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Reproductive cycle of the bivalve clams" *Donax variabilis* (Say, 1822)" in New Damietta shore, Egypt

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Abstract
New Damietta shore is one of the important areas for collection of the clams as well as mullet, seabass and seabream larvae which are reliable for marine aquaculture in Egypt. The reproductive cycle of the clam; *Donax variabilis* (Say, 1822), in this shore was investigated during the period from May 2008 to July 2009. Reproductive maturity was estimated by assessment of gonad index and microscopic examination of gonadal smears and sections. The different stages of the reproductive cycle were characterized by histological examination. Six distinguishable gonad stages were determined in both male and female clams viz., early active, late active, ripe, partially spawning, completely spawning and spent stage. Both males and females showed synchronism in gonadal development. Spawning occurred between May and August with one major spawning phase. *Donax variabilis* is a dioecious species without sexual dimorphism or sex reversals. The ratio of males to females (1 M: 0.85 F) was statistically non-different from the theoretical sex ratio of 1:1 ($X^2 = 8.70$, $P > 0.05$).

Keywords: *Donax variabilis*, gonad stages, spawning periods, reproductive cycle, New Damietta, Egypt

1. Introduction
Bivalves can acquire an important role in solving the problem of shortage and high price of animal protein in Egypt. Egyptian beaches has extended over long distances and it has become necessary to look at how the exploitation of bivalves inhabiting these beaches.

Family Donacidae is restricted to distinct zoogeographical domains [1]. Some species inhabit regions with an overlap of subtropical and temperate zones. Only 5% of the 64 species are found in cold temperate areas (>5 ºC) [2] with lowest species diversity on the West Coast of Africa [1]. The Coquina clam; *Donax variabilis* (Say, 1822) and the carpet shell clam; *Donax trunculus* (Linnaeus, 1758) are the most prevalent species, especially in warm water [1]. *D. variabilis* is the dominant macro fauna on many of the exposed beaches of the south-east United States [3]. It is found from Virginia south to Florida and round into the Gulf of Mexico to Texas [4].

Clams are important recreational and commercial resources in many countries [5]. *Donax sp.* spreads in the northern coast of Egypt and is the favorite food among the population in coastal cities. Inshore waters of the Damietta region (North coast of Egypt) support nursery (larval or fry) areas for several commercially important species, especially the common mullet, sea bream and sea bass [6].

The study of bivalve reproduction forms the basis of most ecological studies because it provides important data relating to population structure and also enables accurate predictions to be made concerning recruitment to population [7]. Knowledge of certain aspects of reproduction such as the reproductive cycle and the number of spawning events is fundamental to the management of shell fishery [8]. Thus, the present study aims to determine gonad stages, reproductive cycle, spawning pattern and sex ratio of *D. variabilis* in New Damietta shore, Egypt.

2. Materials and methods
2.1 Study area
The study area (New Damietta shore) is an important part of the Egyptian coast of the Mediterranean Sea characterized by several important characteristics [9]. New Damietta shore extends about 19.5 km from the Maritime Damietta Port in the east to the limits of Gamssah city in the west (Fig. 1).

This shore is one of the important areas for collection of the clams as well as mullet, seabass and seabream larvae which are reliable for marine aquaculture in Egypt. Fluctuation in the environmental qualities is due to their proximity to the channel which reaches the Nile River with the sea as well as draw close to the Damietta Maritime Port.

2.2 Sampling

In order to determine the extent of *Donax variabilis* populations and also to select suitable sampling site, a general survey of the New Damiettia shore using quadrates was undertaken in May, 2008. Consequently, a site was chosen east of New Damietta adjacent to Damietta Maritime Harbor (31º 28.577 N and 31º 44.408 E) (Fig. 1).

Samples of *D. variabilis* were collected at monthly intervals between May 2008 and July 2009 by dragging from a depth of nearly 2 meters. The sediments were washed out carefully in situ through one mm mesh size sieve. The materials retained by the sieve were put in labeled containers filled with 6% neutral formalin. A subsample (12–18 specimens) was fixed monthly in Bouin's solution for histological studies.

