



Reproductive Aspects of Two Octopus Species, *Amphioctopus Aegina* and *A. Membranaceus* in the Gulf of Suez, Northern Red Sea, Egypt

Ayman S. Ahmed^{1,*}, Azza A. El Ganainy¹,
Mohamed F. Osman² and Magdy T. Khalil³

¹National Institute of Oceanography and Fisheries, NIOF, Cairo, Egypt

²Faculty of Agriculture, Ain Shams University, Cairo, Egypt

³Faculty of Science, Ain Shams University, Cairo, Egypt

*Corresponding Author: aahmed.niof@yahoo.com

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ABSTRACT

The reproductive characteristics (sex ratio, maturity stages, gonadosomatic index and length at first sexual maturity) of *Amphioctopus aegina* and *Amphioctopus membranaceus* were reported from the Gulf of Suez, northern Red Sea, Egypt. A total of 833 individuals of *A. aegina* and *A. membranaceus* were dissected and examined between September 2017 and April 2018. The sex ratios (males: females) were 1: 1.03 and 1: 1.46 for *A. aegina* and *A. membranaceus*, respectively. Five maturity stages for both sexes of *A. aegina*, four maturity stages for males and five maturity stages for females of *A. membranaceus* were recorded. Two peaks of spawning have been recorded, the first occurred in March-April and the second peak was in September-October for both species. Dorsal mantle length at first sexual maturity (L_m) was estimated at 6.4 and 7.0 cm for males and females of *A. aegina*, respectively, while for *A. membranaceus*, it was 3.7 and 3.9 cm for males and females, respectively.

INTRODUCTION

The Gulf of Suez (Fig. 1) lies between latitudes 27° 36' and 29° 56' N and longitudes 32° 30' and 33° 30' E. The sides are mostly rocky and bounded by interrupted fringing coral reefs of different widths, but these tend to be narrow towards the north. The bottom of the Gulf is relatively flat, formed of sand as well as a considerable content of silt and clay (REMIP, 2008).

The increasing exploitation of finfish resources and the depletion of a number of major fish stocks that formerly supported industrial-scale fisheries forces continued attention to the once-called 'unconventional marine resources', which include numerous species of cephalopods (Jereb & Roper, 2010). Global cephalopod catches have steadily increased in the last 45 years, from about 1 million metric tons in 1970 to more than 4 million metric tons in 2016 (FAO, 2018). Among the commercial cephalopod catches in

the Gulf of Suez is the octopus species, which is mainly caught as bycatch of the bottom trawl fisheries.

The genus octopus consists of more than 100 species (**Ignatius & Srinivasan, 2006**). From these, few species inhabit the Egyptian waters, and they were reported in many studies, such as; *Octopus robsoni*, *O. horridus* and *O. macropus* (**Adam, 1959**), *O. cyaneus* (**Adam, 1960**), *O. vulgaris* (**Riad & Gabr, 2007**), *O. defilippi* (**El-Ganainy & Riad, 2008**), *O. vulgaris*, *O. macropus*, *O. defilippi*, *O. aegina* and *O. membranaceus* (**Riad, 2008**), *O. vulgaris* (**Ebraheem, 2009**), musky Octopus *Eledone moschata* (**Riad & Kilada, 2012**) and *O. aegina* (**Osman, 2013**).

Biological information on octopuses in the Gulf of Suez is very scarce; only **Riad and Gabr (2007)** studied some morphometric relationships and the natural mortality of *O. vulgaris*; **Riad (2008)** investigated the morphological and taxonomical on some cephalopods; **El-Ganainy and Riad (2008)** examined the population structure of *O. defilippi* and **Osman (2013)** addressed the biological and morphological of *O. aegina*.

The knowledge of the reproductive biology of a marine species is based on the study of its spawning season, for which the reproductive pattern represents a key factor for implementing possible management measures to maintain sustainable exploitation of this resource. The current work was designed to address the lack of information about the reproductive biology of common octopus species in the Gulf of Suez (*Amphioctopus aegina* and *Amphioctopus membranaceus*), including estimation of the sex ratios, gonado-somatic index (GSI) and details of seasonal variation in maturity stage. In addition, this study documented the spawning seasons and the length at first sexual maturity of the species under investigation.

