oocytes size in females or peripheral spermatocytes layer in males and the amount of nutritive tissues in both sexes.

RESULTS

1. Maturity cycle

In females, the maturity stages were determined according to the gametogenic phase of the oocytes that dominated most of the ovary. The percentage of occurrence of different gonadal stages to the total examined oocytes are given in Fig. (3A). The pre-vitellogenic stage was first appeared in May in low percentage of 7%, indicating the onset of oogenesis in few females. The most active phase of gametogenesis mainly started at August. The vitellogenic oocytes started to appear in August with small percentage (25%). It increased until reaching 91% of the examined individuals in September. October could be considered as the onset of spawning season. In November, a 100 % of the examined females showed postvitellogenic oocytes, and the gonads texture changed to loosen ones which were easily releasing their gametes on touching.

In males, the sex could be determined externally during the spawning season. It released white threads of concentrated gametes on handling indicating its sex. The percentage of occurrence of each gonadal stage are shown in Fig. (3B). The pre-mature stage started to appear in May in a small percentage (20 %), whereas the rest of individuals were in the resting phase. During June and July, all the examined individuals were in premature state. The percentage of maturing stage started to increase in August as the percentage of individuals that entered maturing stage were 71%. In September, the most dominant individuals were in the maturing state (86%). This maturing stage lasted for two months. In October, the ripe gonads were noticed declaring the mature stage (100%), it continued till February.

2. Histological description

Histological examination of gonads for both sexes gave the opportunity to confirm the distribution of maturity stages. It illustrated the four stages of maturation as follows:

Female gonads

Pre-vitellogenic stage. At the beginning of oogenesis, the oocyte diameter was about 4 microns. It has obvious large nucleus and nucleoli inside the germinal vesicle. The oogonia appeared to be invaginated in the acini wall. At this stage, the ovary is dominated by nutritive phagocytes (Fig. 4A).

Vitellogenic stage. A yolk supply was clearly seen as the development proceeds. The reproductive cells continued to appear around the periphery of the acini and started to increase in both size and number. The oocytes which filled with egg granules formed stalks then detached from the acini wall migrating to the lumen forming the vitellogenic stage. At this stage, the ovary showed different developmental phases of oocytes as there were few numbers of mature oocytes. The germinal vesicle started to move asymmetrically toward the periphery which was named later as animal pole (Fig. 4B).