2.3 Gonad index

In the laboratory, *D. variabilis* specimens were measured for shell length (SL) with a precision of 0.1 mm using a vernier caliper. The length of the clams was defined as the longest distance between the anterior and posterior shell margins. The flesh was dissected out of the shells. Gonads were carefully separated from somatic mass. In both sexes, the gonad is intertwined with visceral mass which is composed of stomach and the intestinal loop. Flesh weight (FW), gonad weight (GW) were determined separately for each specimen using top-loading digital balance (precision of 0.0001 g). Weight measurements were restricted to adult clams as they are likely to exhibit more variations (due to spawning activity) than juveniles.

Gonad index (GI) is the most widely used quantitative method for estimating the reproductive activity of marine bivalves [10]. GI was calculated individually as the ratio between GW and FW x 100 [11]. GI was calculated monthly and expressed as mean± S.D.

2.4 Histological studies

Gonads of mature individuals fixed in Bouin's solution were dissected, dehydrated in alcohol, cleared in xylene, embedded in paraffin wax and sectioned (6–8 *u*m) at a standard point. Tissue sections were stained in Ehrlich's hematoxylin and Eosin Y according to procedures outlined in [12]. Histological sections of each specimen were staged on the basis of the degree of development of germ cells. Female and male specimens were categorized into six stages. The recognition of these stages was based upon the classification cited by Drummond *et al.* [13] and Nabuab & del Norte-Campos [14] with some modifications based upon preliminary histological observations of the gonad. The description and criteria for each stage for males and females are summarized in the results. The relative frequency of each stage was calculated monthly and graphed for both sexes.

2.5 Sex ratio

Smears of the sexual products were examined at 100x magnification. Each specimen was sexed and the sex ratio (expressed as the number of males per female, F: M) was determined. Statistically significant deviations from the expected sex ratio of 1:1 were assessed by Chi-quare (X^2) analysis with one degree of freedom [15].

3. Results

3.1 Gonad index

Average monthly variation in the mean gonad index (GI) of both males and females of *D. variabilis* are presented in Fig. 2. Both sexes showed more or less similar trends in the mode of monthly variations. GI of males and females showed three major peaks through the study period. In males, peaks were observed in May (26.0), July (26.6) 2008 and April (33.0) 2009, while in females they found in May (26.4), July (26.6) 2008 and May (36.5) 2009. As well as a sharp decline in GI of both males and females were evident in June (7.8 and 8.0), September (9.5 and 9.9) 2008 and July (22.1 and 22.1) 2009, respectively.

3.2 Stages of gonadal development

Six different stages of maturation were assessed in both males and females of *D. variabilis* when the gonad condition was examined histologically. These stages are described as follows:

3.2.1 Male gonad

Stage 1 (early active): Islands of male follicles appeared in well-developed interfollicular space. The interfollicular space contains well developed interfollicular connective tissue rich in nutritive inclusions (Fig. 3A). The follicles were blocked with initial stages of spermatogenesis. Spermatogonia were centripetal to follicle walls. Spermatocytes were numerous and move towards the center of the follicles. Few numbers of spermatids appear in the center of some follicles. Spermatozoa were nearly absent in this stage.

Stage 2 (late active): Testicular follicles became more closely packed and the interfollicular space was reduced (Fig. 3B). There was a marked increase in the number of spermatocytes and spermatids in the center of the follicles as compared to early active stage. Spermatozoa were identified but made up a relatively minor portion of the lumen contents.

Stage 3 (ripe): The gonad attained its fully ripe condition during this stage. Male follicles were fully extended and became highly compressed together. The interfollicular space nearly disappeared (Fig. 3C). Spermatozoa occupied nearly all the available spaces of the lumen (about 90% of the lumen volume). Ripe stage differed from late active stage in the great reduction of early stages of spermatogenesis.

Stage 4 (partially spawning): The arrangement of spermatozoa mass in the ripe stage was found to be disorganized in appearance as a considerable number of spermatozoa (about 40%) were discharged. This partial discharge of spermatozoa resulted in reducing follicular size and the appearance of a distinctive lumen for each follicle (Fig. 3D).

