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Reproductive characteristics of the blue swimming crab, *Portunus pelagicus* (Decapoda, Brachyura: Portunidae) from the Bitter Lakes, Suez Canal, Egypt

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ABSTRACT

The reproductive characteristics of crabs play an important role in their fisheries management. The blue swimming crab *Portunus pelagicus* is one of the most commercially important crustaceans distributed along the Egyptian Coast; it has a great demand for their esteemed food. The spawning season of *P. pelagicus* was estimated based on three parameters: the monthly variation in both the maturity stages and in the gonado-somatic index (GSI) beside the histological examination of the ovarian structure. *P. pelagicus* was capable of continuous spawning throughout the year, where the mature stage was found to be distributed along the whole year indicating that crabs may be spawn at different times. *P. pelagicus* spawning mainly takes place twice, January-February (winter period) and June-August (summer period). As the monthly variation in GSI exhibit two distinct peaks in winter and summer. The mean size at which *P. pelagicus* became potentially reproductive (L_{m50}) was estimated to be 9.32 cm CW for males and 8.45 cm CW for females. To achieve the sustainable utilization, it is recommended to increase the minimum fishing size of this crab to at least 11.0 cm CW to protect the stock biomass and allow young individuals to reach the suitable marketable size.

Keywords*:* Bitter Lakes, *Portunus pelagicus*, ovarian histological criteria, Spawning period, Size at maturation

INTRODUCTION

Portunid crabs are a tropical to sub-temperate crustacean group and are widely distributed around the world (Garth & Stephenson, 1966). Portunidae abundance may be differs from one region to another according to the ecology and life history of each species which can be vary extremely with various environmental condition (Zainal, 2013). The blue swimming crab (*Portunus pelagicus*, Linnaeus 1758) is a true crab (Brachyuran) belonging to the family Portunidae. They are found in the Indo tropical coastal to West Pacific region (Ikhwanuddin *et al.*, 2012). *P. pelagicus* is one of the most commercially important crabs along the Egyptian coast and has a great demand for their esteemed food delicacy and also supporting the value of fishery (Redzuari *et al*., 2012). Due to the high demand in local market, the blue swimming crab is heavily exploited and rapidly decreasing in the population size (Kunsook *et al.*, 2014). The crab culture is one of the alternative explanations to meet the increasing demand for crab meat and to reduce the negative effects of over-exploitation (Josileen & Menon, 2004). The population characteristics of *P. pelagicus*, particular the reproductive pattern, are very important input data in the stock assessment and management purpose (Froese, 2004). Extensive studies of blue crab reproductive pattern were undertaken in different area around the world; (Dineshbabu *et al*., 2008) pointed the length at first maturity of *P. pelagicus* from Karnataka coast, India and (Kamrani *et al*., 2010) observed the monthly variation in sex ratio of *P. pelagicus* at the

Persian Gulf. Moreover, Nitiratsuwan *et al.* (2013) and Hisam, *et al.* (2018) pointed on reproductive characteristics of *P. pelagicus* at Thailand. Seasonal variation, sex ratio, length distribution, maturity stages and length at maturity of *P. pelagicus* in the Persian Gulf, Iran were investigated by Zulkifli *et al.* (2017) at Tegal, Central Java. Emam (2011) studied the ecological and population dynamic studies on some crab species in Bardawil Lagoon, Egypt. Abdel Razek *et al*. (2019) studied the spawning and maturation of *P. pelagicus* at the Mediterranean Sea (eastern part).

Although *P. pelagicus* is considered to be a significant economic species, its reproductive biology information is still lacking in Egypt especially in the Bitter Lakes at the Suez Canal Coast. Therefore the present study aims to investigate the blue swimmer crab reproductive biology particularly size at maturity, breeding and spawning seasons. Achieving these objectives can be used as indicators in the proper management of this crab fishery resource in the Bitter Lakes.

MATERIALS AND METHODS

Sampling area

The Great and Small Bitter Lakes are hypersaline lakes that lay between the north and south part of the Suez Canal. They connected to the Mediterranean Sea and the Red Sea via the Suez Canal (Fig. 1). They constitute the Bitter Lakes fishing area and provide an intermediate harbor for ships traversing the Canal. The Bitter Lakes are considered to be the largest water body along the Suez Canal and have a surface area of about 250 km² at coordinates of 30° 20′N 32° 23′E. The depth ranged from 5.0 m to 28.0 m (El-Serehy *et al*., 2018).