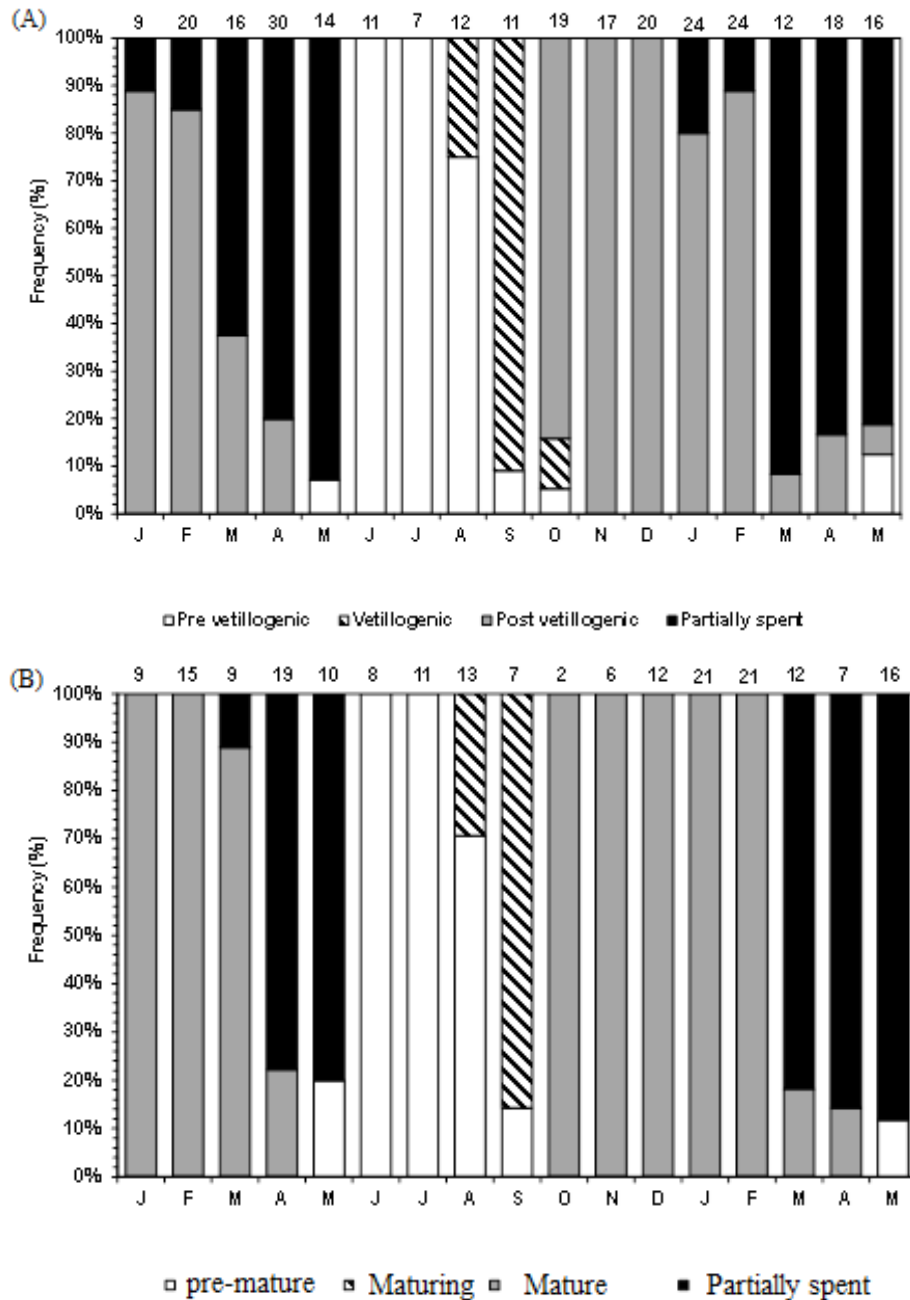


Fig. 3. Annual gametogenesis cycle of *T. gratilla* between. (A) Females; (B) Males. Numbers at the top of each column indicate the number of individuals analyzed monthly per sex.

Post-vitellogenic stage. The mature oocyte showed granules in the cytoplasm, missing the germinal vesicle and the distinctive nuclei. They represent homogenous cytoplasm. The full-grown oocytes became closely packed in the acini center and assumed different shapes, polygonal, triangular and rectangular. There were residual traces of nutritive phagocytes. The oocytes gave maximum size and number. Newly formed oogonia could be noticed in the acini wall. This stage was recognized by the onset of the spawning season (Fig. 4C).

Partially spent stage. During this stage, the ovary showed empty spaces in the lumen and became less condensed which indicated the partially spawning of *T. gratilla*. Informality in the acinal wall and oocytes could be obviously seen. The nutritive phagocytes are nearly absent at this stage. By the end of the spent stage, relicts of irregular oocytes are seen (Fig. 4D).

Male gonads

Pre-mature stage. The acinal wall in this stage is lined by thin layer of spermatogonia which are characterized by a dense stained large nucleus seen submerged in the acini wall. The lumen is occupied by nutritive phagocytes. At the end of this stage, the number of reproductive cells developed and migrated to the center replacing the nutritive phagocytes (Fig. 4E).

Maturing stage. The number of spermatogonia decreased in the acinal wall. The reproductive cells turned into spermatocytes, and the cells started to migrate into the center of the gonads. The number of spermatocytes increased while the number of nutritive phagocytes decreased sequentially (Fig. 4F).

Mature stage. On the accomplishment of the mature stage, the spermatozoa occupied most of the lumen, arranged radially in the center forming pyramids of spermatozoa. The centrally packed spermatozoa are ready for spawning. The nutritive phagocytes became exhausted and underwent a substitutional depletion in both number and size. Once reaching this stage, the sex of the sea urchin could be recognized by the white color of the released gametes (Fig. 4G).

Partially spent stage. During proceeding in the mature stage, the acini showed empty areas that increased sequentially indicating partial spawning. The spermatozoa became less concentrated, the acini looked empty with messy appearance. Very few numbers of peripheral spermatogonia appeared to be invaginated in some parts of the acinal walls (Fig. 4H).