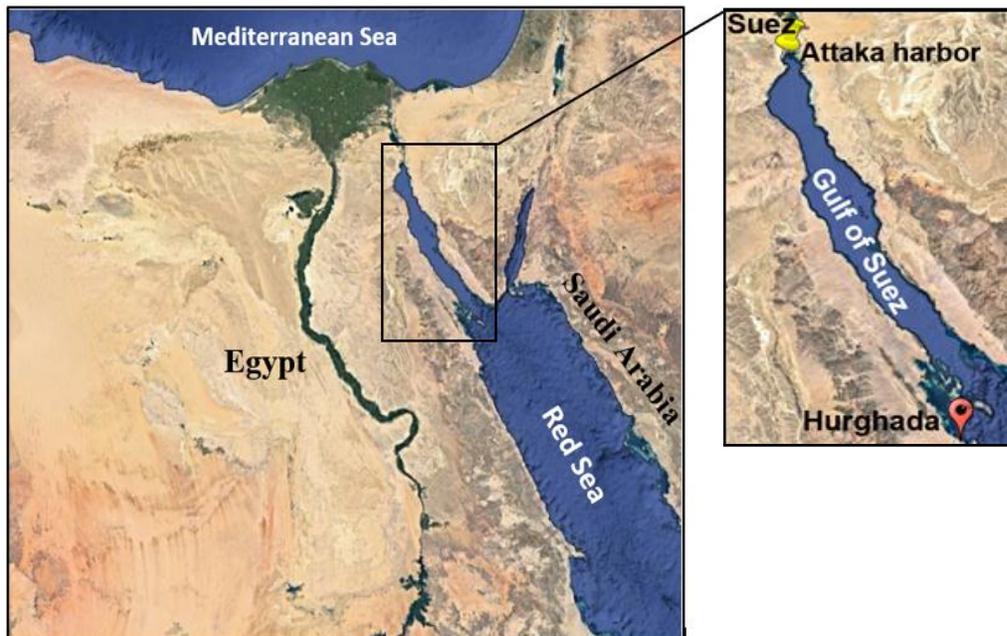


Fig. 1. Map of the studied area (Gulf of Suez) showing the position of Attaka harbor

MATERIALS AND METHODS

A total of 833 individuals (450 of *A. aegina* and 383 of *A. membranaceus*) were randomly collected every month from the commercial fishing trawlers at Attaka harbor, the Gulf of Suez on the northern Red Sea of Egypt (Fig. 1) from September 2017 to April 2018. Since the fishing season closes annually from May to August, the fishing data were not available at these months. Samples were transported to the Fisheries Biology Lab. at the National Institute of Oceanography and Fisheries (NIOF), Suez and Aquba Branch. Individuals were dissected and examined to determine the sex and maturity stage. A single set of a divider, ruler and an electronic balance were used for all samples. All measurements were conducted by one author to avoid unnecessary variations. Samples were weighed to the nearest 0.1gm and their lengths were measured to the nearest 0.1cm, dorsal mantle length. The gonad was weighed (GW) for both sexes to the nearest 0.01g.

The sex was determined by assessing the sexual organs visually. It was calculated as the percentage of males to females (M: F). This ratio was determined according to dorsal mantle length and on a monthly basis to see if there were any variations in sex or not.

Maturity scale used by the IEO C.O. Canaries (Spain) for *O. vulgaris* was based on a general scale defined in the studies of **Guerra (1975)**, **Dia (1988)** and **Perales-Raya (2001)** when assessing maturity stages through the macroscopic examination of the gonads (**ICES, 2010**). This method was used to assess the gonads and their development with increasing maturity, with some modifications. Five stages were defined for both males and females: stage I (immature), stage II (maturing), stage III (mature), stage IV (spawning) and stage V (post-spawning). In males, stages were defined based on size and appearance of the testicles, development of spermatophoric complex and spermatophoric sac and the presence or absence of spermatophores. While in females, the size and color of ovaries, appearance of oviducal glands, the oviducts development, and the presence or absence of fully mature oocytes were recognized to define stages.

Monthly variations in maturity stages were used to determine the spawning season and the occurrence of mature males and females throughout the period of study (Sep. 2017 – Apr. 2018).

The gonado-somatic index is an indirect method for expressing the variation of gonads condition for males and females in different periods of the year. Such parameters are like the percentage of weight of the gonad, whether testicles or ovaries, in the total octopus wet weight. It was calculated using the following equation (**Otero et al. 2007**):
$$GSI = GW * 100 / TW$$

Where: GW is the gonad weight, and TW is the total weight in grams.

To estimate the minimum size at first maturity, individuals were grouped into DML intervals in a 0.5cm- sized group. The percentage of immature and mature stages in each size group was evaluated and plotted against size intervals. The DML, at which 50% of the animals were mature, was taken as the length at first maturity (L_m), and determined

for males and females according to **Pauly (1983)** by plotting the cumulative curve for probability of capture by length.

RESULTS

Sex ratio

In the total samples of *A. aegina* and *A. membranaceus*, the sex ratio (M: F) were 1: 1.03 and 1: 1.46, respectively. The total of 833 individuals (450 of *A. aegina* and 383 of *A. membranaceus*) were collected. Males formed 49.3% and 40.7%, while females formed 50.7% and 59.3% of *A. aegina* and *A. membranaceus*, respectively. The monthly sex ratios are given in Table (1). *A. aegina* males and females were similar during all study months except for September 2017, when males were extremely prevailing. *A. membranaceus* males outnumbered females only in September 2017 and January 2018. However, females were dominant in the other months.

The sex ratio in relation to mid dorsal mantle lengths are displayed in Tables (2, 3), showing that females totally dominated in some dorsal mantle length intervals; 1.8-2.2 cm and from 7.3-7.7 to 9.8-10.2 cm, and both sexes had nearly the same ratio in mid dorsal mantle length interval, recording values from 2.3-2.7 to 6.8-7.2 cm for *A. aegina*. In addition, females dominated totally in dorsal mantle length interval from 5.3- 5.7 to 8.3- 8.7cm, and both sexes recorded nearly the same ratio in mid dorsal mantle length interval (from 2.3- 2.7 to 4.8- 5.2cm) for *A. membranaceus*.

Generally, there is a tendency for more females than males. The sex ratio was not constant throughout the different months and mid dorsal mantle length interval. This means that, males and females are found in different numbers with an obvious deviation from the expected ratio (1: 1). For both studied species, females dominated the large length groups.

