The gametogenic cycle and spawning of the Short-Necked Clam, Paphia undulata Born, 1778 from Timsah Lake, Suez Canal, Egypt

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Abstract

Background

The Short-Necked Clam *Paphia undulata* is a marine edible bivalve consumed all over the world and locally in Egypt. In the past decade, its population declined across the Egyptian fisheries due to overexploitation and pollution. The gametogenic cycle and spawning studies of *P. undulata* were carried out during the period from January 2020 to December 2020 along Timsah Lake, Suez Canal, Egypt.

Results

Shell lengths of the collected clams ranged from 2.31 cm to 6.22 cm in males, 2.05 cm to 5.97 cm in females and 3.70 cm to 4.36 cm in hermaphrodite clams. These clams are functionally dioecious wherein male and female sexes are separate, with very low incidences of hermaphroditism. The sex ratio (male: female: hermaphrodite) of the clam population was 1.0: 1.07: 0.04. The onset of sexual maturity of both males and females was 2.3 cm and 2.7 cm respectively.

Conclusions

Reproductive studies revealed that this species has a prolonged spawning season not restricted during a certain period.

Introduction

The Bivalvia clam *Paphia undulata* (Born, 1778) is economically and ecologically important with respect to a food source, biomass and effects on communities. They are considered the largest proportion of the shellfish markets in many coastal residents' countries all over the world. Worldwide consumption of marine bivalves has increased with growing interest in the nutritional and health benefits of these products. So, people obtain a great part of their animal protein from bivalves [1, 2].

It's a good source of protein, reaches of approximately 68.77% crude protein "dry weight basis" [4, 5, 6, 7], and it was content with high proteins especially essential amino acids which considered an essential source of nutrients for many people, especially in developing countries [8]. It was noticed that clam is consumed locally in Egypt and the population declined across the Egyptian fisheries in the past decade. This is mostly attributed to overexploitation, pollution and parasites [3, 10]. Egyptian Clam fishermen mentioned that clam populations in Egypt have declined in the past decade [11]

In industries, the activated *P. undulata* shells waste can be utilized as a cost-effective catalyst for biodiesel synthesis from non-vegetable oil, and the fly ash-supported CaO catalyst derived from the waste shell of *P. undulata* is used for transesterification of palm oil to yield biodiesel [12, 13). Bioceramic derived from *P. undulata* waste shell is used as alternative bone material for the vertebral bones that consist of cervical, lumbar and spongy bones [14].

The awareness of the culture of the clams was initiated in the 1970s after it was noticed that the worldwide population of clams hastily declined. Increasing coastal populations, improved harvesting efficiency and pollution were some of the reasons that were contributing to the decreasing and maybe the destruction of the clams' local stock from their geographical range [15].

Gametogenesis is the process in which gametocytes undergo cell division and differentiation to form haploid gametes. Asynchrony in the maturation of the male and female gametes during gametogenesis leads to synchronous spawning, whereas discrepancy in gamete development leads to asynchronous spawning [16]

The sex ratio is a fundamental indicator for reproduction success in dioecious species. Nearly equal numbers of males and females are produced in most gonochoristic species, giving a balanced sex ratio, whereas the sex ratio can skew towards one sex in hermaphroditic species. [17, 18]. Size at sexual maturity is defined as the smallest shell length staged with mature

gonads. The size at first maturity of bivalves is the percentage of maturity stages plotted against shell length (size group) showing the shell length at which 50% of clams matured [19].

Due to the commercial importance of *P. undulata* and its poly-culture potential, there is a need to re-assess its status considering that there are still no known management policies governing its exploitation. This study aims to characterize the reproductive cycle, gametogenesis, and spawning season of *P. undulata*. This information can be used to support large-scale artificial breeding and the development of artificial breeding techniques.

Materials And Methods

Sample collection

Two hundred and eighty-four random samples of the clams were collected from Timsah Lake sandy shore at Ismailia city (Fig. 1) during the period from January 2020 to December 2020, (lat. 32° 17E; $long$, $30^\circ 35$·N).

Timsah lake is a small and shallow lake located near Ismailia city at the midpoint of the Suez Canal and about 80 km south of Port Said city. It lies between 30º 33 and 30º 35 N latitude and 30º 16 and 30º 19 E longitude. Its surface area is about 16 km with depth variation between 3 and 16 m. From the western side, it is attached to a small and shallow Western lagoon which is nearly brackish. The significant-high variation of salinity in Timsah lake from 40‰ in summer to 7.3‰ in winter was due to the presence of many freshwater sources such as Ismailia freshwater canal and the wastewaters discharge from the Western lagoon [20].

The clams were transferred alive to the laboratory of the National Institute of Oceanography and Fisheries and prepared for morphometric analysis. Data concerned with the shell length and shell height were measured with vernier callipers. The total shell weight in grams was recorded using an electronic balance accurate to two decimal places. The soft tissues were removed from the shell to obtain the gonad-visceral mass, the gonad and visceral mass are not easily separated. The gonad mass was used to distinguish the sex, and evaluate the reproductive cycle. Gonads were fixed in Bouin's solution and prepared for histological analysis.