Sampling plan

A total of 1562 samples of the blue swimming crab *P. pelagicus* were collected monthly and randomly from the Bitter lakes fishing port at the Suez Canal Coast, during the period from September 2016 to October 2017. In the laboratory, crabs were sexed and separated into males and females based on the morphological differences between them. The carapace width (CW) and total crab weight (Twt) were measured for each sample to the nearest 0.1 mm and 0.1 gm, respectively. Ovaries and testes were extracted and weighed to the nearest 0.01 gm. Gonads were classified into different maturity stages using the scales suggested by De Lestang *et al*. (2003) and Soundarapandian *et al.* (2013) with some modification into three stages as: rudimentary, developing (maturing) and full mature.

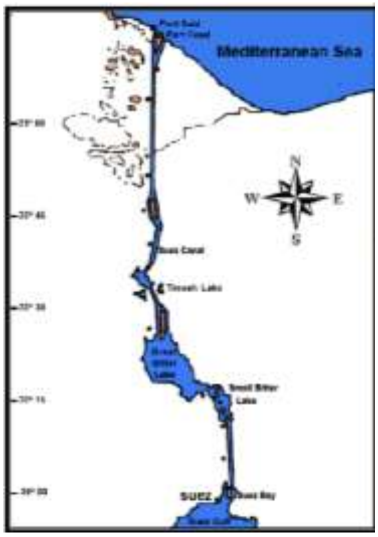


Fig. (1). Map of the Suez Canal including sampling area

Histological studies of ovaries:

The determination of the annual activity of the ovaries of the blue swimming crab, *P. pelagicus*, and the characterization of the developmental stages of the oocyte are fundamental parameter for an efficient management of the crab fishery, so the present study focus on the seasonal variations of the ovaries. Stages of the ovarian maturation were determined based on the histological examination which was studied previously by Quinitio *et al.* (2007) and Ikhwanuddin *et al*. (2012). For light microscopy, small ovarian samples were fixed in freshly prepared aqueous Bouin's fluid for 24 h. After fixation, the samples were dehydrated in ascending grades of ethyl alcohol, cleared in terpineol, and then embedded in pure molten paraplast. Sections of 5-6 µm thickness were cut and mounted on slides and finally were stained with Harris hematoxylin and eosin (Humason, 1972). Thin sections were carefully examined and the photomicrographs were made as required using Sony Cyber-shot digital camera 5.1 MP and measurements were carried out using the eye piece micrometer, calibrated by the slide micrometer.

Gonado somatic index (GSI):
Average GSI was estimated monthly for males and females according to the equation: GSI = Weight of gonads (gm) x100/ Total crab weight (gm).

Spawning period: The spawning times of *P. pelagicus* was estimated from the gonado-somatic index trends exhibited throughout the year, the gonad maturity stages progression, beside the histological examination of the oocyte pattern development.

Length at first maturity (L_{m50}): the size at first sexual maturity (L_{m50}) for males and females crabs was estimated according to King (1995) as the percentage frequency of immature and mature crabs that grouped into 1 cm CW interval and then the maturity curves were fitted to estimate the size at L_{m50}.

Sex Ratio: Sex ratio was found out regarding to all months and sizes. Their chi-square values were recorded to test whether they significantly differ from 1:1 ratio (Snedecor & Cochran, 1967).

RESULTS

The present study is based on 1562 samples of blue swimming crab, *P. pelagicus*; 865 males with a carapace width length ranged from 5.0–16.0 cm and 697 females with CW ranged from 5.0 to 15.0 cm.

Ovaries and testes morphological criteria

Gonad developmental stages of *P. pelagicus* males and females were identified based on the color and the relative size of the gonads in relation to the hepato-pancreas. Moreover, vas deferens was examined in males (Table 1).

Table (1). Gonad developmental stages of male and female blue swimming crab, *P. pelagicus* from the Bitter Lakes.

