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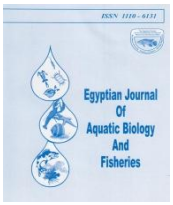

Reproductive Biology of *Callistoctopus macropus* from the Egyptian Mediterranean Waters

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ABSTRACT

Throughout the current study, sampling of *Callistoctopus macropus* was achieved monthly from January to December 2019. The study examined various biological characteristics, including sex ratio, fertility, length at first maturity, maturity stages, gonado-somatic index, hepato-somatic index and fecundity. The fecundity of *Callistoctopus macropus* was addressed; the number of the eggs ranged between 205682 eggs for females, with an average mantle length of 8.5cm to 61946 eggs for females, with an average mantle length of 14.5cm; they were observed to be fertile. Fecundity generally rose with increasing mantle length, yet there were dissembled differences in the fecundity of the same female mantle length. For males and females, the length values at the initial maturity were 7.8 and 8.3cm, respectively. There are four stages of maturity for females: immature, maturing, ripe and spent in addition to three stages for males: immature, ripe and spent. From March to July, there were plenty of ripe males; and from March to August, there were enough females. According to the gonado-somatic index data, the female's spawning season lasts from June to October, whereas the male's spawning season is from June to September. Hepato-somatic index data values for both sexes reach their peak before the spawning season, however, during the spawning season, the lowest values were noted. These could result from the digestive gland's lipid reserves being depleted over the protracted spawning and breeding season. Males outnumber females throughout the year except March and August. The ratio between males to females was 1.8:1.

INTRODUCTION

Two suborders, eleven families, roughly forty genera and about two hundred species make up the order Octopoda **(Nesis, 1987)**. In general, octopuses made up 14.6% of the world's cephalopod fishery's total catch; cuttlefish made up 13.6%, and squids made up 71.8% **(Roper *et al*., 1984)**. According to **Riad (2022)**, there are four octopod species living in the Egyptian Mediterranean waters, *Callistoctopus macropus* is one of them, and the other three are *Octopus vulgaris, Eledone moschata,* and *Macrotritopus defilippi*. The Red Sea and the Mediterranean waters in Egypt are home to *Callistoctopus macropus* **(Riad, 2020)**.

In worm seas, *Callistoctopus macropus* is found worldwide **(Roper *et al*., 1984)**. It is prevalent in diverse regions, including the Gulf of Aqaba, the Central and Western Pacific Oceans, the Indian Ocean, and the North Atlantic **(Adam,**

1960), the Sea of Adrian **(Riedle, 1970)**, the Mediterranean regions-western and eastern, and the Coast of North Africa **(Fischer, 1973)**. Based on the information provided by **Anon (1987, 1998)**, octopuses account for 2.12% on an average of all fish landings. Seldom of these refers to other species. An interest in well, fishing for this species, there are no available catch statistics. It probably makes up a minor percentage of *Octopus vulgaris* catch records in the Mediterranean **(Jereb *et al*., 2014)**. According to **Fischer (1973)**, the species are found in holes and crevices on rocky bottoms and occasionally on vegetative substrates.

Callistoctopus macropus's biological cycle is poorly understood, and up until recently, accurate information on spawning, embryonic development, and post hatching lifecycle is lacking **(Hochberg *et al*., 1992)**.

MATERIALS AND METHODS

During the months of January through December 2019, *Callistoctopus macropus* samples were collected every month from fishing trawlers operating in the Egyptian Mediterranean waters; the samples were collected from the muddy and sandy bottoms in Sidi Abd El Rahman, west of Alexandria, to Rosetta, east of Alexandria (Fig. 1). Octopod specimens totaling 327 (209 males and 118 females) were gathered. Up until they were processed, every animal was kept frozen following thawing at room temperature; the total body weight (T.WT.) was recorded to the nearest gram, and the dorsal mantle length (ML) was measured to the nearest 0.1cm. In the lab., samples were inspected while they were still fresh.

Fig. 1. The Egyptian Mediterranean Sea (Google Earth)

RESULTS

I- Fecundity

Fecundity is a key concept in the study of octopus reproduction.Its modification seems to be a part of the system regulating population density, making it an adaptive characteristic **(Begnal, 1960, 1963)**. Individual female octopuses may experience direct environmental impacts leading to variations in fecundity

(Scott, 1962; Hodder, 1963). The information in Table (1) illustrates the assessment of fecundity with mantle length. The linear model equation (Fig. 2) was the most appropriate for characterizing the relationship [Y=5673.5X-24839]. Where, Y= Fecundity (eggs no.), and X= Mantle length in cm.