Histological studies

The procedures for histological preparation of *P. undulata* gonad tissues followed that [18, 21]. The protocol included: fixing 1 cm pieces of tissue in Bouin’s fluid for 24 h, dehydrated them in a graded series of alcohol and xylene, and embedding them in paraffin wax. Sections of 5 µm thickness were prepared, mounting a microscope slide with neutral resin, staining with haematoxylin and counter-staining with eosin. The histological slides were observed and recorded with a light microscope (Optica B-193).

Gametogenic cycle

Gonadal development of *P. undulata* in both males and females was described and classified into five stages: (1) early development, (2) late development, (3) ripe or mature (4) partially spent or spawning and (5) spent, with some modification, based on the criteria of *P. undulata* [22, 23]. Because the time of gonadal development differed between sexes, the reproductive cycles of the sexes were analyzed independently.

Results

Sex ratio and size at sexual maturity

The visual examination of *P. undulata* showed that both male and female gonads are creamy in colour regardless of sex, stage, and size. Thus, it is not possible to determine the sex and characterize the gonad development of this species through external morphological examination. These clams are functionally dioecious wherein male and female sexes are separate, with very low

incidences of hermaphroditism. However, this species does not exhibit sexual dimorphism. But in ripe gonads, they occupied almost the whole tissue of the mantle.

The results of 285 examined individuals of these clams showed that 135 (47.4%) clams were males, 144 (50.5%) were females, and only 6 individuals were hermaphrodites thus representing (2.1%) of the studied sample (Fig. 2). The hermaphrodite specimens were collected during January and February in the winter season and from June to August in the summer season. The sex ratio (male: female: hermaphrodite) of the clam population was 1.0: 1.07: 0.04 (Table 1). Shell lengths of the collected clams ranged from 2.31 to 6.22 mm (4.64 ± 0.83) in males, 2.05 to 5.97 (4.55 ± 0.9) in females and 3.70 to 4.36 (4.19 ± 0.3) in hermaphrodite clams. The average size at sexual maturity in *P. undulata* was 2.3 cm in males and 2.7 cm in females (Fig. 3).

Table 1
Monthly distribution of the different maturity stages and sex ratio for males, females and hermaphrodite (Herma) of *P. undulata*.

Months	Male					Total No.	Female					Total No.	Herma	Sex ratio
	EAS	LAC	RS	PSS	SS		EAS	LAC	RS	PSS	SS			M:F:H
Jan 2020	6	3	7			16	3	7	3			13	1	1:0.81:0.08
Feb	1	3	2			6	1	1	3			5	1	1:0.83:0.20
Mar		3	5	4		12	1	8	6	5		20		1:1.67:0.00
Apr		4	6	3		13		4	9	1	1	15		1:1.15:0.00
May		5	5	3	2	15		4	5	3	2	14		1:0.93:0.00
Jun		3	5		1	9		1	3	2	1	7	1	1:0.78:0.14
Jul		2	3	2	1	8		2	5	1	1	9	1	1:1.13:0.11
Aug		1	6		1	8		5	4	1	2	12	2	1:1.50:0.17
Sep		3	5	5	1	14		2	3	5	1	11		1:0.79:0.00
Oct			2	8	2	12		5	6	2		13		1:1.08:0.00
Nov			3	2		5		4	2			6		1:1.20:0.00
Dec	2	8	4	3		17	9	8	1	1		19		1:1.12:0.00
Total No.	9	35	53	30	8	135	14	51	50	21	8	144	6	1:1.07:0.04

Gametogenesis

Histological study of the gonads, under light microscopy, can distinguish the sexes of *P. undulata*. It was found that gametogenesis of the calms in both males and females (Fig. 4) can be categorized into five stages as;

Stage 1, Early active stage (EAS), at this stage, sex can be distinguished and gonad proliferation began. In females; oogonia are at different stages of development. Oocytes are attached to the edges but starting to fill the follicles, free oocytes absent in the lumen. In males; rounded to expanded tubules with spermatogonia and spermatocytes were present except for spermatids and spermatozoa (Fig. 4A).

Stage 2, Late active stage (LAS), in females; Follicles are filled with mature and free oocytes in the center of follicles with prominent nucleus and nucleolus. There are free oocytes present in the center of follicles but the amount is less than half of the total oocytes present in the follicles; attached oocytes are equally abundant. In males: spermatogonia, spermatocytes, spermatids and spermatozoa are present in the follicles in less developed gonads dominant cell type cannot be observed, but in more developed gonads, most of the follicles are full of spermatids and spermatozoa (Fig. 4B);

Stage 3, Ripe (Maturing) Stage (RS), in females: the gonad occupies a large surface area, many oocytes are free in the center of follicles, the shape of follicles is in a polygonal configuration and their walls are thin; in males: the follicles are mainly composed of matured spermatozoa with their flagellum pointing towards the center of the follicle, to form concentric bands or plugs; In very ripe specimens, spermatozoa bands are close to the follicle wall; the appearance of follicles are neat and orderly (Fig. 4C);

Stage 4, Partially Spent (spawning) Stage (PSS), in males: spermatozoa are clearly visible in a swirling shape and account for the greatest portion of cells in the follicle; there is empty space in some follicles due to the release of mature spermatozoa; in females: free oocytes in each follicle are reduced; some follicles are empty due to the releasing of gametes; the follicle walls are broken (Fig. 4D).