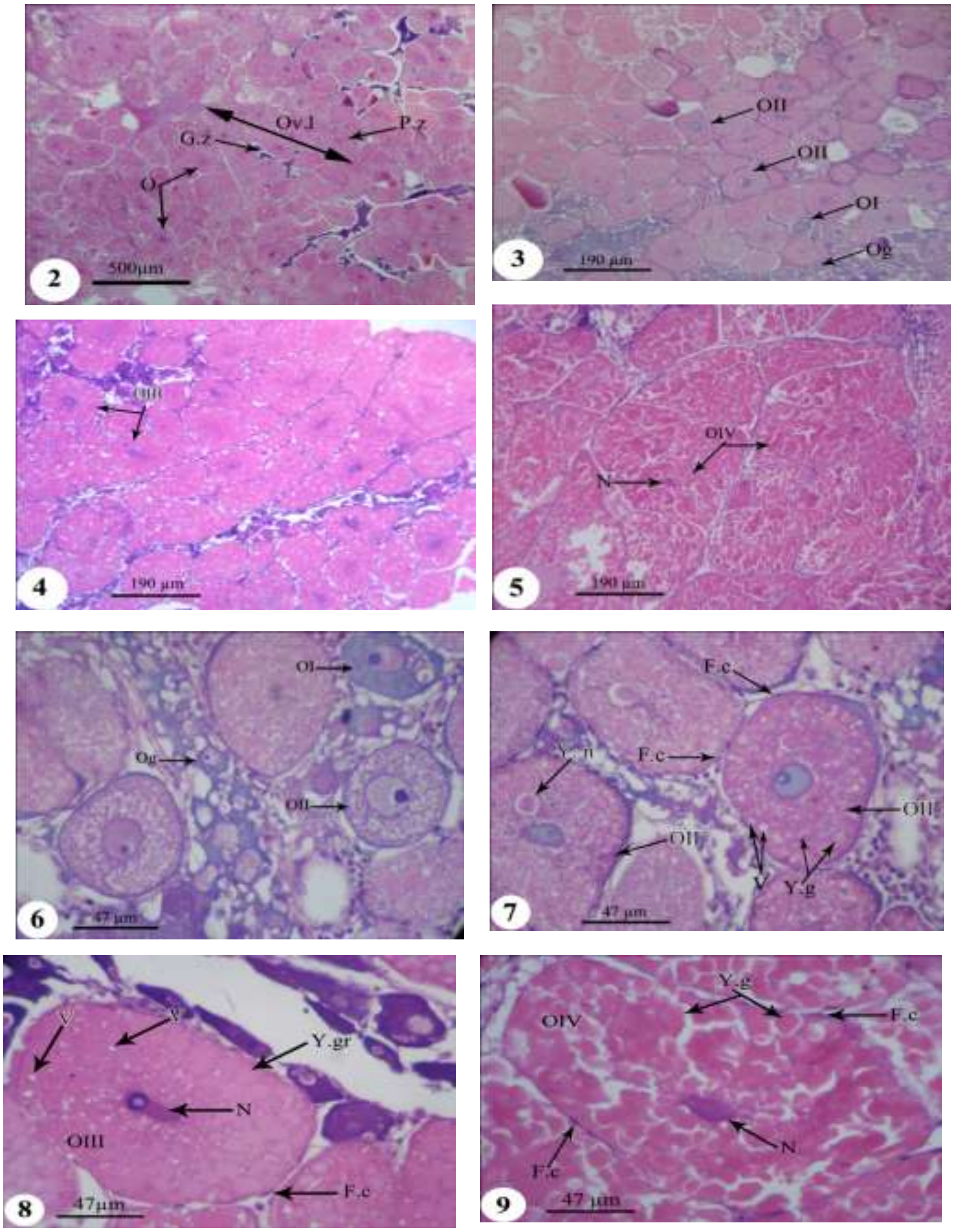
Maturity stages	Males	Females
Rudimentary	Testes are small and creamy or off whit in color. Testes and vas deference are not clearly differentiated.	Ovaries appear as pale yellow or translucent thread like structures and they occupy small area of the body cavity
Developing	Testes are well developed and can be clearly differentiated from the vas deference.	Ovaries are light yellow in color, and somewhat enlarged, but do not extend into hepatic region
Full mature	Testes appear as enlarged and coiled white structures and the vas deference occupies distinct area.	Ovaries are dark yellow/orange with well developed lobes extending into hepatic region and grey egg masses are attached to the pleopods