Stage 5 (completely spawning): There was a general reduction in the area of the visceral mass occupied by male follicles. These follicles collapsed, decreased in size and had a large lumina with few un-discharged strands of spermatozoa which loosely filled the follicles. The inner periphery of the follicles was lined with narrow bands of spermatogonia and spermatocytes (Fig. 3E). Interfollicular space was larger than that of the partially spawning stage.

Stage 6 (spent): Spent stage was usually regarded as a stage of low activity. At this stage, there was still further reduction in the area of visceral mass occupied by gonadal tissues. The gonad contains very few follicles, which were sparse and very small in diameter. There were many empty spaces between and within the follicles (Fig. 3F). The lumina of the follicles contained yellow brown fragments as the result of cytolysis of the residual gametes. Phagocytic cells were very abundant inside and outside the follicles. The nutritive inclusions occurred in great abundance.

3.2.2 Female gonad

Stage 1 (early active): This stage was characterized by the onset of oogenesis after the end of spent stage. Small separate ovarian follicles were embedded in interfollicular connective tissue. Numerous oogonia, small oocytes and growing oocytes arose along the walls of the follicles. Ripe ova were nearly absent in this stage (Fig. 4A).

Stage 2 (late active): A considerable increase in the follicle mass and a reduction of the interfollicular space were clear in this stage (Fig. 4B). Ripe ova appeared in the center of follicles which were occupied mainly by growing oocytes. Early stages of oogenesis; oogonia and small oocytes, were seen attached to follicle wall.

Stage 3 (ripe): The area of the visceral mass occupied by ovarian follicles was large. The follicles were fully distended and confluent with the absence of interfollicular space (Fig. 4C).The lumens of follicles were filled with ripe ova compacted into polygonal shapes. There was a great reduction in the early stages of oogenesis. However, the follicles still contained a considerable number of growing oocytes.

Stage 4 (partially spawning): In this stage, there was a marked decline in the number of ripe ova filling the follicles. These follicles were partially empty. The follicular lumens and the interfollicular spaces became wide (Fig. 4D). The remaining ova were relatively rounded off as the pressure within the follicles is reduced following partial emission. Growth of the interfollicular connective tissue tacked place immediately at this stage.

Stage 5 (completely spawning): This stage was characterized by a great reduction of ripe ova compared to the previous stage. Ovarian follicles were almost empty except few residual ova in the center of the follicles. Interfollicular space became very noticeable (Fig. 4E). Some follicles were invaded by phagocytic cells, which degenerate the unspent ova. Degenerate ovum was easily detected by the loss of its round shape, which was characterized of residual ova in completely spawning stage.

Stage 6 (spent): The criteria of spent stage for female gonads were similar to that in males. However, the onset of oogenesis (the formation of small oocytes) was observed in very few follicles of some individuals. This may enable the sex of the spent clams to be determined (Fig. 4F).

3.3 Reproductive cycle

Monthly proportions of the clams classified in each gonadal stage are represented in Fig. 5A for males and in Fig. 5B for females. Both sexes showed monthly variation in the percentage occurrence of the different stages. The gametogenic cycles were synchronous, as evidenced by the simultaneous occurrence of one or two stage in most of the monthly samples. Ripe stage was recorded in 71.4% of the investigated males during May 2009. However, the occurrence of this stage was 42.9%, 50.0% and 66.6% of the total examined females in April, May and June 2009, respectively. Spawning in both sexes appeared on a large scale, as suggested by the presence of partially and completely spawning individuals, in the period from May to August. Large percentages of early active stage occurred during October–March indicating the beginning of oogenesis. For both sexes, spent stage appears in large percentages (18.8% to 100%) during the period from August to November 2008.

3.4 Sex ratio

Out of 1354 clams examined microscopically, 56.1% were sexed as males and 43.9% as females. The overall ratio of males to females (1 M: 0.85 F) was statistically non-different from the theoretical sex ratio of 1:1 (X^2 = 8.70, P > 0.05).