Stage 5, Spent Stage (SS), the follicles appear broken, scattered, and relatively empty; in males: in advanced spent individuals, only residual spermatozoa are found and are undergoing resorption; there is a presence of phagocytes; in females: only residual oocytes can be found in the follicles, with most of them are undergoing resorption; Many phagocytes are present (Fig. 4E).

Gonadal cycle

P. undulata has a continuous breeding season as supported by the co-occurrence of different reproductive stages in all the monthly samples of the population (Table 1). The presence of mature gonads in almost all months and the infrequent occurrence of spent stages are consistent with this species having a prolonged reproductive cycle (Figs. 5&6). However, variation in the intensity of the reproductive activities results in peaks or periodicity in the reproductive phases.

The annual gonad development of male *P. undulata* was represented in Fig. 5. Male gonads in the early active development stage were observed from December to February although the late active development stage was observed throughout the year except in October and November. The ripe (maturing) stage was observed throughout the year without exception. Spawning activity began in March and continued into December except in June and August. The annual gonad development of female *P. undulate* showed in Fig. 6. Female gonads in the early active development stage were observed from December to March but the late active development stage and the ripe (maturing) stages were observed throughout the year. Spawning activity prolonged from April to September.

Hermaphroditism

In the current study, six specimens out of 286 specimens of *P undulata* were found to be hermaphrodite thus representing (2.1%) of the studied sample. In all specimens, testicles and ovaries were found next to each other but separated, Fig. 7 showed that the hermaphrodite gonad contains testicles and ovaries in the partially spent stage.

Discussion

The sex ratio (male: female) of the short-neck clam, *P. undulata*, was 1:1.07, which indicates that *P. undulata* is a dioecious organism. Some hermaphrodites were also encountered in this study but in a low percentage. Males and females are equally represented in the population. This means that the population is still in equilibrium, wherein inbreeding and competition for mates do not occur [22, 23]. In general, the ratio of the sexes for dioecious bivalves is approximately 1:1 [24], with the number of females often being slightly higher than the number of males [22, 25]. Previous studies on bivalve reproduction have found that the sex ratio was 1:1, including for *Gafrarium pectinatum* [25], *Gafrarium tumidum* [19], *Donax trunculus* [27], *Anadara granosa* [28], Blood cockles *Anadara inaequivalvis* [29], *Scrobicularia plana* [30], and Noah's ark shell *Arca noae* [31].

Histological study of the gonads can distinguish sexes of *P. undulata*. It was found that *P. undulata* developed their gametes in different stages similar to gametogenesis in other bivalves e.g. cockle, hard clam and manila clam etc. [23]. But the duration of spawning was different for example most of the clams in temperate areas spawned in the summer months while the clams in tropical areas spawned throughout the year.

P. undulata is a dioecious organism, with small numbers of hermaphrodites (2.1%). Nabuab et al. [22] found a small percentage (0.4%) of hermaphrodite short-necked clam *P. undulata* in the Central Philippines, but that species is still functionally dioecious.

Drummond et al. [32] mentioned that the occurrence of hermaphrodites in a dioecious mollusc illustrates that this is an unusual incidence. The unnatural condition of the environment may have caused hermaphroditism of the clams. The sex change of clams has not been reported in short-necked clams *P. undulata* [19, 22].

P. undulata has a protracted or continuous breeding season as supported by the co-occurrence of different reproductive stages in all the monthly samples of the population. The presence of mature gonads in almost all months and the infrequent occurrence of spent stages are consistent with this species having a prolonged reproductive cycle. However, variation in the intensity of the reproductive activities results in peaks or periodicity in the reproductive phases. Protracted breeding seasons with periodicity over an annual cycle are commonly exhibited by tropical bivalve species such as *Scapharca inequivalvis* [33], *Anodontia edentula* [34], and *Gari elongata* [35]. This is because seasonal patterns of gonad development result from responses to within-year changes in environmental factors [36, 37], although seasonal variation in the tropics may be minimal.

The average size at first sexual maturity of *P. undulata* in Timsah lake was 2.7 cm and 2.3 cm for females and males respectively. This value is greater than that of Sutthakorn and Tuaycharoen [38] who found that the smallest size at first maturity of *P. undulata* along the western coast of Thailand is 1.43 cm, which is different from the Gulf of Thailand as in Trat province the size at first maturity is 4.25 cm but it is decreased to 3.2 cm as reported in Chatananthawej [39] in the same site. while in Suratthani province it is 3.06 cm. Jindalikit [25] stated that the size at first mature of *P. undulata* in Mahachai Bay, Samut Sakhon province was 3.16 and 3.19 cm in males and females, respectively while its size at first maturity found in Southern Negros Occidental, Central Philippines was 4.23 cm for males and 4.48 cm for females [40] and it was 4.26 cm for males and 4.48 cm for females [22]. The average size at sexual maturity in *P. undulata* was greater than 4.0 cm in Thailand [41, 42].