3. Gonadosomatic Index (GSI)

The gonadosomatic index increased from August to October 2018. It showed a remarkable increase during this period and reached the peak in October when the highest GSI values were recorded. Then, it started to decrease till February indicating that the spawning season occurs from October to February. The period between March and May seemed to be steady or resting phase. Both males and females showed the same trend. This indicates that both showed synchronization in the reproductive cycle (Fig. 5A).

The surface sea water temperature (SSWT) at the study site had maximum values in summer months (July-August) and minimum values in winter months (January to March) (Fig. 5B). Chl a monthly values recorded during our study period were lower between July and August, and two peaks were recorded: one in February 2018 (0.50 mg m^{-3}) and one in February 2018 (0.54 mg m^{-3}). No significant relationships between GSI and SSWT and Chl a were observed. However, the spawning season coincided with the time period of an increase levels of Chl a.

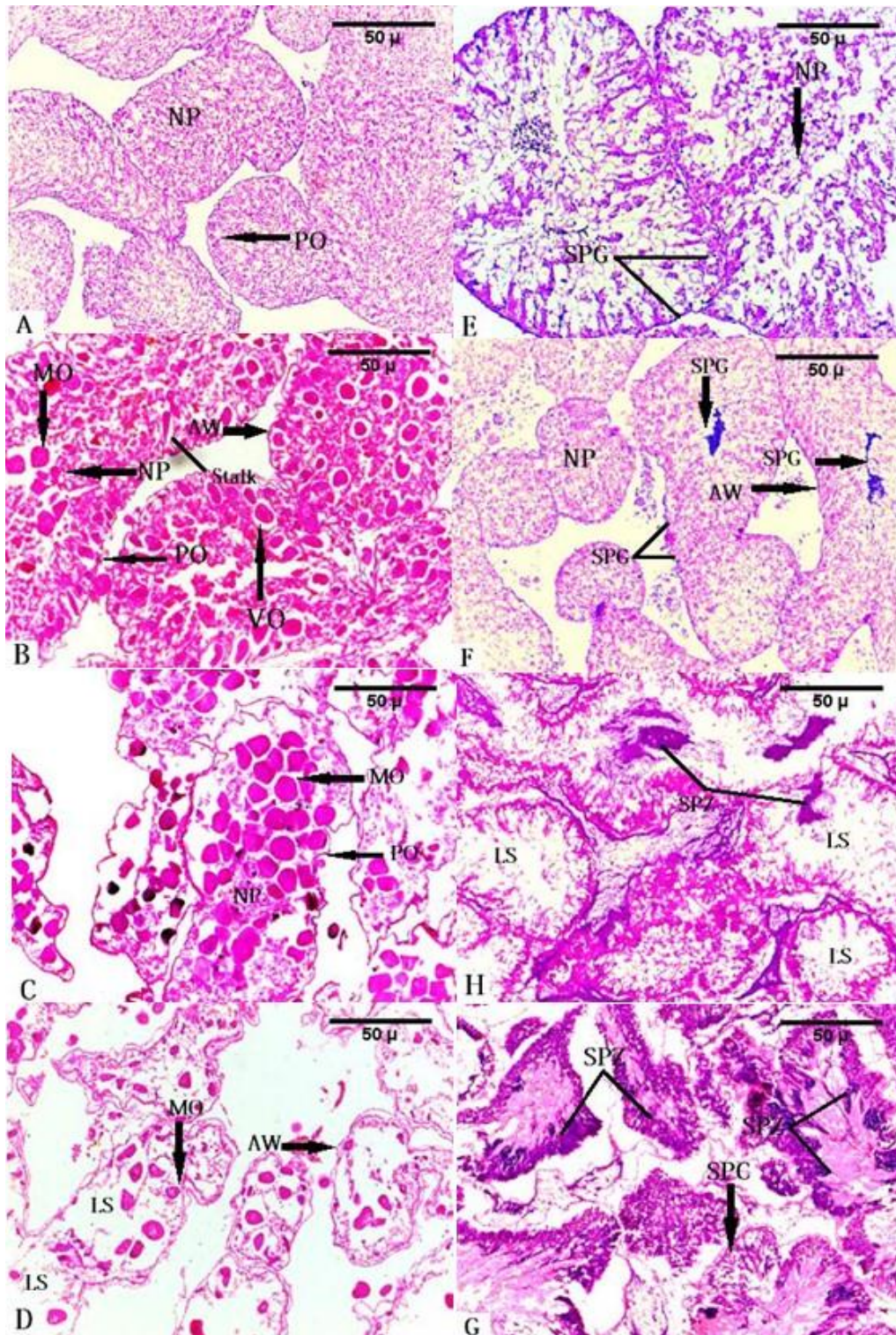


Fig. 4. The histological figures of different maturity stages of ovary (A-D) and testis (E-G) of *T. gratilla* showing several types of cells as, nutritive phagocytes; NP, vitalogenic oocyte; VO, mature oocyte; MO, pre-vitellogenic oocyte; PO, spermatozoa; SPZ, spermatocytes; SPC and acinal wall; AW (400 X).

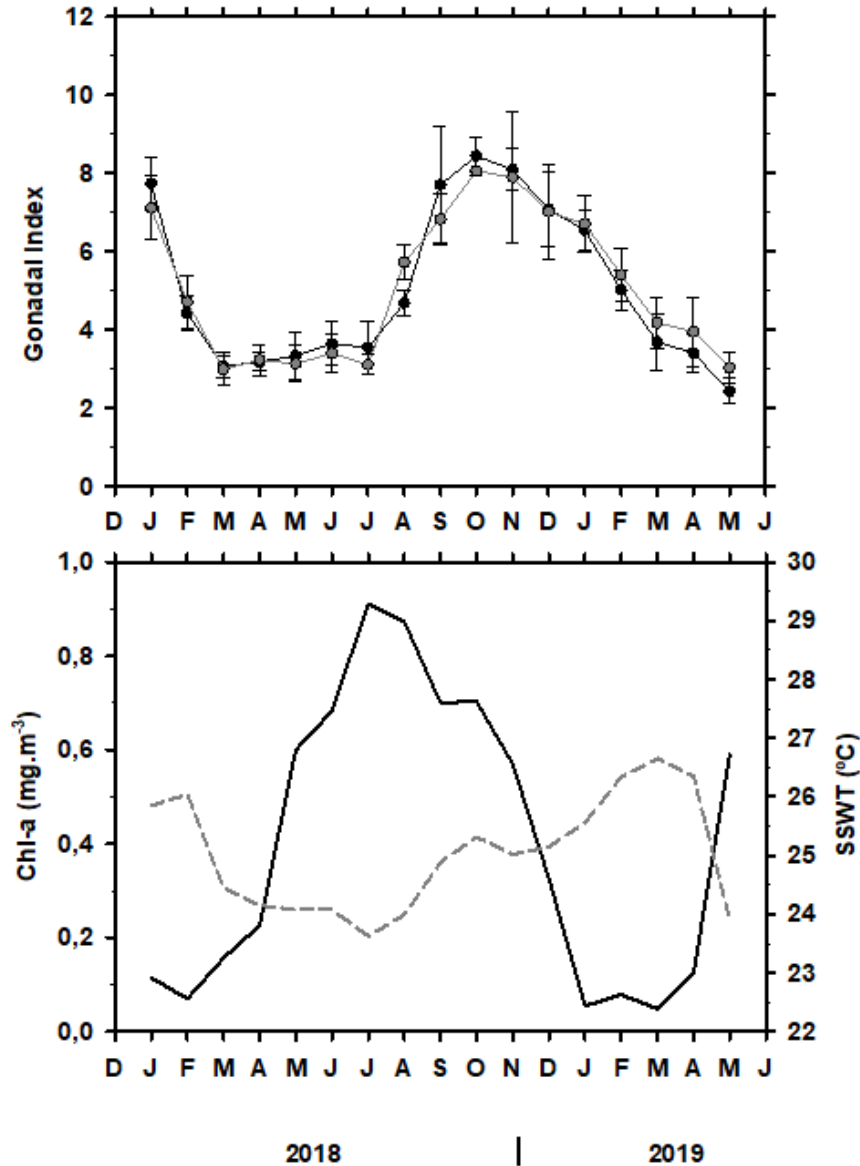


Fig. 5. (A): Monthly changes in gonadosomatic index (GI, mean, +SE) throughout the period of study. Black line indicates the female GSI while the grey dashed line indicates the male GSI. (B): the grey dashed line indicates monthly mean values of chlorophyll a concentration (Chl a), whereas black line indicates monthly mean of sea surface water temperature (SSWT); from January 2018 to June 2019.