Table 1. Monthly variations in sex ratios of *A. aegina* and *A. membranaceus* collected from the Gulf of Suez during the period from September 2017 to April 2018

Month	<i>A. aegina</i>			<i>A. membranaceus</i>		
	Male %	Female %	Sex ratio	Male %	Female %	Sex ratio
Sep. 2017	86.8	13.2	1:0.15	52.3	47.7	1:0.91
Oct. 2017	55.8	44.2	1:0.79	41.1	58.9	1:1.44
Nov. 2017	47.3	52.7	1:1.12	35.2	64.8	1:1.84
Dec. 2017	44.8	55.2	1:1.23	31.3	68.8	1:2.20
Jan. 2018	37.3	62.7	1:1.68	54.8	45.2	1:0.83
Feb. 2018	43.8	56.3	1:1.29	36.4	63.6	1:1.75
Mar. 2018	51.1	48.9	1:0.96	25.7	74.3	1:2.89
Apr. 2018	45.8	54.2	1:1.18	46.7	53.3	1:1.14
Means	49.3	50.7	1:1.03	40.7	59.3	1:1.46

Table 2. Sex ratio in relation to dorsal mantle length for *A. aegina* from the Gulf of Suez during 2017/2018

Mid DML	Total No	Male		Female		Sex ratio
		No	%	No	%	
1.8-2.2	1	0	0.0	1	100.0	0:1
2.3-2.7	4	1	25.0	3	75.0	1:3
2.8-3.2	10	4	40.0	6	60.0	1:1.5
3.3-3.7	14	8	57.1	6	42.9	1:0.75
3.8-4.2	29	15	51.7	14	48.3	1:0.94
4.3-4.7	41	20	48.8	21	51.2	1:1.05
4.8-5.2	58	31	53.4	27	46.6	1:0.87
5.3-5.7	66	43	65.2	23	34.8	1:0.53
5.8-6.2	82	52	63.4	30	36.6	1:0.58
6.3-6.7	63	32	50.8	31	49.2	1:0.97
6.8-7.2	35	13	37.1	22	62.9	1:1.69
7.3-7.7	17	2	11.8	15	88.2	1:7.5
7.8-8.2	11	1	9.1	10	90.9	1:10
8.3-8.7	8	0	0.0	8	100.0	0:8
8.8-9.2	5	0	0.0	5	100.0	0:5
9.3-9.7	4	0	0.0	4	100.0	0:4
9.8-10.2	2	0	0.0	2	100.0	0:2
Total	450	222	49.3	228	50.7	1:1.03

Table 3. Sex ratio in relation to dorsal mantle length for *A. membranaceus* from the Gulf of Suez during 2017/2018

Mid DML	Total No	Male		Female		Sex ratio
		No	%	No	%	
2.3-2.7	7	6	85.7	1	14.3	1:0.17
2.8-3.2	27	17	63.0	10	37.0	1:0.59
3.3-3.7	46	33	71.7	13	28.3	1:0.4
3.8-4.2	86	46	53.5	40	46.5	1:0.87
4.3-4.7	76	36	47.4	40	52.6	1:1.11
4.8-5.2	52	15	28.8	37	71.2	1:2.47
5.3-5.7	25	1	4.0	24	96.0	1:24
5.8-6.2	19	1	5.3	18	94.7	1:18
6.3-6.7	16	0	0.0	16	100.0	0:16
6.8-7.2	16	1	6.3	15	93.8	1:15
7.3-7.7	6	0	0.0	6	100.0	0:6
7.8-8.2	4	0	0.0	4	100.0	0:4
8.3-8.7	3	0	0.0	3	100.0	0:3
Total	383	156	40.7	227	59.3	1:1.46

Maturity stages

Throughout the sampling period (September 2017 to April 2018), Five maturity stages for both males and females of *A. aegina*, adding to four maturity stages for males and five maturity stages for females of *A. membranaceus* were recorded and classified as follows:

Stage I (immature)

Males: Transparent, small whitish testicles and spermatophoric complex, which is small and without spermatophores.

Females: Small homogeneous and white ovaries, transparent and lacking granular structure.

Stage II (maturing)

Males: Creamy-white testicles with visible structure, distinguished from the spermatophore sac. Few spermatophores can be seen.

Females: White-yellowish ovaries with granular structure clearly visible and fine granular appearance. Oviducal glands distinguishable from the oviducts.

Stage III (mature)

Males: Large, creamy-white testicles. Numerous spermatophores in the spermatophore sac and in the spermatophoric duct.

Females: Large, more voluminous ovaries, white-yellowish in color and thick granular appearance. Oviducal glands tend to be light orange in color.

Stage IV (spawning)

Males: Very large testicles, creamy in color and spermatophore sac full of spermatophoris.

Females: Very big, developed and creamy-yellow ovaries. Oocytes highly developed and oviducal glands with maximum development.

Stage V (post-spawning)

Males: Heterogeneous and flaccid testicles without spermatophores or with few spermatophores stored.

Females: Small, shrunk and flaccid ovaries with no eggs inside. Oviducal glands similar than in maturity stage IV but smaller.