The resulting curve is displayed in Fig. (2). For *Callistoctopus macropus*, the observed fecundity varied from 205682 to 61946 eggs (Table 1) per female. On the other hand, a significant difference in fertility was noted across the individuals of the same mantle length. The predicted fertility of *Callistoctopus macropus* spans from 205682 eggs for female, with an average mantle length of 8.5cm, to 61946 eggs for female, with an average mantle length of 14.5cm. In general, we can observe an increase in fecundity with increasing mantle length (Table 1 & Fig. 2).

Table 1. Evaluation of fecundity with mantle length for *Callistoctopus macropus*

Examined sample	Mantle length group in cm	Average observed fecundity	Calculated fecundity
4	8.5	205682	23385
14	9.5	29750	29059
28	10.5	31374	34732
24	11.5	35924	40406
4	12.5	46500	46079
4	13.5	51820	51763
10	14.5	61946	57426

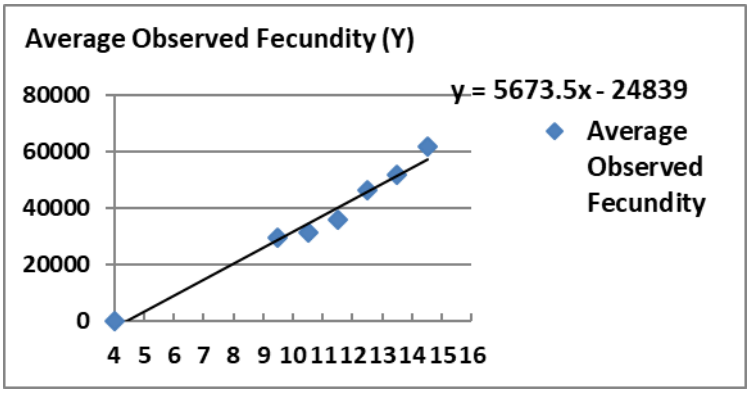


Fig. 2. Relation between mantle length and fecundity for *Callistoctopus macropus*

II- Maturity stages

According to **Mangold (1983a, b)** and **Whitaker (1991)**, maturity stages were classified into three maturity stages and four maturity stages for males and females, respectively.

A- Maturity stages for males

There are three maturity stages for males *Callistoctopus macropus*:

1- **Immature:** The testes are clearly identifiable from the outside and the Needham's sac has no spermatophores.
2- **Ripe:** The Needham's sac contains spermatophores, which range in size from 1 to 3.5cm.
3- **Spent:** Spermatophores in Needham's sac are extremely few.

B- Maturity stages for females

There are four maturity stages for females *Callistoctopus macropus*:

1- Immature: There are no ovisac eggs in the tiny, whitish ovaries.
2- **Maturing:** The ovaries generally turn yellow and grow larger very quickly.
3- Ripe: Loose eggs in ovisac are found in the large ovaries.
4- Spent: The ovaries have a bright color and are contractile.

Monthly variations in maturity stages

Tables (2, 3) summarize the monthly fluctuations in the developmental stages of *Callistoctopus macropus* for males and females, respectively, over the course of the year, whereas Fig. (3) provides a graphical representation of the data. The abundance of ripe males and female's fish in the capture peaked from March to July for males and from March to August for females (Tables 2, 3 & Fig. 3).

Ripe males disappeared around August, whereas ripe females were absent between September and November. The spawning and spent conditions for males also coincided with the presence of immature females (Tables 2, 3 & Fig. 3). Males immature individuals are highly represented from March to December, while females immature individuals are well represented from August to December (Tables 2, 3 & Fig. 3). Female individuals in the maturing period are well represented from January to June (Tables 2, 3 & Fig. 3).