4. Sex ratio

In the whole sample, females generally outnumbered males. Out of the total count of 339 examined animals, 208 (61%) were recognized as females, compared to 131 as males (39% of the total sample). The overall sex ratio of *T. gratilla* (male: female) was 1:1.6 ($P < 0.05$). Chi-squared test showed significant difference ($P < 0.05$) between both sexes. The larger the size intervals, the higher the female occurrence rather. The percentage occurrence of males in *T. gratilla* population was twice the females at the smallest length

interval (6-6.99 cm). However in larger sizes (8-8.99, 9-9.99 and 10-10.9), females were more frequent than males.

For seasonal level, females outnumbered male in winter, spring and autumn with sex ratios of 1:1.4, 1:1.4 and 1:3, respectively. It is noticeable that the lowest occurrence of males was recorded in autumn. The highest occurrence of males was recorded in summer where males slightly outnumbered females (1 males: 0.8 females).

5. Oocytes sizes frequency distribution

The examined egg diameter showed monthly variation depending on the gametogenic stage. Hence, the oocytes sizes frequency distribution referred to the monthly gametogenic status. The eggs diameter ranged from 4 to 60 micron that were distributed all over the gametogenic cycle (Fig. 6). The least diameter ranges were 4 to 14 micron that were recorded mostly in June and July. This period was equivalent to the onset of gametogenesis. However, the three following months; August, September and October witnessed a gradual increase in the occurrence of bigger- sized ova. Whereas, the maximal recorded an ova size ranging between 50 to 60 micron and showed a highly occurrence in the period between October and February. This confirmed that the echinoid in the present investigation underwent post vitalogenic stage in this period.

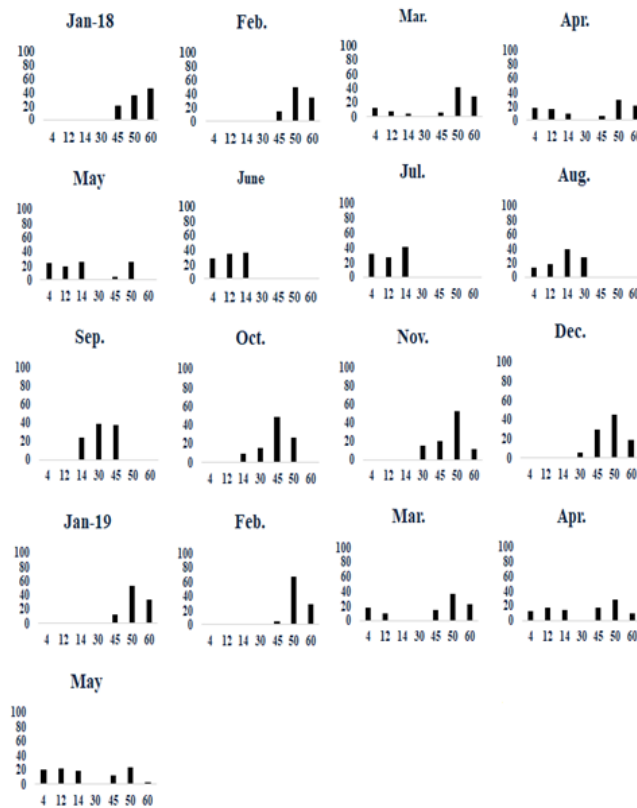


Fig. 6. The monthly occurrence percentage of ova of *T. gratilla* in each diameter class throughout the period of study. In each graph, X-axis is the diameter of ova in micron and Y- axis is the percentage of occurrence each diameter among the examined individuals.