Spawning season

For *A. aegina* males, immature individuals (stage I) occurred in all months except April. Stage II (maturing) occurred in all fishing months, and the maximum numbers was

recorded in November. Mature individuals (stage III) were dominant in all fishing months, except April during which they appeared in small numbers, while spawning (stage IV) appeared in September and from January to April. Stage V (post-spawning) was recorded in October and March-April (Fig. 2). For *A. aegina* females, the maximum numbers of immature individuals (stage I) occurred in all fishing months during 2017/2018 except during September. Stage II (maturing) and stage III (mature) appeared with few numbers in all fishing months except September. Spawning (stage IV) was recorded in all months except November. It should be mentioned that, all individuals collected in September were in spawning condition (stage IV). Stage V (post-spawning) was found in October, March and April (Fig. 2).

For *A. membranaceus* males, immature individuals (stage I) occurred in all months except December, February and April. Stage II (maturing) appeared in all fishing months and was dominant in December. Mature individuals (stage III) were recorded in all fishing months but were absent in November and December, while spawning (stage IV) just occurred in few numbers in October (Fig. 3). For *A. membranaceus* female's immature individuals (stage I) were recorded in all fishing months during 2017/2018 except September and April. Stage II (maturing) occurred in all fishing months. Stage III (mature) appeared from September to April but disappeared in March. Spawning (stage IV) occurred during September-October and from February to April and appeared slightly in January and disappeared in November-December. While, stage V (post-spawning) was found slightly in March-April and strongly in September (Fig. 3).

Generally, maturation and spawning for males and females of *A. aegina* and *A. membranaceus* approximately occur throughout all months, with a greater reproductive activity in March-April. Besides, there is a second spawning period in September-October.

Gonado-somatic index (GSI)

By studying the GSI for *A. aegina* and *A. membranaceus*, it was found that there was a big difference between the values of GSI for males and those of females, this is because the testicles weight was less than the ovaries weight. The values of GSI for *A. aegina* males were nearly similar in all fishing months, and the highest values were recorded during March-April, while the lowest values were shown in October-November. The highest values of GSI for *A. aegina* females were recorded in September and March-April, while the lowest value was in November (Fig. 4).

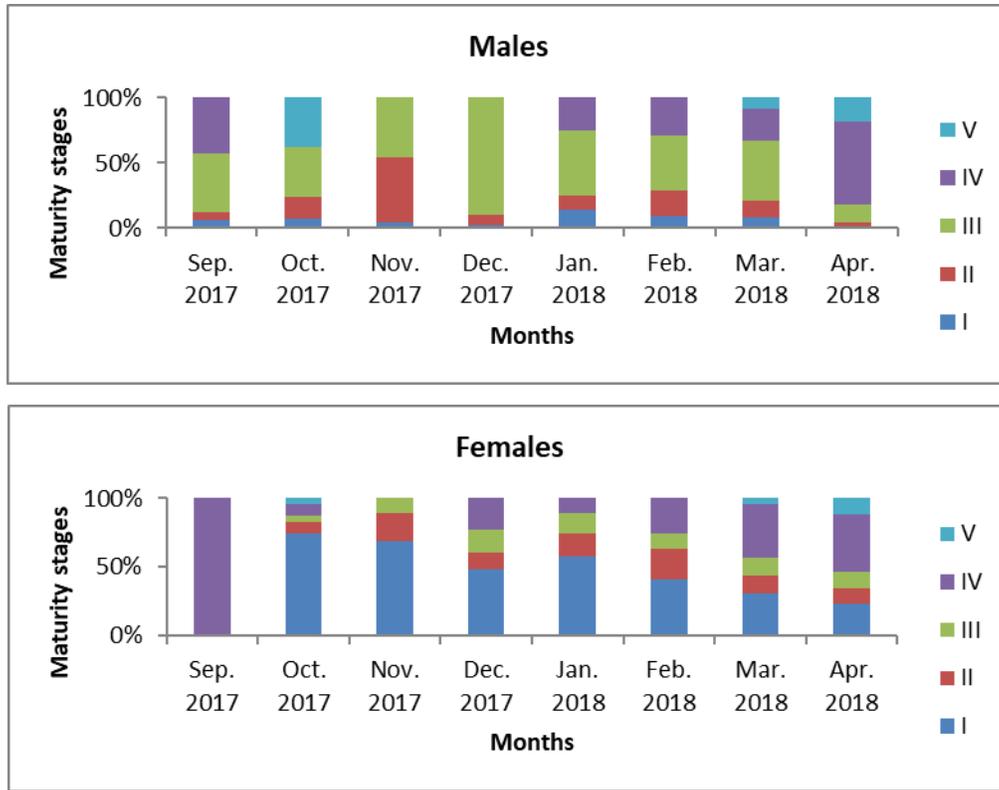


Fig. 2. Monthly distribution of different maturity stages for males and females of *A. aegina*