Ovaries histological criteria

The ovary of *P. pelagicus* is formed of numerous ovarian lobes that encompass the oocytes. Each lobe consists of two zones, namely: the central or germinal and peripheral zones (Fig.2). The germinal zone is mainly built up of oogonia and oocytes I, while the peripheral zone has the oocytes II, III and IV. The ovarian maturity stages of *P. pelagicus* are classified into three stages, namely: the rudimentary, developing and mature stages (Figs. 3-5). Besides, the oogenesis of *P. pelagicus* is divided into five stages. These stages are: the oogonia, oocytes I, oocytes II, oocytes III, oocytes IV. Rudimentary ovary contains oogonia, oocytes I and oocytes II, while developing ovary mainly contains oocytes III. In addition, the mature ovary mainly contains oocytes IV (Figs. 3-5).

The oogonia are more or less rounded in shape with their mean diameter is about 11 µm and have darkly stained cytoplasm. The nucleus of oogonium is rounded (the mean diameter is about 7 µm) and has a prominent nucleolus (Fig. 6). The oogonia are characterized by a high nucleo-cytoplasmic ratio.

The oocyte I is oval in shape with the mean long axis is about 47 µm and has a rounded nucleus and basophilic cytoplasm. The nucleus is rounded with the mean diameter is about 17 µm and possesses a large nucleolus (Fig.6). The oocyte II is mainly oval in shape with the mean long axis is about 90 µm and has acidophilic cytoplasm. The nucleus is rounded with the mean diameter is about 19 µm and possesses a large nucleolus (Figs. 6 &7). The acidophilia of this stage is due to the accumulation of yolk granules. The cytoplasm possesses numerous vacuoles and is characterized by presence of the yolk nucleus. The homogenous cytoplasm gradually increases in volume and the nucleo-cytoplasmic ratio starts to decrease. The oocytes II are enclosed by follicular cells, which are flattened cells with elliptic nuclei (Fig. 7). The oocyte III is oval in shape with the mean long axis is about 122 µm and has an irregular nucleus. The cytoplasm is homogenous and contains numerous large acidophilic yolk granules and vacuoles. In addition, the oocytes III are enclosed by the follicular cells (Fig.8). The oocyte IV is oval in shape with the mean long axis is about 185 µm and is enclosed by the follicular cells. The cytoplasm of this stage is homogenous and contains numerous acidophilic large yolk granules (Fig. 9). The nucleus of the oocytes IV is darkly stained with irregular shape; and it is usually difficult to visualize because the cytoplasm is filled with large yolk granules (Figs. 5 & 9). This stage has a lower nucleus-cytoplasm ratio. The rudimentary stage extends from September to November and from March to May, while the developing stage dominates in December and June. In addition, the mature stage dominates in January, February, July and August.



Figs. 2-9: T. S of the ovary of *P. pelagicus Stained* Hx-E. (Mag. 40X) showing:
Fig.2 : oocytes (O) in the ovarian lobes (Ov.l), germinal (G.z) and peripheral zones (P.z).
Fig. 3: oogonia (Og), oocytes I (OI) and oocytes II (OII) at the rudimentary stage.
Fig. 4: oocytes III (OIII) at the developing stage.
Fig. 5: oocytes IV (OIV) at the mature stage.
Fig. 6: oogonia (Og), oocytes I (OI) and oocytes II (OII) at the rudimentary stage.
Fig. 7: oocytes II (OII) yolk nucleus (Y.n), yolk granules (Y.g) and vacuoles (V) and the follicular cells (F.c) at the rudimentary stage.
Fig. 8: oocytes III (OIII) nucleus, yolk granules (Y.g) and vacuoles (V) and the follicular cells (F.c) at the developing stage.
Fig. 9: oocytes IV (OIV) nucleus (N) and yolk granules (Y.g) and the follicular cells (F.c) at the mature stage.

Monthly variation of Maturity stages with the GSI trend

The monthly variation in maturity stages of the blue swimmer crabs showed that the immature (rudimentary stage), the developing and the mature stages were distributed throughout the year. The mature stage was defined by high percentage in January (50.0%) and in June (49%) in males (Fig. 10); however in females the mature stage exhibited a high percentage in February (55.5%) and in July (48%) (Fig.11).