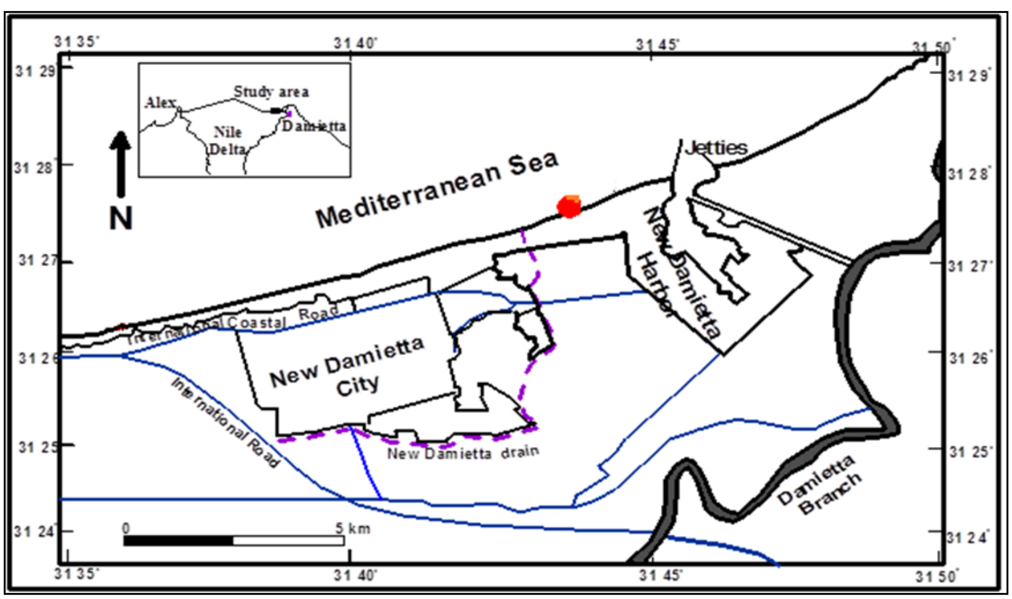


Fig 1: Map of the study area showing sampling site detected from land sat image.

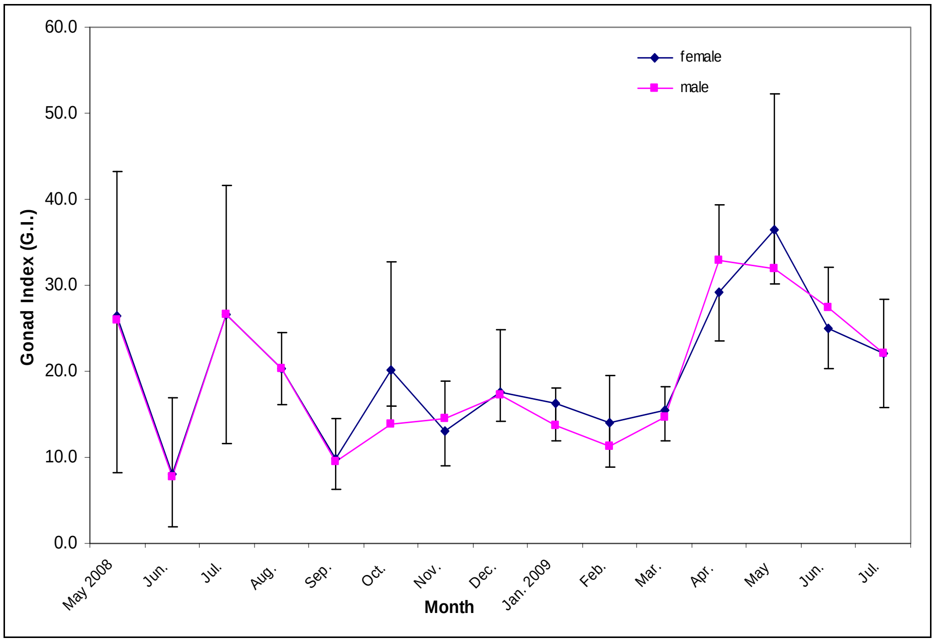


Fig 2: Monthly changes in mean gonad index (± S.D.) in male and female *D. variabilis* collected from New Damietta shore.