Conclusion

In conclusion, the reproductive cycle, gametogenesis, and spawning season of *P. undulata* from Timsah Lake were done around one year. It was found that this species developed its gametes in different stages, and has a prolonged reproductive cycle. *P. undulata* is a dioecious organism with small numbers of hermaphrodites. The sex change of clams has not been reported. This information can be used to support large-scale artificial breeding and the development of artificial breeding techniques.

Declarations

Ethics Approval and Consent to Participate

The study did not involve human participants or welfare of animals. The sampled species is not endangered and was not collected in protected area.

Consent for Publication

Not applicable

Availability of data and materials

Data and Materials are available from Mostafa A. M. Mahmoud on reasonable request.

Competing interests

The authors declare there are no competing interests.

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Author Contributions

MY designed the study, collected the specimens and prepared wax blocks. MM carried out the slide sections preparation and analyzed the data. MM wrote the first draft of the manuscript MY reviewed the manuscript. All authors read and approved the final manuscript.

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Figures

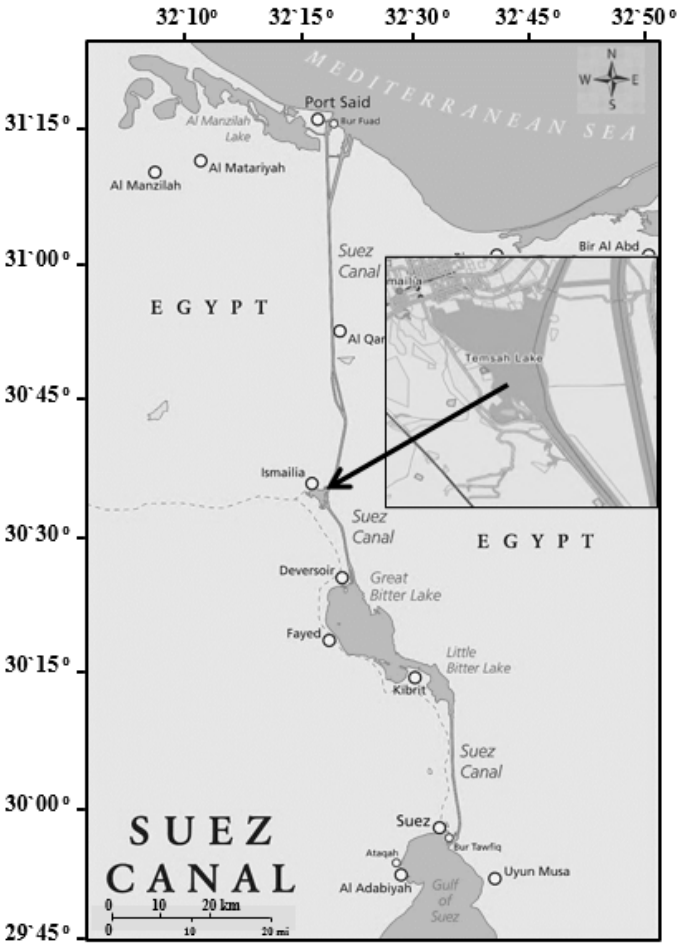

Figure 1

Sampling site of *P. undulata* in Timsah Lake, Ismailia.