Monthly average of gonado-somatic index of males ranged from the smallest value 0.13 in May to the biggest one in January (1.34) and in June (1.35). In females the monthly average of GSI ranged from minimum value (1.77) in September to maximum value (6.11) in February and (5.85) in July. The increasing in gonado-somatic index (GSI) value occurred simultaneously in both sexes and coincided with the mature stage value increases, (Figs.10 and 11).

There were two main GSI peaks in both sexes. In males the GSI first peak appeared in January and the second one occurred in June (Fig. 10), while females reached the first highest GSI peak in February and the second in July (Fig. 11). Males reached their highest GSI levels much earlier by one month than females.

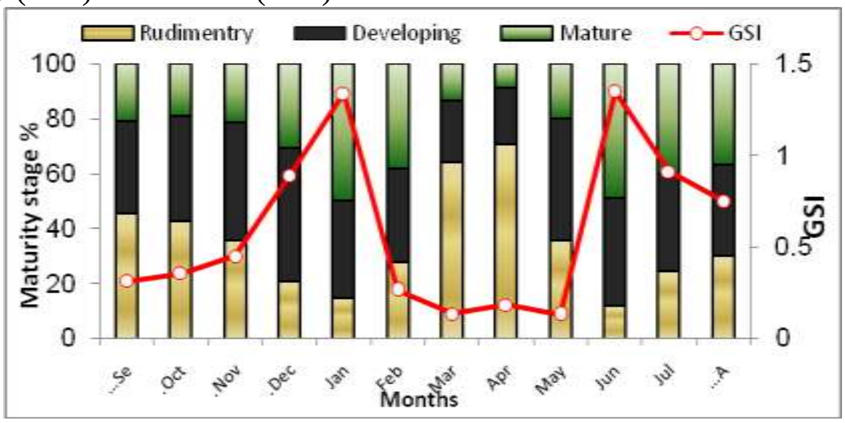


Fig. (10). Monthly variations in maturity stages and the GSI trend in *P. pelagicus* males from the Bitter Lakes

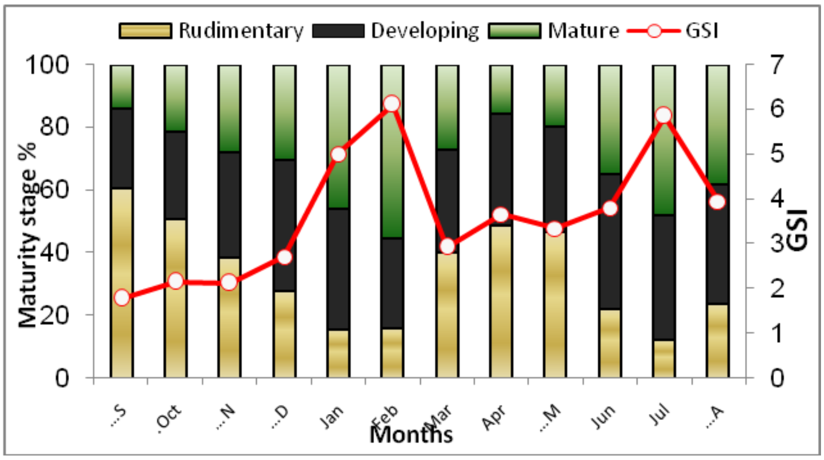


Fig. (11). Monthly variations in maturity stages and the GSI trend in *P. pelagicus* females from the Bitter Lakes

Spawning Season

The spawning season of *P. pelagicus* was estimated based on three changeable parameters: the analysis of the gonad maturation, the monthly variation in the gonado-somatic index (GSI) beside the histological ovarian examination. The present study revealed that the *P. pelagicus* spawning takes place twice through the year, January- February (winter period) and June-August (summer period).

Size at first maturation (L_{m50})

In the present study the smallest observed mature male and female was found to be 5.5 cm and 5.0 cm respectively. Data analysis revealed that males of *P. pelagicus* crabs attained sexual maturity (L_{m50}) when they reached the size range of 9.0-9.9 cm CW with an average of 9.32 cm, however females attained their sexual maturity at length range of 8.0-8.9 CW with an average of 8.45 cm (Fig.12). It is clear that (L_{m50}) of males are smaller than that in females by about 1.1 cm.