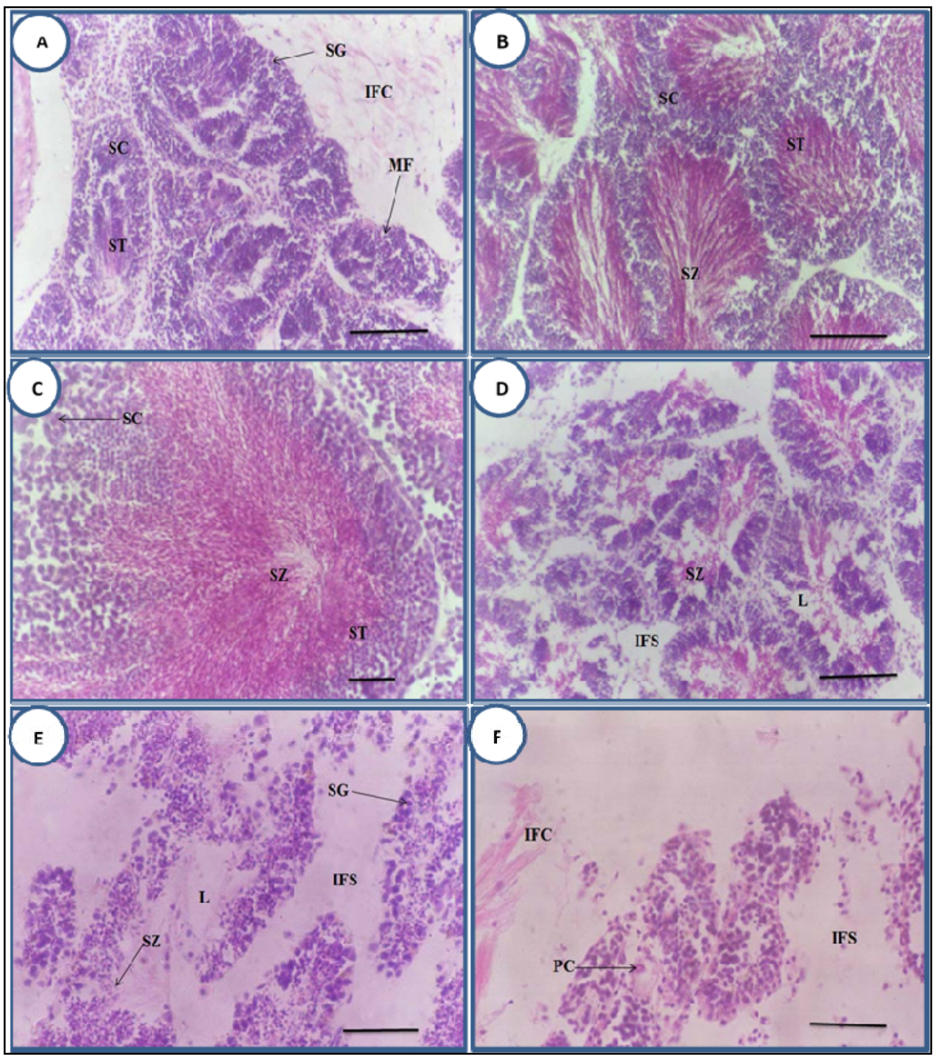


Fig 3 A–F: Gonad developmental stages for male *D. variabilis.* (A) Early active stage. (B) Late active stage. (C) Ripe stage. (D) Partially spawning stage. (E) Completely spawning stage. (F) Inactive stage. *Abbreviations*: FW, follicle wall; IFC, interfollicular connective tissue; IFS, interfollicular space; L, lumen; MF, male follicle; NI, nutritive inclusion; PC, phagocytic cell; PS, perivitelline space; SC, spermatocytes; SG, spermatogonia; ST, spermatids; SZ, spermatozoa. Scale bar = 4*u*m.