III- Length at first maturity

The practical use of initial sexual maturity size information is in determining the minimum acceptable size that may be required to protect a spawning portion of an octopus's population over the course of the year. The maturity of *Callistoctopus macropus* was investigated. Table (4) provides the percentages of mature *Callistoctopus macropus* at 0.5cm. Mantle length intervals for both sexes are visually illustrated in Fig. (4), depicting with mantle length. According to the statistics, every *Callistoctopus macropus* individual that is smaller than 6cm in length in males and 7.5cm in females is regarded as immature. For males and females, the smallest mature sizes were 6 and

7.5cm, respectively. According to **Snedcor (1956)**, the time at which 50% of the animals under study develop a certain trait can be interpreted as the time at which that trait first appears. This means that for males *Callistoctopus macropus*, the length at first maturity is reached at a length of 7.8cm, and for females, it occurs at 8.3cm (Fig. 4).

Table 2. Monthly variations of maturity stages of *Callistoctopus macropus* males

Month	Sample NO.	Immature		Ripe		Spent	
		No.	%	No.	%	No.	%
January	23	5	21.74	13	56.52	5	21.74
February	-	-	-	-	-	-	-
March	9	3	33.33	6	66.67	-	-
April	12	-	-	5	41.67	7	58.33
May	6	-	-	3	50	3	50
June	11	-	-	7	63.64	4	36.36
July	8	-	-	4	50	4	50
August	4	1	25	-	-	3	75
September	15	6	40	3	20	6	40
October	39	21	53.85	11	28.21	7	17.95
November	28	10	35.71	11	39.29	7	25
December	54	20	37.04	26	48.15	8	14.81
Total	209	66	31.58	89	42.58	54	25.84

Table 3. Monthly variations of maturity stages of *Callistoctopus macropus* females

Month	Samples no.	Immature		Maturing		Ripe		Spent	
		No.	%	No.	%	No.	%	No.	%
January	10	-	-	4	40	3	30	3	30
February	-	-	-	-	-	-	-	-	-
March	13	-	-	3	23.1	9	69.2	1	7.7
April	4	-	-	2	50	2	50	-	-
May	2	-	-	-	-	1	50	1	50
June	7	-	-	3	42.9	4	57.1	-	-
July	6	-	-	-	-	3	50	3	50
August	7	3	42.9	-	-	3	42.9	1	14.3
September	-	-	-	-	-	-	-	-	-
October	15	8	53.3	-	-	-	-	7	46.7
November	11	6	54.5	5	45.5	-	-	-	-
December	43	26	60.5	8	18.6	9	20.9	-	-
Total	118	43	36.4	25	21.2	34	28.8	16	13.6