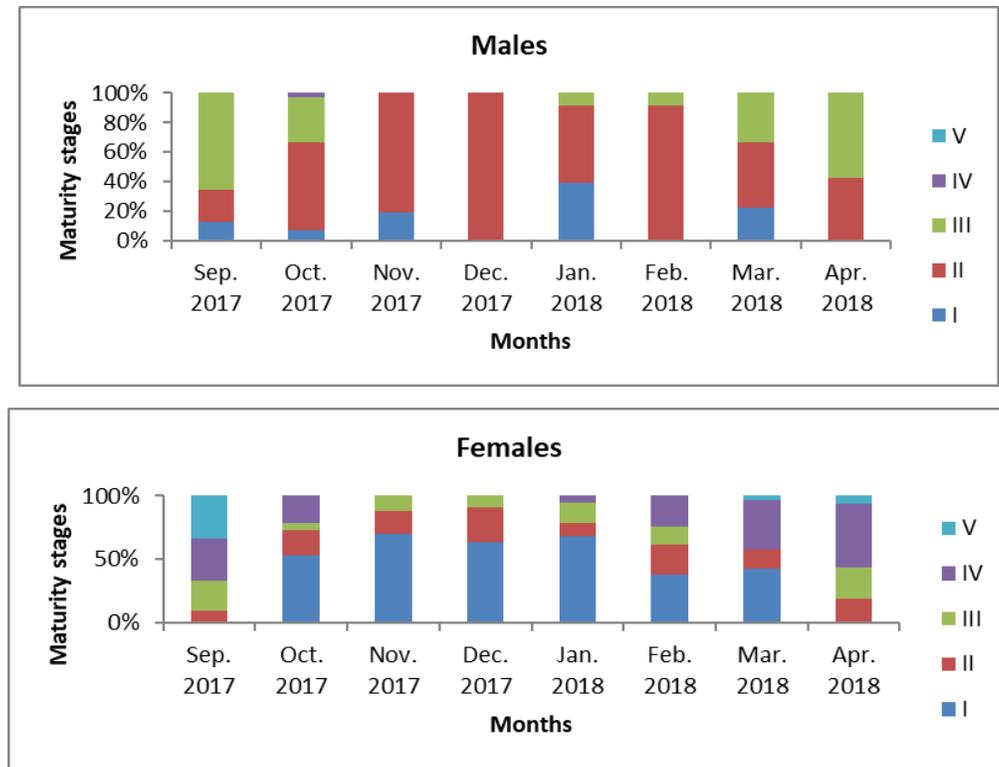


Fig. 3. Monthly distribution of maturity stages for both sexes of *A. membranaceus*

The values of GSI for *A. membranaceus* males were similar steadily in all fishing months, and the highest values were recorded in February, March and April, whereas the lowest value was in January. On the other hand, the highest values of GSI for *A. membranaceus* females were recorded during September and March-April, whereas the lowest values were registered during November-December (Fig. 5). These results revealed that the peak of spawning season occur in March-April, and there is a second spawning period in September-October.