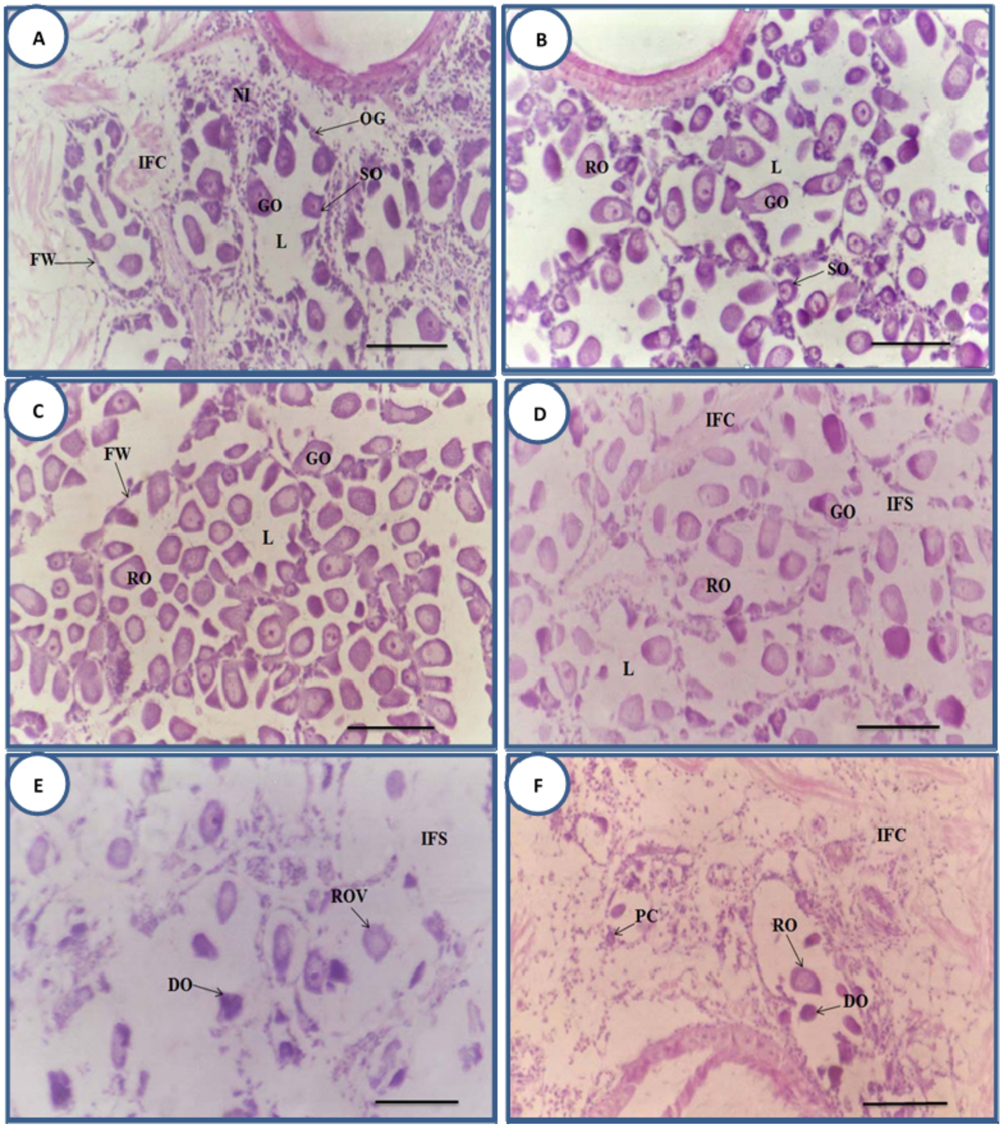
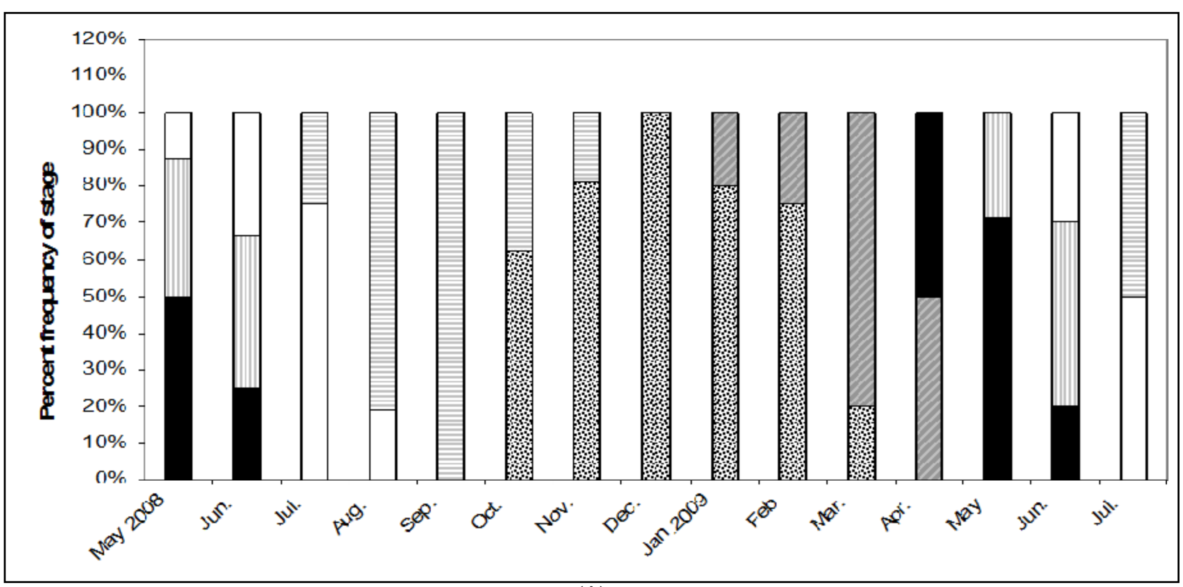