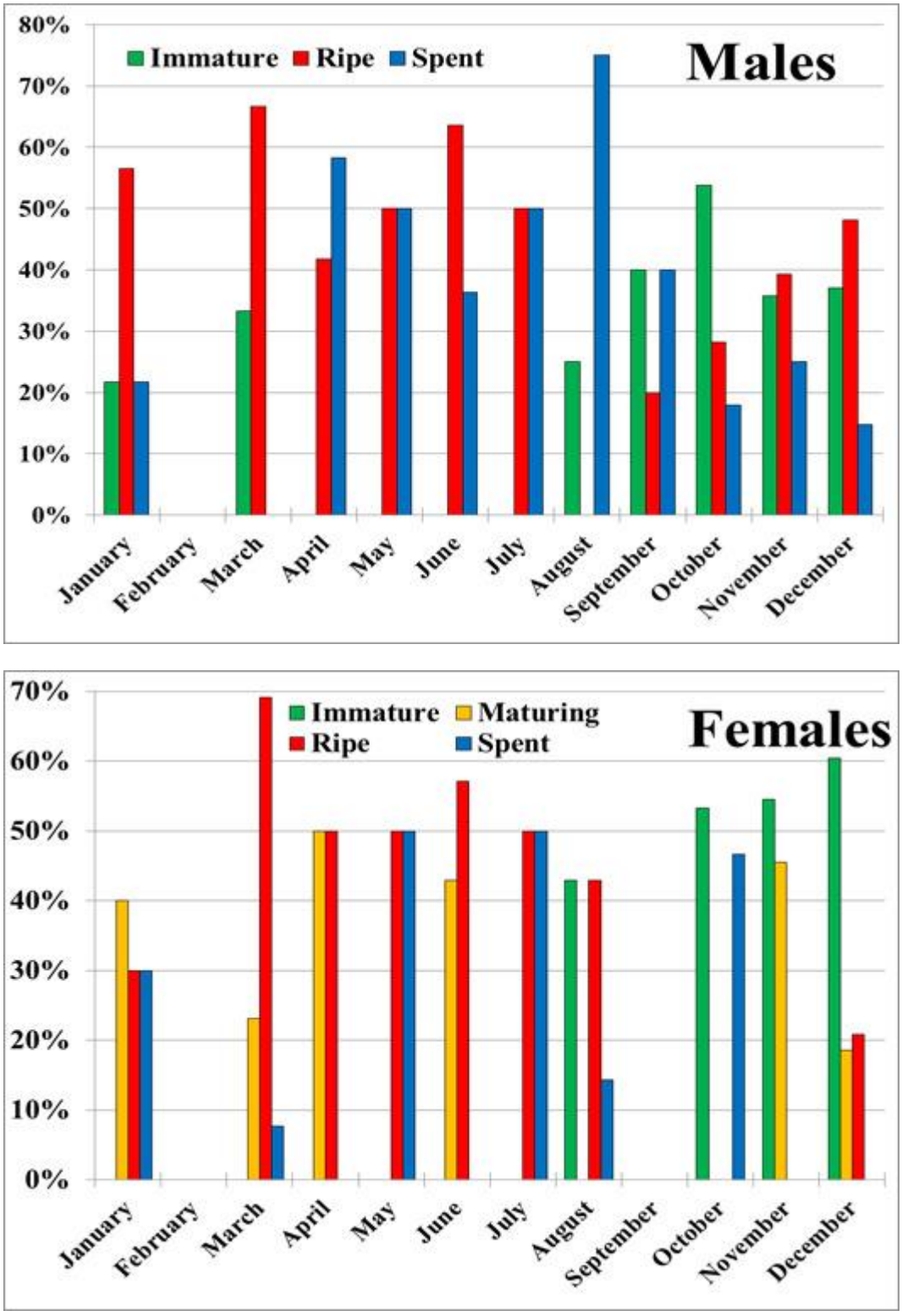


Fig. 3. Monthly variations of the different maturity stages for males and females of *Callistoctopus macropus* specimens

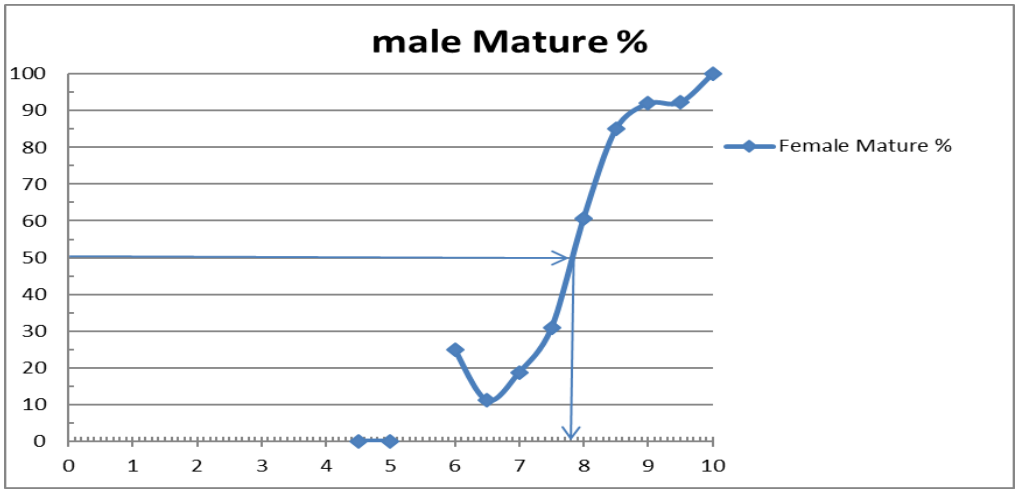
Table 4. Percentage distribution of mature males and females of *Callistoctopus macropus* in each mantle length group

	Mantle length group (cm)	**Male**		**Female**	
		Samples examined	**Mature%**	**Samples examined**	**Mature%**
	4.5	-	-	-	-
	5	**2**	**0.0**	**5**	**0.0**
	5.5	**3**	**0.0**	**5**	**0.0**
	6	**45**	**25.0**	**8**	**0.0**
	6.5	**12**	**11.1**	**11**	**0.0**
	7	**21**	**18.7**	**15**	**0.0**
	7.5	**33**	**30.8**	**24**	**18.2**
	8	**36**	**60.7**	**36**	**42.1**
	8.5	**26**	**85.0**	**34**	**81.2**
	9	**32**	**92.0**	**17**	**100.0**
	9.5	**17**	**92.3**	-	-
	10	**22**	**100.0**	-	-