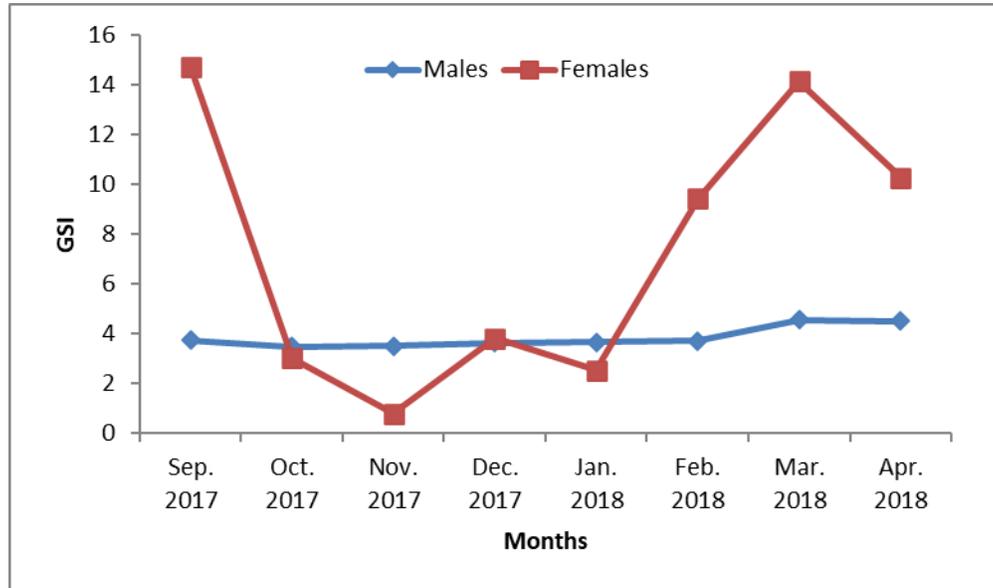


Fig. 4. Gonad-somatic index of *A. aegina* from the Gulf of Suez

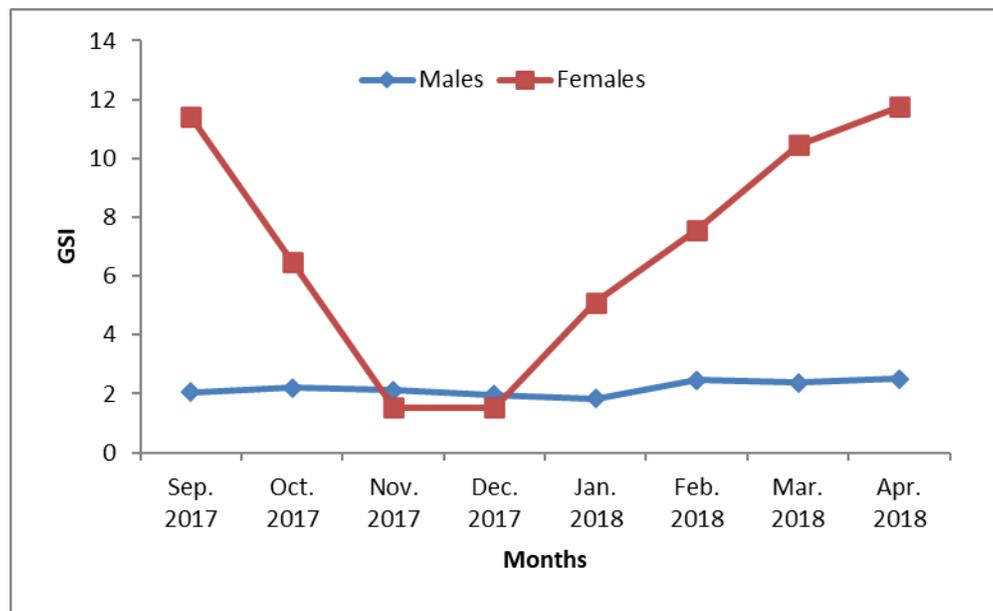


Fig. 5. Gonad-somatic index of *A. membranaceus* from the Gulf of Suez

Length at first sexual maturity (L_m)

L_m is defined as the length at which 50% of the individuals are maturing or mature (Bakhayokho, 1983). L_m of *A. aegina* and *A. membranaceus* were studied by grouping both males and females in different mid lengths into immature and mature individuals. Figs. (6, 7) illustrate the relationship between the proportion of mature stages and DML of *A. aegina* and *A. membranaceus* males and females. Figs (6,7) are based on 185 and 211 individuals collected throughout the sampling period of *A. aegina* and *A. membranaceus*, respectively. The data indicate that the males of *A. aegina* and *A. membranaceus* mature at smaller size (L_m) and attain a smaller adult length than those (size & length) of the females. Additionally, the males and females of *A. membranaceus* mature at smaller size (L_m) and attain a smaller adult length, compared to the *A. aegina*. The mean length at which 50% (L_m) of *A. aegina* maturity was 6.4 and 7.0 cm for males and females, respectively. For *A. membranaceus*, the mean length at which 50% (L_m) of maturity was 3.7 and 3.9 cm for males and females, respectively.

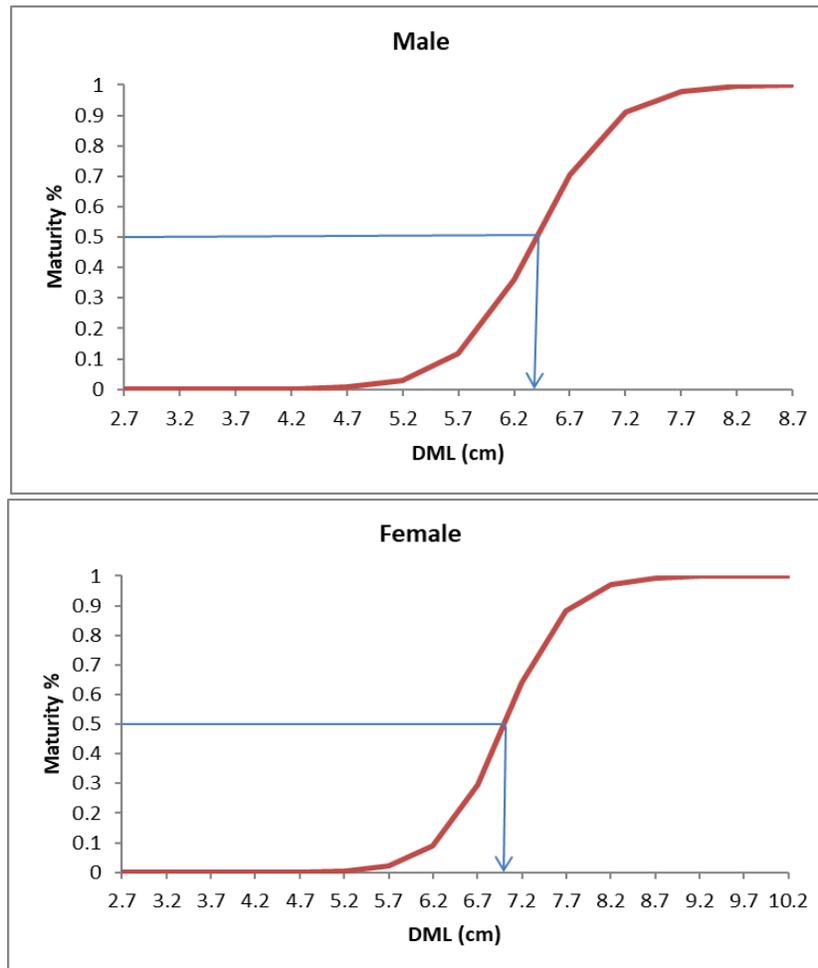


Fig. 6. The length at first sexual maturity (L_m) for both sexes of *A. aegina* collected from the Gulf of Suez