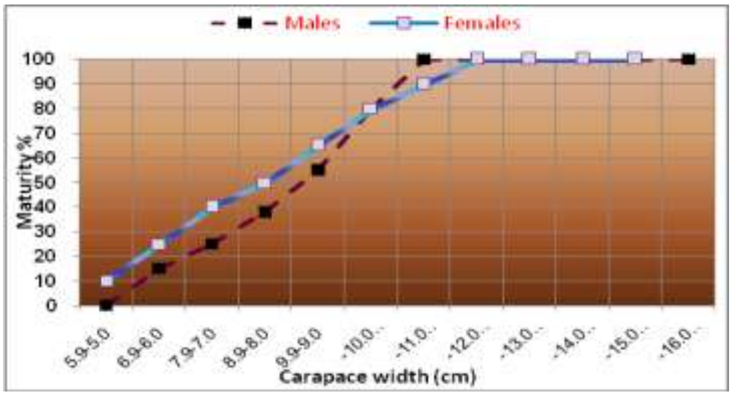


Fig. (12). Size at first maturity of *P. pelagicus* from the Bitter Lakes

Sex Ratio

Total sex ratio of *P. pelagicus* during a year of study was estimated as M: F = 1.24:1 in favor to males. The highest abundant of males was found in November (62%) and May (66%), while the highest percentage of females was found in January, February, June to August, constituting about 50% of the total monthly sampling (Fig. 13).

Table (2). Monthly sex ratio of *P. pelagicus* male to female from the Bitter Lakes

Month	Total samples	Male No.	%	Female No.	%	Sex ratio (M/F)		Chi-square (X^2) value
						♂	♀	
Sep. 016	101	53	52.47	48	47.53	1.104	1	0.248
Oct.	98	54	55.10	44	44.90	1.227	1	1.020
Nov.	201	124	61.69	77	38.31	1.610	1	10.990
Dec.	209	117	56.00	92	44.00	1.272	1	2.990
Jan. 017	**172**	**84**	**48.84**	**88**	**51.16**	**0.955**	**1**	0.043
Feb.	**116**	**57**	**49.10**	**59**	**50.90**	**0.966**	**1**	0.034
Mar.	101	59	58.42	42	41.48	1.405	1	2.861
Apr.	123	68	55.28	55	44.72	1.236	1	1.374
May	128	85	66.41	43	33.59	1.977	1	13.781
Jun.	**103**	**50**	**48.54**	**53**	**51.46**	**0.943**	**1**	0.037
Jul.	**111**	**55**	**49.55**	**56**	**50.45**	**0.982**	**1**	0.009
Aug.	99	59	59.60	40	40.40	1.475	1	3.646
Total	1562	865	55.38	697	44.62	1.241	1	18.069

The sex ratio monthly variation revealed that the ratio of males and females (Fig.13 and table, 2) is tended to be 1:1 in (January, February) and (June, July) months, i.e. during the spawning period, crabs males and females tend to be one to one. Table (2) shows the Chi-Square analysis between males and females through different months. There were no significant difference in all months (X^2 ranged from 0.093 to 13.781and $P>$ 0.05), except in January, February, June and July were exhibited a significant difference (X^2 ranged from 0.009 to 0.043 and $P<0.05$). Chi-Square analysis of the total samples exhibit a non significant difference ($X^2=18.069$ and $P>0.05$).