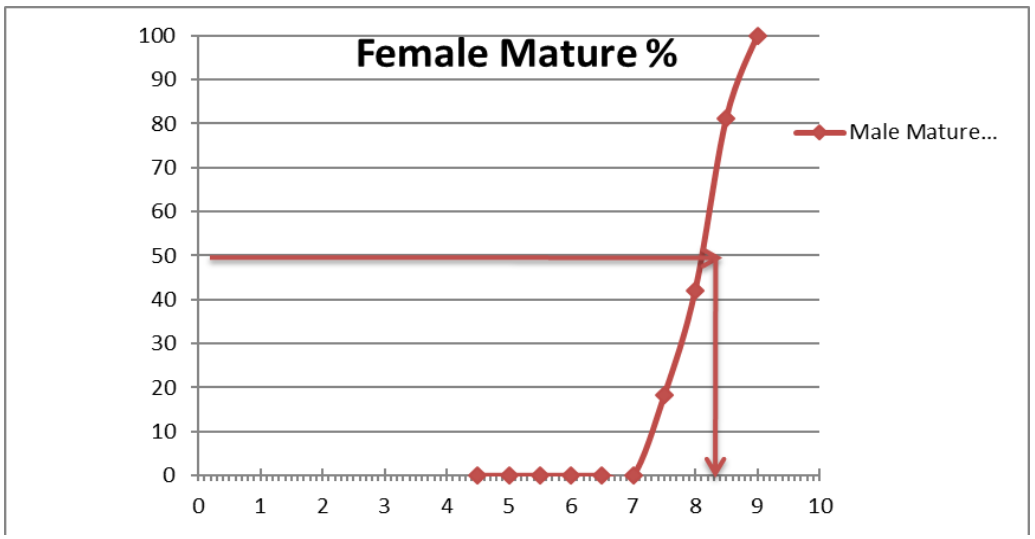
IV- Gonado-somatic index (G.S.I)

The spawning season can be predicted based on changes in gonadal size throughout the year. The gonado-somatic indexes' initial, progressive increase marks the start of the breeding season. The period of breeding is when the G.S.I reaches its maximum values. Table (4) and Fig. (5) show the monthly variations of *Callistoctopus macropus* G.S.I for males and females. For females, the minimal G.S.I was 0.31 in October, whereas for males, in September it was 0.44.

For males, the G.S.I begins to rise in March (0.76), reaching its highest point (0.89) in June, indicating a single peak and suggesting that the spawning season spans from June to September. The G.S.I for females *Callistoctopus macropus* begins to rise in March (0.63) and reaches its highest point in June. Individuals are found between August and December (0.89). The time frame for the spawning season is from June to October. The G.S.I changes with maturity stages can be summed up as follows: Male immature individuals are found between September and December, while female immature individuals are found between August and December. The spawning activity of ripe females and males of *Callistoctopus macropus* is at its peak when the G.S.I values reach their lowest points, occurring from March to June. *Callistoctopus macropus* was found in the catch all year long, with male abundance peaking from March to August, when gonadal activity is at its peak from March to June. On the other hand, female abundance peaked from March to August, with the height of gonadal activity in April and June.



Mantle length



Mantle Length

Fig. 4. Relationship between mantle length and percent of mature males and females of *Callistoctopus macropus* (The length at first maturity is shown at 50%)

In summary, male and female immature individuals possessing the lowest G.S.I values were present year-round and peaked in abundance from June to September for males, and from June to October for females.

V- Hepatopancreas-somatic index

Table (6) summarizes monthly variations in the H.S.I values for males and females of *Callistoctopus macropus*, while Fig. (6) shows the changes graphically. For males, the maximum H.S.I value was reached in April, June, and November, and for females, it was reached in May. Throughout the spawning season, which runs from June to September for males and from June to October for females, the minimal values were noted (Table 6 & Fig. 6). This could result from the digestive gland's lipid supplies being depleted over the protracted spawning and breeding season.