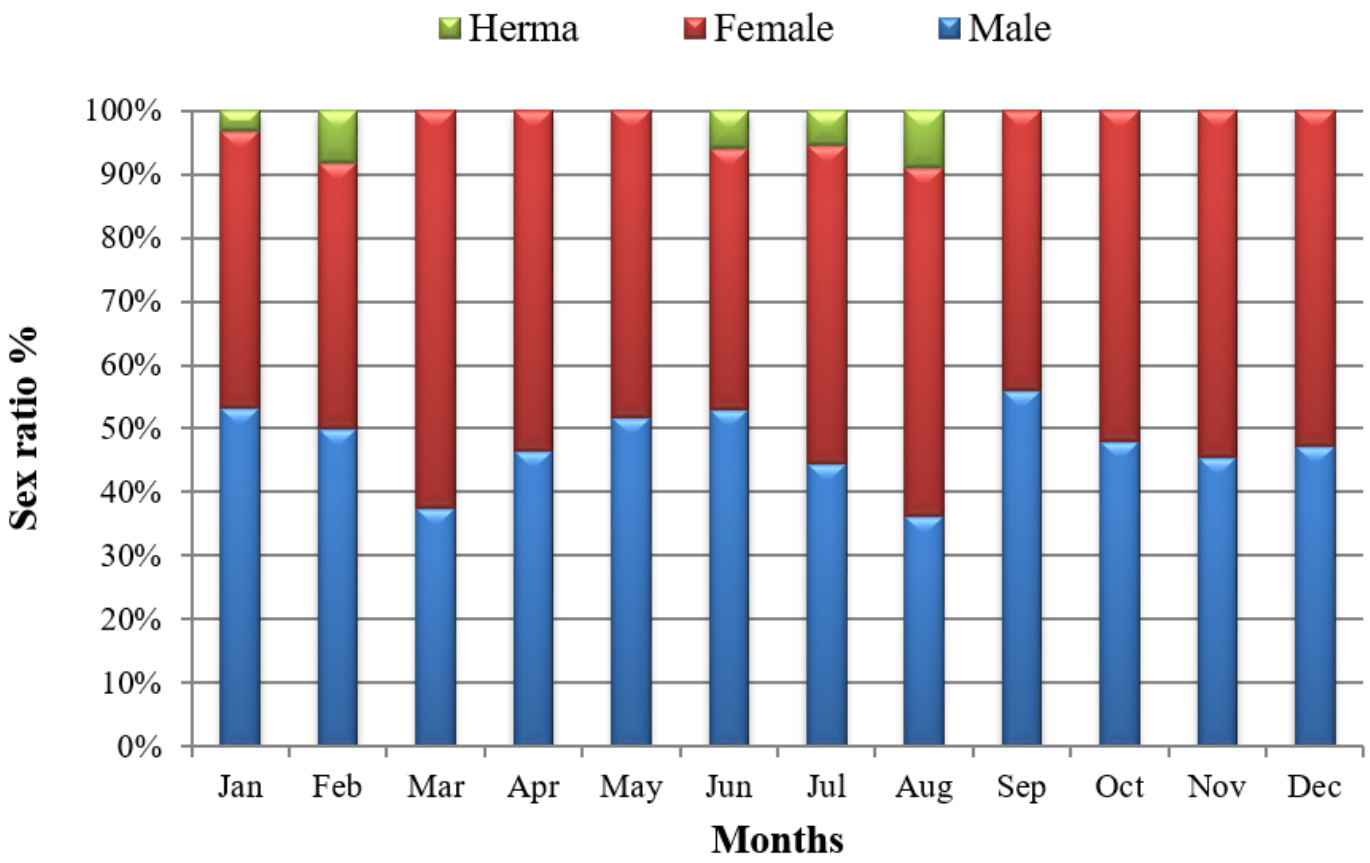

Figure 2

Monthly sex ratio of ratio for males, females and hermaphrodite (Herma) *P. undulata.*