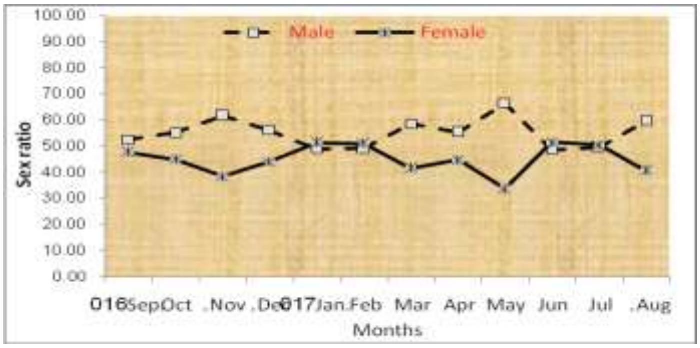


Fig. (13).Monthly variation in Sex ratio of *P. pelagicus* from the Bitter Lakes

DISCUSSION

Blue swimming crabs are commercially and economically crucial in the Egyptian fishery and received extraordinary attentive as an objective seafood resource. Little is distinguished about the reproductive criteria of the blue swimmer crabs at the Bitter Lakes, Suez Canal fishing area. Gonad maturation, gonado-somatic index and the size first sexual maturity are essential tools for effectively estimating the species reproductive criteria. *P. pelagicus* sizes limit observed in the Bitter Lakes were 5.0 to 16.0 cm for males and 5.0 to 15.0 cm for females. Males were larger than females, that reached to 16.5 cm and they were dominated the whole collected samples by 55%. The predominance of male crab may be due to that they are more vulnerable to the fishery, while the female face a fishing stress as it is the highest price in local markets than males and it is the more demand. Zulkifli *et al.*, (2017) premeditated the sex ratio of *P. pelagicus* in central Java, Indonesia and reported the inverse of our results as the female be master the catch by 55% than the male 45%. This is may be due to the difference in sampling time and area.

The histological evaluation of the ovaries of *P. pelagicus* confirms that the oocytes are contained within an ovarian lobe and as the oocytes mature, they move to the periphery of the ovarian lobe, and only oogonia and oocytes I are found closer to the center of the ovarian lobe. This area, where the younger stages originate, is known as the germinal zone or germaria, which is represented as specialized germ cell areas. Similarly, Lee *et al.* (1996) found that the germ cells of various developmental stages were present simultaneously within the ovaries of the blue crab, *Callinectes sapidus*. The oogenesis of *P. pelagicus* is divided into five stages. These stages are the oogonia, oocytes I, oocytes II, oocytes III, oocytes IV. The rudimentary ovary contains oogonia, oocytes I and oocytes II, while the developing ovary mainly contains oocytes III. Moreover, the mature ovary mainly contains oocytes IV. These observations are in accordance with those of Santos *et al*. (2009) in *Armases rubripes* The cytoplasm of oocyte II of *P. pelagicus* is characterized by presence of the yolk nucleus that disappear later by dispersing in the cytoplasm as the oocyte develops further. Moreover, Brown (2009) stated that the peri-nuclear yolk complex of the oocytes of *Callinectis sapidus* aids in the future production of yolk. All oocyte stages, except the oogonia and oocyte I, are surrounded by the follicular cells. This observation is accordance with that of Brown (2009) who stated that the follicular cells are more apparent during the later stages of oocyte development of *Callinectis sapidus*. However, Ando & Makioka (1999) did not observe these follicular cells surrounding the oocytes throughout oogenesis of the freshwater crab, *Potamon dehaani.* Brown (2009) stated that the Tampa Bay blue crab, *Callinectis sapidus*, had a continuous reproductive cycle throughout the year. The previous author added that the mature oocytes of Tampa Bay blue crab occurred more frequently from December to March than that any other time of the year. Moreover, the mature oocytes were also observed in specimens collected during July, August, and September. In addition, October was the only month during this study in which no the mature oocytes were observed. During April to June, oocytes in most females were undergoing primary growth or early secondary growth, and no oocytes in the late secondary growth stage were observed in any of the specimens.

The present study showed that *P. pelagicus* was capable of continuous spawning throughout the year, where the mature stage was found to be distributed along the whole year with different percentage values indicating that females may be spawn at various time through the year. Abdel Razek *et al*. (2019) reported this phenomenon in the *P. pelagicus* from the Mediterranean Sea. In the present study there were two peaks of spawning and breeding season seem to be occur, one through winter (January and February) and the other at summer (June to August). These results disagree with those of Zairion & Achmad (2015) in *P. pelagicus* in the East Lampung Coastal Waters of Indonesia where, they reported that the reproductive pattern was partially spawners, seasonal-continuous throughout in the year with the peak spawning and breeding in April to June and October to November. Hamid *et al*. (2016) and Tureli & Yesilyurt (2017) studied the reproductive biology of the blue swimming crab, in Indonesia and in Turkey respectively, however they concluded that the peak of spawning season seems to be occur three times through the whole year. Differences in results may be related to the difference in the regional water temperature, it is well known that the species reproduction is corresponsive with the changeable in water temperature and high temperature may be induce the spawning activity and vice verse.