Table 5. Monthly variations of gonado-somatic index for *Callistoctopus macropus*

Month	Male G.S.I	Female G.S.I
January	**0.67**	**0.37**
February	**-**	**-**
March	**0.88**	**0.62**
April	**0.82**	**0.75**
May	**0.85**	**-**
June	**0.89**	**0.89**
July	**0.3**	**0.87**
August	**0.57**	**0.78**
September	**0.44**	**0.57**
October	**0.51**	**0.31**
November	**0.58**	**0.43**
December	**0.63**	**0.45**

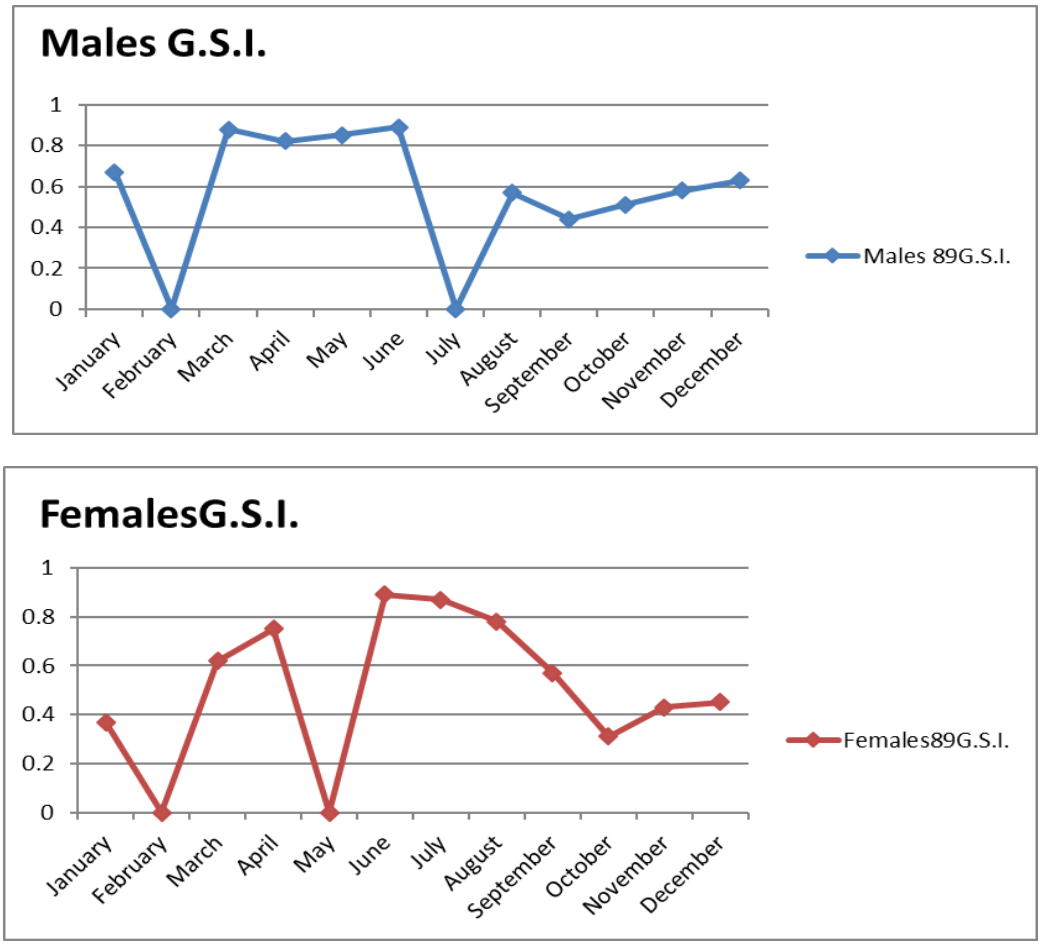


Fig. 5. Monthly gonado-somatic index (G.S.I) for males and females of *Callistoctopus macropus*

Table 6. Monthly variations of hepato-somatic index (H.S.I) for males and females of *Callistoctopus macropus*

Month	Male H.S.I	Female H.S.I
January	4.54	5.76
February	4.21	5.38
March	3.40	4.73
April	4.05	4.54
May	3.18	4.85
June	3.37	4.10
July	3.25	4.00
August	2.92	3.25
September	3.14	3.18
October	4.24	4.01
November	4.72	4.08
December	4.68	5.73