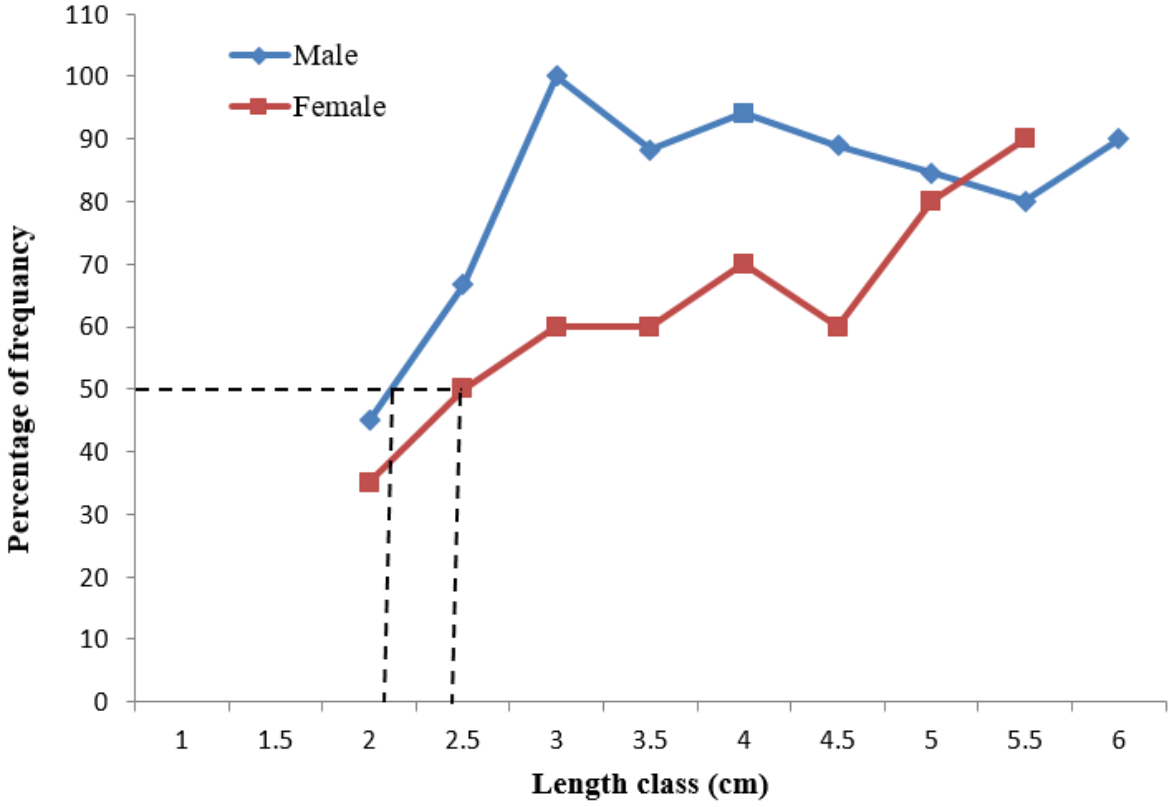

Figure 3

Length at the onset of maturity of *P. undulata* from Timsah Lake

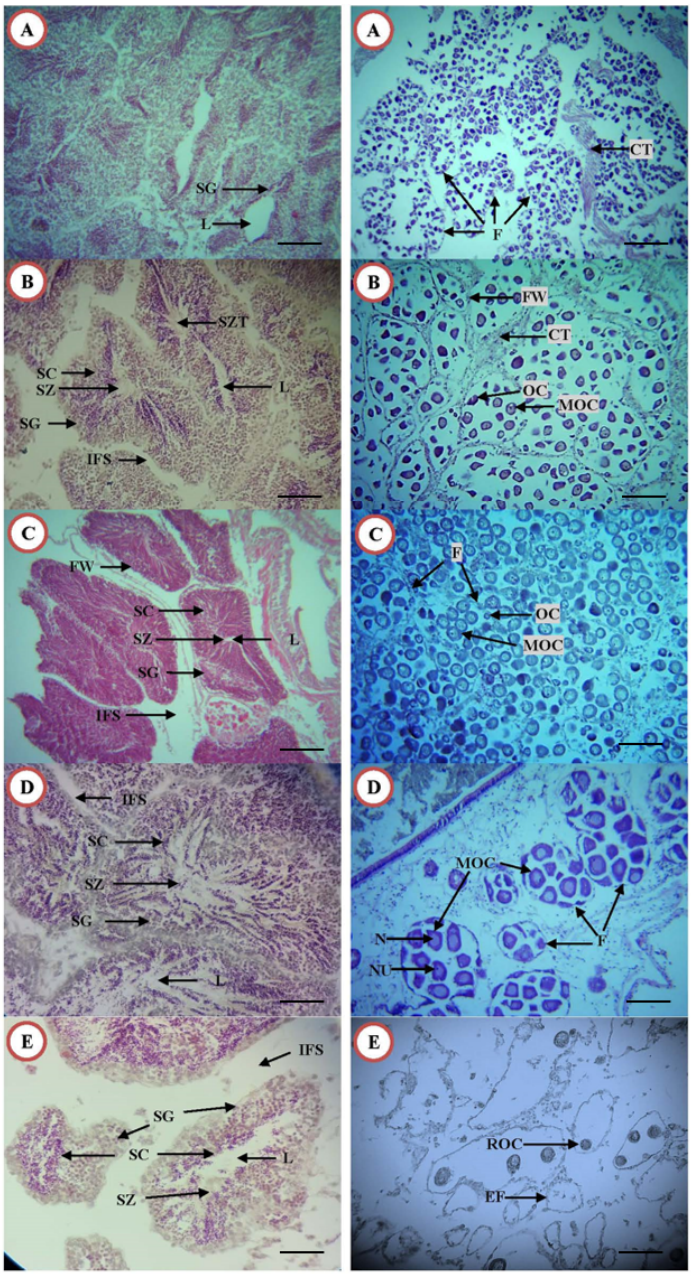

Figure 4

Gonadal development stages of *P. undulata* in male and female (100×). Left panels: male; Right panels: female.

A: early active stage; B: late active stage; C: ripe stage; D: partially spent stage; E: spent stage; CT: connective tissue; EF: empty follicle; F: follicle; FW: follicle wall; IFS: interfollicular space; L: lumen; MOC: mature oocyte; N: nucleus; NU; nucleulus; OC: mature oocyte; ROC: residual oocyte; SC: spermatocyte; SG: spermatogonia; SZ: spermatozoa; SZT: spermatozoa tail. Scale bar=100µm.