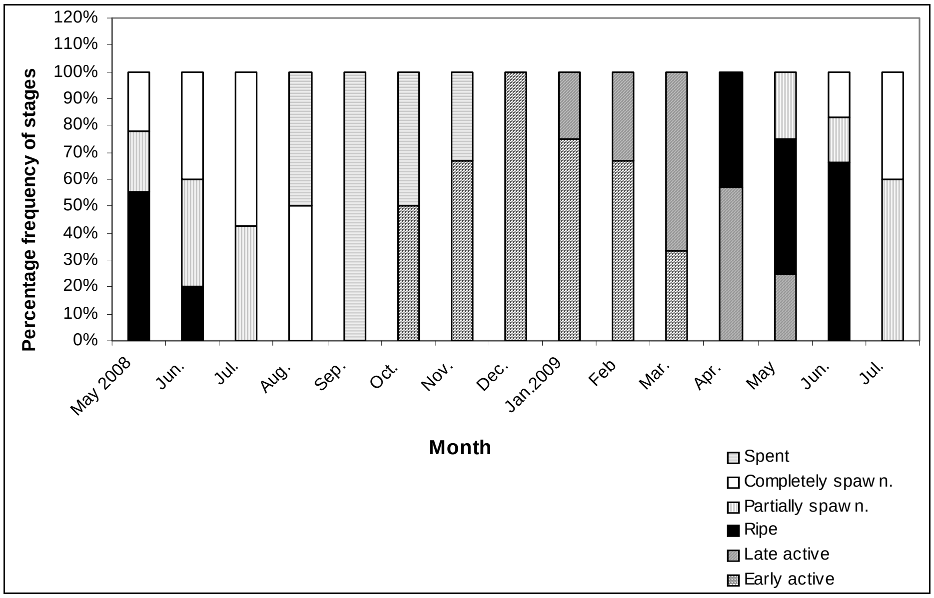