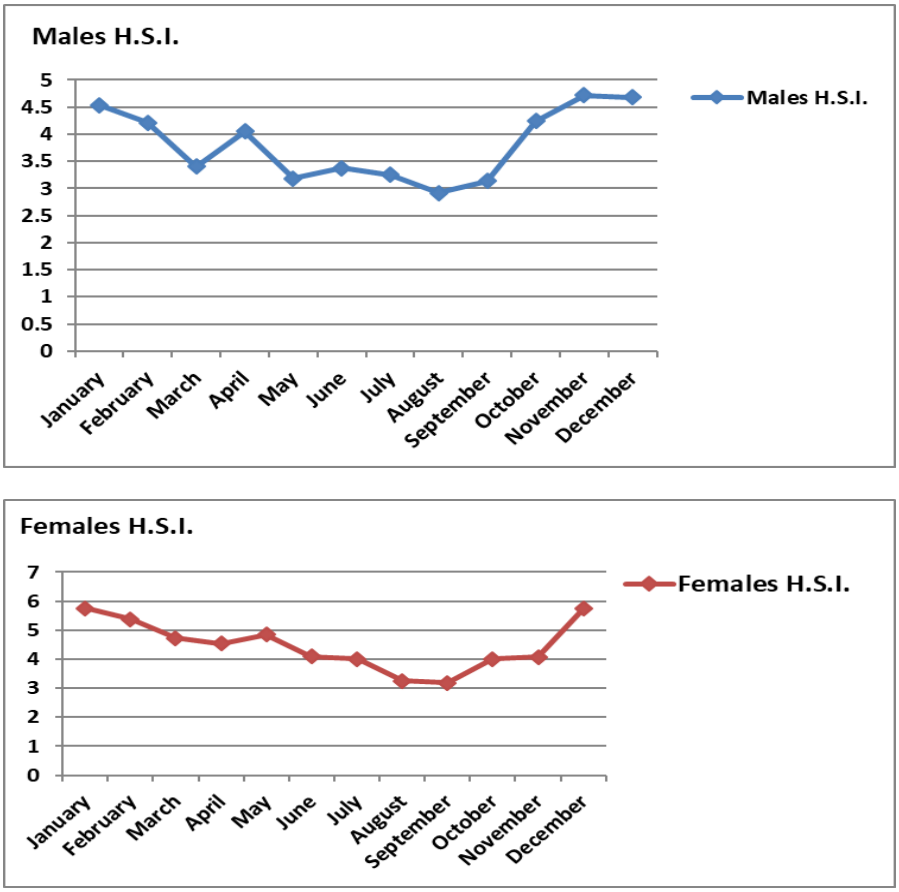


Fig. 6. Monthly hepatopancreas– somatic index (H.S.I) for males and females of *Callistoctopus macropus*

VI- Sex ratio

Table (7) displays the monthly ratio of males to females, and Fig. (7) exhibits the monthly percentage of males and females of *Callistoctopus macropus*. It appears that throughout the year, the number of males exceeds that of females, especially in the months of January (69.7%), April (75%), June (61.1%), September (71.8%), October (72.2%), November (71.8%), and December (55.7%), whereas the females outnumber males in March (59.1%) and August (63.6%).

Table 7. Monthly sex ratio of males to females of *Callistoctopus macropus*

Month	Sample no.	Male		Female		Sex ratio
		no.	%	no.	%	
January	33	23	69.7	10	30.3	2.3 : 1
February		-		-		
March	22	9	40.9	13	59.1	0.7 : 1
April	16	12	75	4	25	3 : 1
May	8	6	75	2	25	3 : 1
June	18	11	61.1	7	38.9	1.6 : 1
July	14	8	57.1	6	42.9	1.3 : 1
August	11	4	36.4	7	63.6	0.6 : 1
September	15	15	100			15 : 0
October	54	39	72.2	15	27.8	2.6 :1
November	39	28	71.8	11	28.2	2.5 : 1
December	97	54	55.7	43	44.3	1.3 : 1
Total	327	209	63.9	118	36.1	1.8 : 1