DISCUSSION

The sea urchin *T. gratilla* from the Red Sea, Egypt develops an annual reproductive cycle with one spawning event in early fall and winter (from October to February). Other sea urchin species in the same geographic area showed a coincident reproductive cycle. For example, *Echinometra* sp. (Bronstein & Loya, 2015) and *Diadema setosum* (Bronstein *et al.*, 2016). However, the spawning season occurs in June and July in *Heterocentrotus mammillatus* (Dotan, 1990); from June to August in *Echinometra mathaei* (Hamza Hasan, 2019). While, *Lovenia elongata* spawn from April to September (Pearse, 1969), and *Eucidaris metularia* spawn from April to September (Campbell, 1987). The number of studies in the reproductive biology of the echinoderms in the Red sea is still scarce. One of the few studies existing recently reported the spawning of several species of echinoderms (*i.e.*, several starfish: *Astroboa nuda*; *Leiaster cf. leachi*, *Mithrodia clavigera* and a sea cucumber: *Pearsonothuria graefei*) together with other marine invertebrates occurred 5 and 6 days before full moon in June (Webb *et al.*, 2021). Karako *et al.* (2002) reported that, the starfish *Asterina butoni* spawns from December to March. On the other hand, Pearse (1968) noted that several sea star species from the Red Sea have been observed to spawn in winter, spring and summer. To summarize, most echinoderm species inhabiting the northern Red Sea show an annual reproductive cycle but variable spawning season (Mortensen, 1938).

T. gratilla reproductive cycle is highly variable along its wide geographical distribution range (Lawrence & Agatsuma, 2013). Frequently, it shows a unique spawning season. This season occurs also in autumn Australia (O'Connor *et al.*, 1978) and Taiwan (Chang-Po & Kun-Hsiung, 1981) and. While, it appears from mid-summer to autumn in Japan (Kobayashi, 1969) and Indonesia (Radjab, 1997) and in summer in Kenya (Muthiga, 2005). Other observations show that *T. gratilla* may spawn throughout the year in the Philippines (Tuason & Gomez, 1978). Whereas, it spawns twice (spring and autumn) each year on the Great Barrier Reef (Stephenson, 1934). Focusing on the Red Sea, previous studies observed *T. gratilla* spawning season in spring in the 1960's. Fouda and Hellal (1990) recorded that, *T. gratilla* spawning takes place from autumn to spring, with a peak in winter in the Gulf of Aqaba in the 1980's. All these previous studies disagree with our findings. The fact that *T. gratilla* has little genetic differentiation between populations that were geographically distant along its distribution range (Lessios *et al.*, 2003; Juinio-Meñez *et al.*, 2008; Brink *et al.*, 2018) implies that this echinoderm has a high reproductive plasticity and that its resilient to changing environmental conditions both in space and time. Variability in reproductive patterns (both spatial and temporal) is common among echinoid species and even within the same species population. This pattern was described for the temperate species *e.g.*, *Strongylocentrotus purpuratus* (Lester *et al.*, 2007), austral sea urchin species such as *Loxechinus albus* (Schuhbauer *et al.*, 2010), *Paracentrotus lividus* (Ouréns *et al.*,

2011). In addition to the afore-mentioned species, tropical species *e.g.*, *Echinometra mathaei* (Pearse & Cameron, 1991) are considered. This variability is explained by the fact that the reproductive cycle (gametogenesis and spawning) is influenced by several abiotic (sea water temperature, hidrodinamism, day length, moon cycle) and biotic factors (food availability and phytoplankton (Pearse, 1974; Lawrence & Agatsuma, 2013). Factors that control the reproduction of *T. gratilla* are still poorly understood. For example, Chang-Po and Kun-Hsiung (1981) observed that gonad growth and spawning of *T. gratilla* was correlated with seawater temperatures in Taiwan and Vaitilingon *et al.* (2007) detected a correlation when temperatures and day length were the lowest in Madagascar. However, other studies concluded that *T. gratilla* reproduction had no relation to seawater temperature and day length (Pearse, 1974, Fouda & Hellal, 1990). Muthiga (2005) suggested that, lunar phase may play a role in the timing of spawning. She observed spawning occurring between lunar day 7 and 21 in Kenya. A relationship with moon cycle was also described in the study of Juinio-Meñez *et al.* (2008) in Philippines. These authors observed that the spawning coincides with the new moon. Johnson and Ranelletti (2017) described spawning during wintertime (December) and near a new moon. Finally, Juinio-Meñez *et al.* (2008) suggested that broad scale major spawning events occurs during the peak in the monsoon. In the current study, no correlation was detected between spawning and seawater temperature neither day length. The synchronization with the moon cycle was not studied, but recently Webb *et al.* (2021) showed a spawning event 5 and 6 days before full moon & broadcast spawning of four echinoderm species in the Red Sea. This suggests that it may play an important role also in *T. gratilla*.