Fig 4: A–F: Gonad developmental stages for female *D. variabilis.* (A) Early active stage. (B) Late active stage. (C) Ripe stage. (D) Partially spawning stage. (E) Completely spawning stage. (F) Inactive stage. *Abbreviations*: DO, degenerated ovum; FW, follicle wall; GO, growing oocyte; IFC, interfollicular connective tissue; IFS, interfollicular space; L, lumen; NI, nutritive inclusion; OG, oogonia; PC, phagocytic cell; RO, ripe ovum; ROV, residual ovum; SO, small oocytes. Scale bar = 4*u*m.



(A)



(B)

Fig 5: Monthly variations in the percentage frequency of gonadal developmental stages for male (A) and female (B) *D. variabilis* collected from New Damietta shore.

4. Discussion

The reproductive cycle of bivalves usually takes the following forms: a progressive development from a condition in which the gonads are undifferentiated, through differentiation of the gonad (gametogenesis and maturation of the gametes), to spawning (with partial or complete release of gametes), and a return to one of these earlier stages [16]. A similar sequence of events has been reported for population of *D. variabilis* in New Damietta shore. However, spawning of the gametes was divided into three stages in both sexes (partially spawning, completely spawning and spent). The end of the spawning season was usually marked by gamete atresia (degeneration and phagocytosis) within the follicles. The reason for oocyte atresia is a self-cleaning process at the end of gametogenic cycle in preparation for the next cycle [17].

Six different stages of gonad maturation were assessed in both males and females of *D. variabilis* namely; early active, late active, ripe, partially spawning, completely spawning and spent stage histological analysis. Gonad development in both male and female clams showed that gonads are active all throughout the year. The highest sexual maturity occurred during spring (April-June). Spawning occurred during late spring (May and June) and summer (July–September). Spent stage was observed during late summer (August and September) and autumn (September–November). The Elongate Sunset clam, *Gari elongate* (Lamark 1818) studied in Banate Bay Area, West Central Philippines also exhibits this pattern of gametogenesis [14]. Despite this pattern however, gonad development in *D. variabilis* is highest during spring (April–June) as shown by the higher frequency of ripe stage during these months.

A sharp decline in mean gonad index (GI) was observed in June (7.8 and 8.0), September (9.5 and 9.9) 2008 and July (22.1 and 22.1) 2009 for males and females, respectively. The trend of decreasing GI indicated spawning in *D. variabilis*. Thus, monthly changes in mean GI were considered a reliable tool for measuring the reproductive activity as reported for the Elongate Sunset clam, *Gari elongate* [14] and the cockle *Cerastoderma glucum* [18].

High biomass (mg/m^3) of phytoplankton (chlorophyll-*a*) was recorded during late spring (May and June) and early summer (August and September) in New Damietta shore [9]. High biomass of phytoplankton during these periods can be attributed to the optimum utilization of sunlight energy for photosynthesis. Energy derived by the clams from the phytoplankton was utilized in gamete proliferation and growth. This can be evident by marked accumulation of gametes as indicated by height frequency of developing gonads during autumn (October–December) and winter (January–March). *D. variabilis* is a dioecious species without hermaphroditic individuals. The overall ratio of males to females (1 M: 0.85 F) was statistically non-different from the theoretical sex ratio of 1:1 (X^2 = 8.70, P > 0.05). The sex ratio in the closely related clam; *D. trunculus* was studied by Lucas [19], Badino & Marchionni [20] and Moueza & Frenkiel-Renault [21]. These authors mentioned that males of this clam made up a slightly higher proportion but they did not show a statistically significant difference from a sex ratio of 1:1.

On the other hand, Nabuab & del Norte-Campos [14] reported that a few specimens of hermaphroditism was observed in the Elongate Sunset clam, *Gari elongate* (Lamark 1818) from Banate Bay Area, West Central Philippines. Based on the Chi square test, the relatively same proportion (1M: 1.04F) of male to female indicates that both sexes are equally represented in the population of this clam. In other dioecious bivalves, the ratio of the sexes is approximately 1:1, with females often slightly more numerous than males [22] and thereby, ensuring the higher number of eggs to be fertilized by the sperms.

5. Conclusion

The results of the present study indicate that *D. variabilis* is well adapted to the specific environmental conditions of New Damietta shore in order to maximize its reproductive success. The study of certain aspects of reproduction such as the reproductive cycle and the number of spawning pattern forms the basis of most ecological studies because it provides important data relating to population structure and also enables accurate predictions to be made concerning recruitment to population. These data is fundamental to the management of shell fishery. However, further research is needed to obtain data on population dynamics.

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