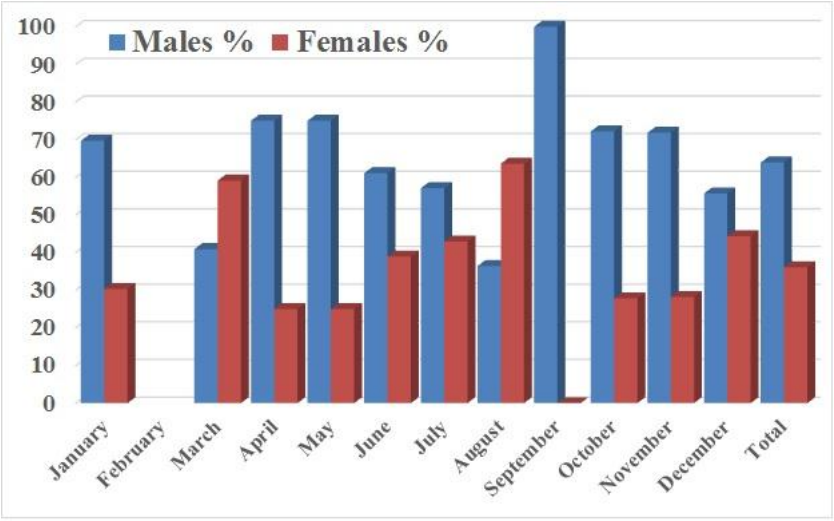


Fig. 7. Monthly percentage of *Callistoctopus macropus* males and females

DISCUSSION

Specimens of *Callistoctopus macropus* were collected from muddy, sandy bottoms in the Egyptian Mediterranean Sea for the current study. According to **Roper *et al*. (1984)**, *Callistoctopus macropus* is a benthic organism found in shallow water that inhabits reef flats, open bottoms, and coral reefs. According to **Fisher (1973)**, the species are found in holes and crevices on rocky bottoms and occasionally on vegetative substrates.

The greatest specimen in the current work has a mantle length of 16.3cm for males and 15.9cm for females. Males measure 138cm in total length, while females measure 131cm total length. In terms of absolute weight, males weigh 880gm, whereas females weigh 625gm. Related species differed in their maximum all-out lengths from 90 to 110cm, according to **Fisher (1973)**. **Roper *et al*. (1984)** calculated a mantle length of 14cm and a total length of 120 to 150cm. The species in the present work was taken offshore from Abu Qir Bay (50- 70m in depth) and El-Agamy (25- 45m in depth) in the Egyptian Mediterranean waters.

Due to an absence of *Callistoctopus macropus,* the breeding habits of Atlantic were unknown for a very long period. According to current research on *Callistoctopus macropus,* the females spawning on lasts from June to October (early summer to autumn). Contrary to this, **Roper *et al*. (1984)** discovered that the western Central Atlantic *Callistoctopus macropus* reproductive season lasts from winter to early spring. This runs counter to the current findings, which show that *Callistoctopus macropus* spawns from June to October (early summer to autumn). The same species' season runs from March to October in the Mediterranean waters off Banyuls-sur- Mer, France **(Boletzky, 1998)**.

For fecundity, the predicted fertility of *Callistoctopus macropus* spans from 205682 eggs for females with an average mantle length of 8.5cm to 61946 eggs for females with an average mantle length of 14.5cm. Generally, increasing fecundity with increasing mantle length can be observed in *Callistoctopus macropus.*

The female octopus ceased feeding as soon as the eggs were laid, which is common for octopus species, and she died soon after the eggs emerged. The planktonic larvae that emerged from the eggs each around fed on zooplankton, including crab larvae **(Naef, 1923)**.

CONCLUSION

In conclusion, the following could be used to outline the *Callistoctopus macropu* 's primary biological reproductive traits:

For length at first maturity, males and females, the smallest mature sizes were 6 and 7.5cm, respectively. For males and females, the length at initial maturity was 7.8 and 8.3cm, respectively.

In the current work, the stages of maturity were divided into four categories for females (Immature, maturing, ripe and spent), and three categories for males (Immature, ripe and spent).

From March to July, there were plenty of ripe males *Callistoctopus macropus*; from March to August, there were enough females.

The G.S.I states that the female *Callistoctopus macropus* spawns from June to October, whereas the males spawn from June to September.

The H.S.I values for both sexes reach their peak before the spawning season; however during spawning season, the lowest values were noted. This could be since the digestive gland's stored lipid was used up throughout the protracted spawning and breeding process.

For fecundity, the predicted fertility of *Callistoctopus macropus* spans from 205682 eggs for females with an average mantle length of 8.5cm to 61946 eggs for females with an average mantle length of 14.5cm. In general, we can observe an increase in fecundity with increasing mantle length.

For sex ratio: Males outnumber females throughout the year except March and August. The ratio between males to females is 1.8:1.

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