Sea urchin spawning events have been observed close to phytoplankton blooms (Starr *et al.*, 1992, Gaudette *et al.*, 2006). *T. gratilla* has a pelagic larval duration between 42 and 52 days (Juinio-Meñez *et al.*, 1998). Spawning (from September to October) coincides with a strong increase of Chl *a* in waters after early and mid-summer when the lowest Chl *a* was recorded. The synchrony between both events maximizes the food availability for the pelagic larvae. Starr *et al.* (1992) reported that the synchronization of the spawning of echinoderms with phytoplankton availability is an advantage adaptation.

No sexual dimorphism was recorded in the present study. This coincides with the findings of Fouda and Hellal (1990) as no hermaphroditism recorded in *T. gratilla* at the northern Red Sea. However, there was an unusual case of hermaphroditism in *T. gratilla* from Eilat, the Gulf of Aqaba (Kidron *et al.*, 1972). The sex ratio is one of the parameters that contribute to maintain the echinoid species' capacity of reproduction. This index represents the abundance of one sex compared to the other. At the level of the present study, there were an imbalance between the sexes in favor of females. On the contrary, Fouda and Hellal (1990) estimated a tendency towards males of *T. gratilla* in the northern Red Sea with ratio 1:2. The imbalanced estimation of *T. gratilla* population

towards females was also reported in the study of **Vaitilingon *et al.* (2007)** in the southwestern Indian ocean. Notwithstanding a 1:1 ratio was recorded in the study of **Muthiga (2005)**. Sex ratios may be affected by the environment and the sexual maturity condition. It has also been reported that the ratio can be affected by different mating behaviors (**McCarthy & Young, 2002**) and different levels of natural predation (**Gianguzza *et al.*, 2009**) between genders.

The gonad is the only organ in sea urchin that can store nutrients as the gut has a very limited storage capacity. Besides, it carries out the function of reproduction. This means that conservation of nutrient is closely related to reproduction (**James *et al.*, 2018**). Hence, the increase in oocyte size confirm that gametogenesis is initiated, as sufficient reserve of nutrients must be accumulated in the gut tissues and in the nutritive phagocytes of the gonads (**Vaitilingon *et al.*, 2007**). **Pearse (1969)** suggested that gametogenesis in echinoids could not be initiated until a critical level of nutrients was available within the storage tissues. This explains the reason of changes in the gonad mass throughout the reproductive cycle.

The increase in oocyte size started in October as the first 50-micron ova were recorded in October; this evidenced that spawning was initiated in this period. This was also confirmed by the released egg size in the induced spawning experiment which ranged from 50 to 60 micron (**AbouElMaaty *et al.*, 2022**). Sexual staging of the gonads and oocyte size of indicates a well-defined annual reproductive cycle for the Red Sea population. The annual gametogenic cycle in both sexes is nearly similar but not identical. This synchrony in the gametogenic cycle is one of the primary ways that broadcast spawners can improve fertilization success (**Johnson & Ranelletti, 2017**). They show restricted breeding season with intermittent spawning. This was confirmed by the histological sections in the gonads. It showed ripe gametes during the whole period of spawning; this was indicated by the presence of free spaces in the acini beside releasing the gametes on handling the gonads. Furthermore, in the present study, the peak of the gonado-somatic index value was in October which synchronize the breeding season in the Red Sea. The gonadosomatic index indicates the cellular changes in the gonad. October witnessed the maximum GSI in, the gonad weight of females was 8.4 % of the body weight while it constitutes about 8 % of the body weight of male. The maximum gonadosomatic index reported for *T. gratilla* was 10–16% (**Tuason & Gomez, 1978; Fouda & Hellal, 1990; Shimabukuro, 1991; Juinio-Meñez *et al.*, 2008**). **Muthiga (2005)** found that, the maximum size of gonads of *T. gratilla* on Kenyan reefs was 5% of wet body weight. Low availability or low nutritional quality of food is probably the explanation for these small gonad sizes (**Lawrence & Agatsuma, 2013**). October was followed by a decline in the percentage of GSI till June (from 8.4% and 8% to 3.6% and 3.4%, for females and males respectively), then it started to increase once again. This coincides with *T. gratilla* at Okinawa where the gonad index decreased from 10–12 to 2% with spawning (**Shimabukuro, 1991**). However, **Vaitilingon *et al.* (2007)** reported a

peak gonad index in *T. gratilla* in Madagascar of 16.1, followed by a decrease to only 11.5 after spawning.

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