The size at the first maturity (L_{m50}) is a fundamental basic parameter in the reproductive biological studies. The maturity size L_{m50} is the common minimum legal size used in many open water crab fisheries but exclusively for female crab, because it is responsible for the seeds production, that development the fisheries resources (Overton & Macintosh, 2002). Fishing pressure may affect marine and crustacean species at population level, e.g. through changing life history parameters such as size at maturity (Sharpe and Hendry, 2009**).** In the current study, the smallest observed mature crab was 5.5 cm CW for males and females. The estimated mean size at first maturation (L_{m50}) of *P. pelagicus* was recorded as 9.32 cm (CW) in males and 8.45 cm (CW) for females. In fishery management, minimum legal size limit is usually determined based on size at maturity, allowing individuals to mate at least once after reaching maturity before they are large enough to harvest in order to protect reproductive potential of the stocks (Stevens *et al.*, 1993)

Table (3), shows the comparing of spawning period and the size at maturity for males and females of *P. pelagicus*, that done by many authors at different regions. It is clear that there was dissimilarity between our finding and the previous studies; however the results appeared to be around the present originating from Bitter Lakes, Egypt. The fluctuation and difference

possibly attributed to the differences in environmental conditions and could be to the difference in the sampling time or to the length range or to the longevity of the species.

Table (3). Size at maturity and spawning period of *P. pelagicus* studied by many authors at different regions.

Region	Size at maturity L_{m50} (CW)		Spawning period	Reference
	Male	Female		
Southwest coast, India	10.5 cm	9.9 cm	Jan, Feb, & Sep	Campbell & Eagles (1983)
Ragay Bay, Luzon, Philippines	----	10.5 cm	Feb-Apr & Jul-Oct.	Ingles & Bkaum (1989)
West coast of Australia	8.8 cm	9.8 cm	Sep & Nov	De Lestang *et al.* (2003)
Serawak, Malaysia	8.5 cm	9.5 cm	---	Ikhwanuddin *et al.* (2009)
South-East Australia	---	9.6 cm	Nov & Dec	Johnson *et al*. (2010)
Persian Gulf & Oman Sea	----	11.3 cm	Sep & Oct	Safaie *et al*. (2013)
East Lampung, Indonisia	9.8 cm	10.3 cm	Mar-Jun & Sep-Nov	Zairion & Achmad (2015)
Lasongko Bay, Indonesia	11.0 cm	11.6 cm	Throughout the year	Hamid *et al.* (2016)
Egypt, Med. Sea	10.76 cm	9.61 cm	Apr-May & Aug-Sep	Abdel Razek *et al.* (2019)
Egypt, Bitter Lakes	9.32 cm	8.45 cm	Jan-Feb & Jun-Aug.	The present study

Conclusion:

The study output exhibits that *P. pelagicus* spawn throughout the year with two distinct spawning peaks occurred in winter and summer. To achieve the sustainable utilization, minimum fishing size of crabs should be limited to at least 11.0 cm (CW) to protect the stock biomass and allow young individuals to reach the marketable size. Also protecting adult crabs must be in consider to provide better spawning chance for females for the good crab fishery management.

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خصائص التكاثر للكابوريا الزرقاء *بروتنيس بلاجيكس* في البحيرات المرة – قناة السويس- مصر

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المستخلص

تلعب خصائص التكاثر الكابوريا دورا" هاما في إدارة مصايده. وتعتبر الكابوريا الزرقاء *بروتنيس بلاجيكس* واحدة من القشريات ذات الأهمية الاقتصادية في السواحل المصرية. ويحدد موسم التزاوج للكابوريا الزرقاء علي ثلاثة أسس وهي : التغير الشهري في مراحل النضوج ،المعامل الجسدي التناسلي وكذلك التغير الهستولوجي لتركيب المبيض. وهي تستطيع التزاوج خلال فترات العام كلها. ويلاحظ وضع البيض خلال مراحل مختلفة من العام و هي تحدث مرتين خلال يناير-فبراير في الشتاء و وخلال يونيو-أغسطس في الصيف حيث يلاحظ قمتين للمعامل الجسدي التناسلي. و قد وجد أن 50% من الكابويا التى تم داستها تصل الى النضوج الجنسى والتزاوج ووضع البيض فى متوسط عرض الدرقة 9.32 سم للذكور و 8,45 سم في الإناث . وتوصى الدراسة بوفع حجم الصيد هذا النوع من القشريات ليكون 11سم للمحافظة علي المخزون منه.