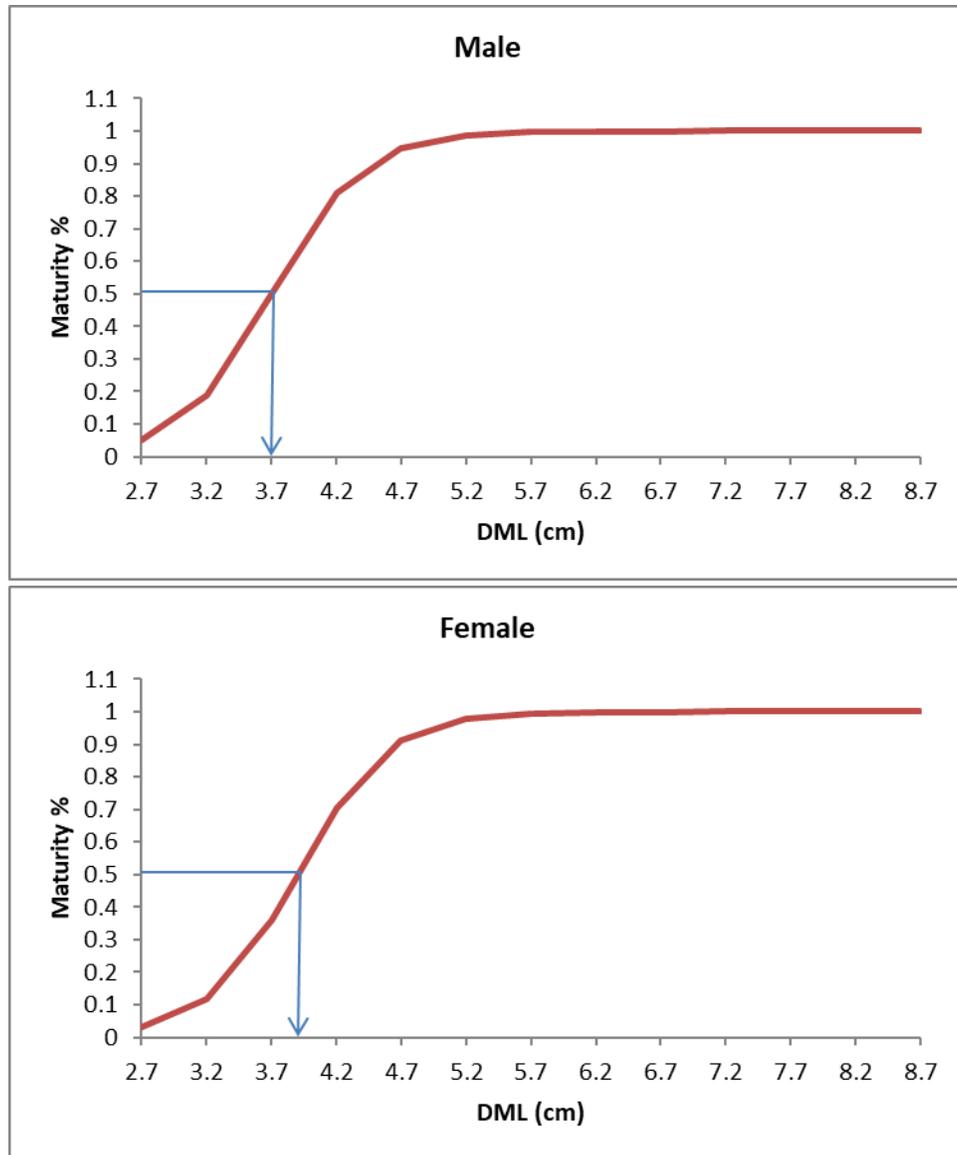


Fig. 7. The length at first sexual maturity (L_m) for both sexes of *A. membranaceus* collected from the Gulf of Suez

DISCUSSION

The sex ratio result of this study agrees with that recorded in the study of **Yedukondala and Mohana (2013)** who studied the monthly sex ratio of *Octopus membranaceus* from Visakhapatnam, East coast of India and reported the dominance of females over males (1.54: 1) during most of the study period except June, August and November; however, it disagrees with that of **Osman (2013)** who studied the sex ratio of *O. aegina* in the Suez Canal, the Gulf of Suez and the Mediterranean Sea; the overall ratio of males to females was 1.5: 1, 1: 1 and 1.5: 1, respectively. **Ignatius et al. (2011)** addressed the monthly sex ratio and the length group (4-10 cm) of sandbird octopus, *Amphioctopus aegina* from Mandapam coastal waters (Palk Bay), southeast coast of

India, where the sex ratio of males to females was 1.71: 1. Moreover, **El-Ganainy and Riad (2008)** studied the sex ratio of *O. defilippi* in the Suez Gulf, and the ratio of males to females was 1: 0.5. The same trend of sex ratio was recorded in the studies of **Hernandez-Garcia *et al.* (2002)** and **Cuccu *et al.* (2013)** for *O. vulgaris* in the Mediterranean Sea. On the contrary, **Garcia-Martinez *et al.* (2018)** revealed that both sexes of *O. vulgaris* in the northern Alboran Sea, western Mediterranean, were present in almost equal ratios 1: 1. The differential sex ratio of octopus is more or less similar to that recorded in previous observations. Generally, the ideal 1: 1 (male: female) ratio rules do not exist in nature as seen from many earlier findings.