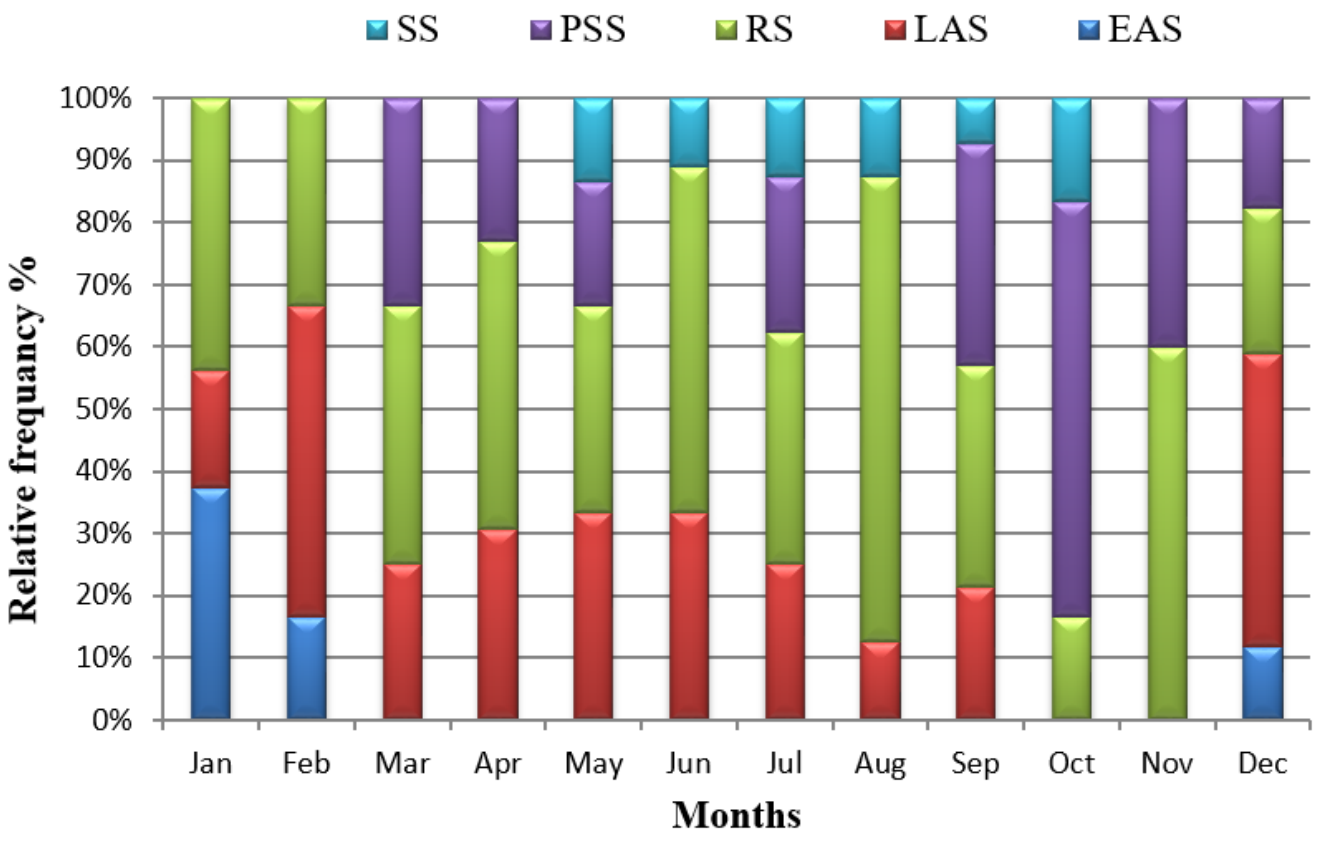

Figure 5

Monthly gonad development of male *P. undulata.*

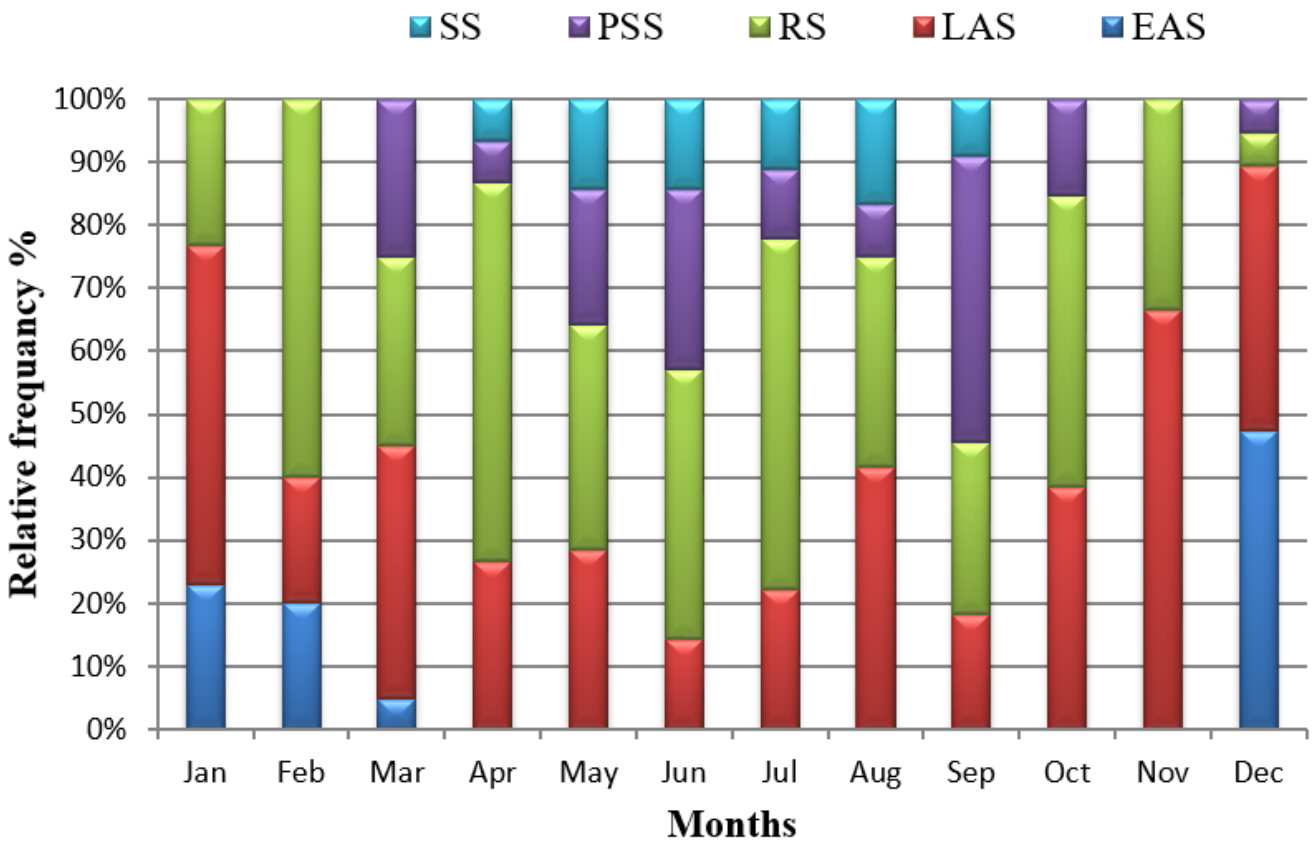

Figure 6

Monthly gonad development of female *P. undulata.*

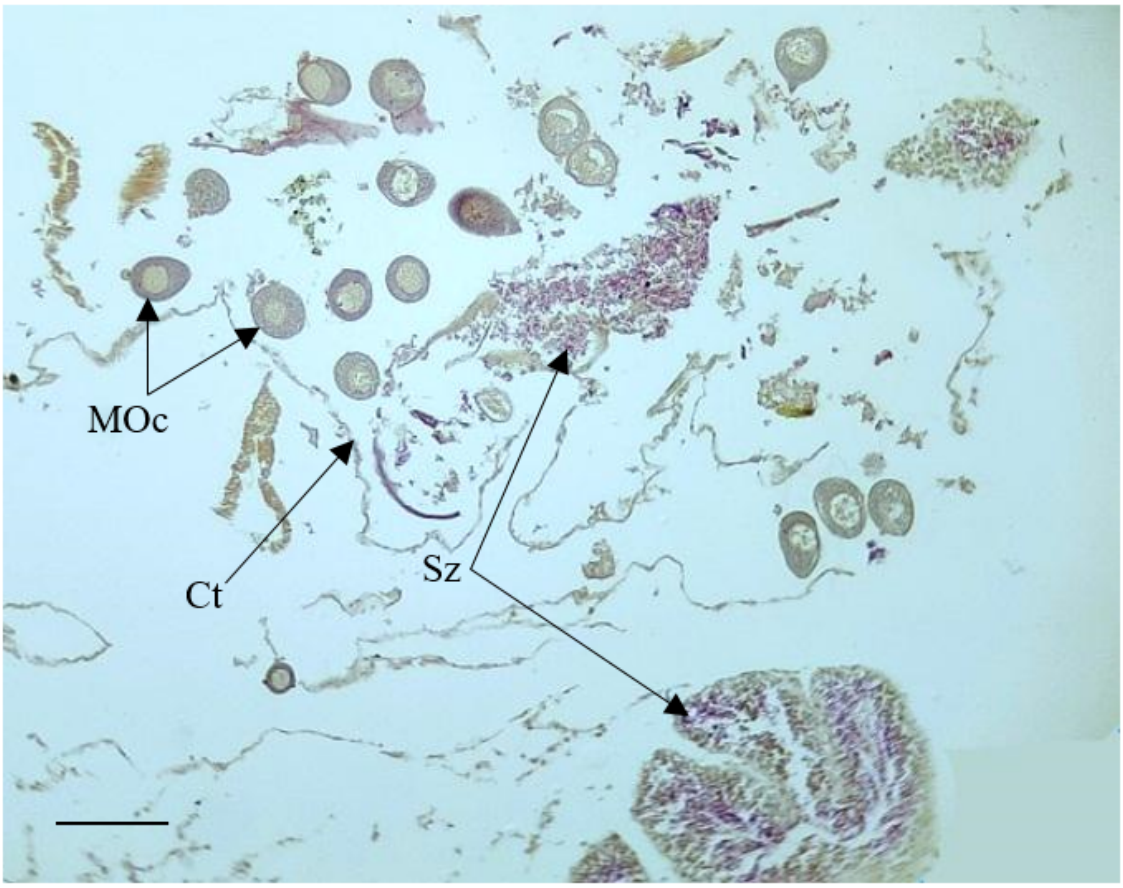

Figure 7

spermatids and mature eggs, respectively, in hermaphrodite *P. undulata* .

Photomicrograph of the hermaphrodite *P. undulata*; Ct: connective tissue; Sz: spermatozoa; MOc: mature oocyte. Scale bar=100μm.