The results of monthly variations in maturity stages are more or less similar to that of **Osman (2013)** who studied the reproductive biology of *O. aegina* from the Suez Canal. Three maturity stages for males and females were visually recognized; immature (I), maturing (II) and mature (III). Male maturity cycle starts in summer with the first occurrence of immature individuals (3.57%), and gradually increasing in maturing stages from summer (67.86%) to winter (100%). Spring was the season in which all males were mature (100%). Winter had the highest percentage of immature females (65.52%), followed by the highest percentage occurrence of maturing stage in spring (87.50%). No mature females were found; however, the successive increase of the maturing stage from winter (68.97%) to spring (87.50%) may imply the presence of mature females in spring and summer seasons. Additionally, **Ignatius *et al.* (2011)** studied the reproductive traits of *A. aegina* from Mandapam coastal waters (Palk Bay), southeast coast of India. Four maturity stages were recognized for females and two for males. Maturation and spawning occur all year round; greater reproductive activity occurred from July to February with a peak during October and another peak during January-February. Furthermore, **Yedukondala and Mohana (2013)** in Visakhapatnam, the east coast of India found that the females of *O. membranaceus* maturity were categorized into four stages; immature (I), maturing (II), mature (III) and spent (IV), while spawning takes place throughout the year with a peak in September.

Moreover, **Cuccu *et al.* (2013)** identified six stages of sexual maturity (immature, developing, maturing, mature, spawning and spent) for both sexes of *O. vulgaris* from the Sardinian waters, the Mediterranean Sea. **Garcia-Martinez *et al.* (2018)** stated that, the spawning of *O. vulgaris* in the northern Alboran Sea, western Mediterranean occurs during June, July and August. In addition, **Riad and Kilada (2012)** studied the reproductive biology of the musky octopus, *Eledone moschata* in the eastern Mediterranean, and they found the peak fecundity occurring during winter, followed by a progressive decline in spring through fall. Males matured faster than females, as more males (about 60%) were fully mature during spring than females, whereas only about 30% of the females were fully mature. It is clear that fully mature individuals were observed throughout the year for males and females.

For the GSI, a great difference was detected between the gonad index in males (3.48-4.55) and that in the females (0.8-14.72) of *A. aegina*, while, values of 1.83-2.51 were recorded for males and 1.53-11.75 for the females of *A. membranaceus*. These differences may be due to the variation between the species and the gonad weight of males and females. Both sexes of *A. aegina* and *A. membranaceus* have a breeding season, which may extend from March to April (the first peak); it may extend through May, June, July and August to September and October during the second spawning period; however, the lack of samples in these months (summer) due to the closed season makes it difficult to confirm these data. These results agree with those of **Osman (2013)** who noticed that, the male of *A. aegina* had the highest average GSI during spring and autumn in the Suez Canal and Mediterranean Sea, while it reached its highest value during summer in the Gulf of Suez. Moreover, the highest seasonal average GSI of females was recorded during spring and summer in the Suez Canal and during all seasons in the Gulf of Suez and during the summer in the Mediterranean Sea. Many studies recorded the same trends in different locations of *O. vulgaris* (**Silva et al., 2002; Smith & Griffith, 2002; Rodriguez de la Rua et al., 2005; Otero et al., 2007; Gonzalez et al., 2011**). Furthermore, **Ignatius et al. (2011)** reported that, in males, no definite seasonal changes were observed in GSI values. In females, there were two peaks in GSI values during October and January-February for *A. aegina* from Mandapam coastal waters (Palk Bay), the southeast coast of India.

The results of L_m are similar to those recorded in the work of **Osman (2013)**, who estimated the size at first sexual maturity of *O. aegina* with 7.0 and 6.0 cm DML for males in the Gulf of Suez and the Mediterranean Sea, respectively. No mature females have been recorded in these two sites. While, these findings disagree with the results of the same species in the Suez Canal. The size at maturity was 8.0cm DML for males. Due to lack in female samples of more than 12.0cm DML, the size at maturity for females was suggested to be 14.0cm DML. In addition, these results are more similar to those of **El-Ganainy and Riad (2008)** who found that $L_m = 6.0$ cm for the combined sexes of *O. defilippi* in the Gulf of Suez. In this respect, **Ignatius et al. (2011)** illustrated that, the smallest mature specimen collected in the sample of *A. aegina* from Mandapam coastal waters (Palk Bay), southeast coast of India, was 4.8cm DML in females and 3.6cm DML in males. All the males were immature up to 3.4cm DML. The maturity percentage increased gradually from 3.5- 3.9cm, and 100% mature animals were found above 7.5-7.9cm DML. The size at first maturity was estimated at 5.70cm DML for males. For all females, <4.4 cm DML were immature at 6.5- 6.9cm DML. There was a sudden increase in maturity, and by 8.5cm nearly 94% of females were mature. The size at first maturity was estimated at 7.17cm DML for females. While, **Yedukondala and Mohana (2013)** mentioned that the mean length at which 50% of the individuals attained maturity was 22.5cm in the females of *O. membranaceus* from Visakhapatnam, the east coast of India, which is much higher than that recorded in the Gulf of Suez (present study).

Length at first sexual maturity (L_m) in this study was more or less similar with that recorded in many other regions. This may be due to the influence of environmental conditions such as temperature, salinity, food availability and water quality factors. In general, the variation to some extent of maturity stages, spawning seasons, GSI and L_m could be attributed to the environmental conditions, growth, mortality, recruitment and fishing pressure in each region. This study provided some important information on the reproductive aspects of *A. aegina* and *A. membranaceus* from the Gulf of Suez, northern Red Sea of Egypt which will be helpful in developing strategies for the management and conservation of these species.

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