

Reproductive Biology of *Sepia pharaonis* Ehrenberg, 1831 (Cephalopoda: Sepioidea) from the Suez Gulf (Red Sea), Egypt.

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ABSTRACT

Reproductive biology of males and females of *Sepia phraonis* were investigated from samples obtained from commercial catch caught by local fishermen using trawling nets. The samples were seasonally collected (individuals) between winter 2014 to autumn 2014. The results of sex ratio indicated that females outnumbered males. The mean size at first maturity was 7cm and 8.6cm dorsal mantle length (ML) for males and females respectively. Based upon morphological features of the gonads and nidamental gland color change in female, five and four maturity stages for males and females were described, respectively. Depending on the proportion of each maturity stage, as well as various maturity indices (gonado-somatic index, G.S.I and nidamental-somatic index, N.S.I) the spawning season was found to take place during spring and summer and sometimes extends to autumn. So, it is recommended to reduce fishing activity during these periods.

Keywords: *Sepia pharaonis*, reproductive biology, Suez Gulf, Red Sea, Egypt

INTRODUCTION

Class Cephalopoda comprises Cuttlefishes, Squids, Octopuses and Nutili. It embraces about 1000 known species and constitutes about 2.07% of phylum Mollusca (Hassan, 1974). Cephalopoda represents a significant worldwide fishery resource. According to Roper *et al.* (1984) the world total catch of cephalopod consists of 71.8% were squids; 13.6% cuttlefish and 14.6% octopuses. Riad (1993) stated that in the Egyptian Mediterranean waters the cephalopods constitute 9.8% of the total fish catch of which 61% are cuttlefishes, 3.5% octopuses and 0.21% squids. On the other hand, in the Red Sea Egyptian waters cuttlefishes constitute about 0.69% from the total fish catch (Riad and Abdelhafez, 2008).

Cephalopod fishery is economically very important, because of its high commercial value in national and international markets (Mehanna *et al.*, 2014). Mehanna *et al.* (2009) stated that the high protein content of cephalopods and their low fats make them an important and healthy element in human diet either as fresh food or processed products. Samiee *et al.* (2013) indicated that the dominant fatty acids in muscle tissue are Docosahexaenoic acid (DHA) (32.89%) and Eicosapentaenoic acid (EPA) (27.68%). DHA and EPA are Omega-3 fatty acids and essential fats.

The cephalopods represent about 3% of the total world capture fisheries production that stood at 3.5 million tons in 2003 (FAO, 2005). Among the cephalopods of the family Sepiidae, the pharaoh cuttlefish *Sepia pharaonis* is widely distributed in the Indo-West Pacific region ranging from Red Sea to Japan and Australia and forms a dominant species in the commercial fisheries (Roper *et al.*, 1984; Nesic, 1987). This cuttlefish is the primary fishery in the Suez Canal and the

most valuable commercial cephalopods in the northern Indian Ocean. However, their reproductive biology, which is essential for fishery management, is poorly known (Gabr *et al.*, 1998).

The major species of cuttlefish in the Gulf of Suez are *S. savignyi*, *S. dollfusi*, *S. pharaonis* and *S. prashadi* (Emam, 1983&1994). It is worth to note that the reproductive biology of the common cuttlefish of the eastern Atlantic and Mediterranean Sea, *Sepia officinalis*, is now well-known in considerable detail. In contrast, little is known about the biology of *Sepia pharaonis*. Therefore, the objective of this study is to investigate the reproductive biology of this important species in the Suez Gulf, Red Sea. Information on maturation and spawning of *S. pharaonis* will contribute to knowledge of their population dynamics and management of the stocks.

MATERIALS AND METHODS

Seasonal specimens of *Sepia pharaonis* were collected during the period from winter 2014 to autumn 2014 (850 individuals) from commercial trawling vessels of Suez Gulf, Egypt. Samples were frozen and stored for investigation. After thawing at room temperature, the sex was determined by checking the presence of the left arm IV hectocotylized (modified arm) in male.

Sex ratio: number of males and females, individuals of each sex, was carried out.

Length at first maturity was determined which is the mantle length at which 50% of the *Sepia pharaonis* are mature (Snedecor, 1956).

The indices of reproduction. The indices of reproductive status were collected according to Joy (1989) and Pierce *et al.*, (1994). Gonado-somatic Index (GSI) for both sexes and Nidamental gland-Somatic Index (NSI) for the females were calculated as follows:

$$\text{GSI} = \frac{\text{G. Wt} * 100}{\text{To. Wt}}$$

$$\text{NSI} = \frac{\text{NG. Wt} * 100}{\text{To. Wt}}$$

Where to. Wt is total body weight; G. Wt is the gonad weight; NG. Wt is the nidamental gland weight.

Maturity stages: According to Mangold (1983) male maturity stages were identified by the presence of spermatophores in spermatophoric sac. On the other hand, female maturity stages were identified according to Richard (1967); the egg diameter was measured, the Nidamental glands length (NL) and the color of accessory Nidamental glands were determined.

Fecundity: For fecundity, egg clusters were removed out of gonads and placed in Petri dish containing water. Large reticulated, eggs were only counted as they considered developing eggs. The total number of eggs was estimated from an exact count of all eggs of each individual visually.

RESULTS

Sex ratio

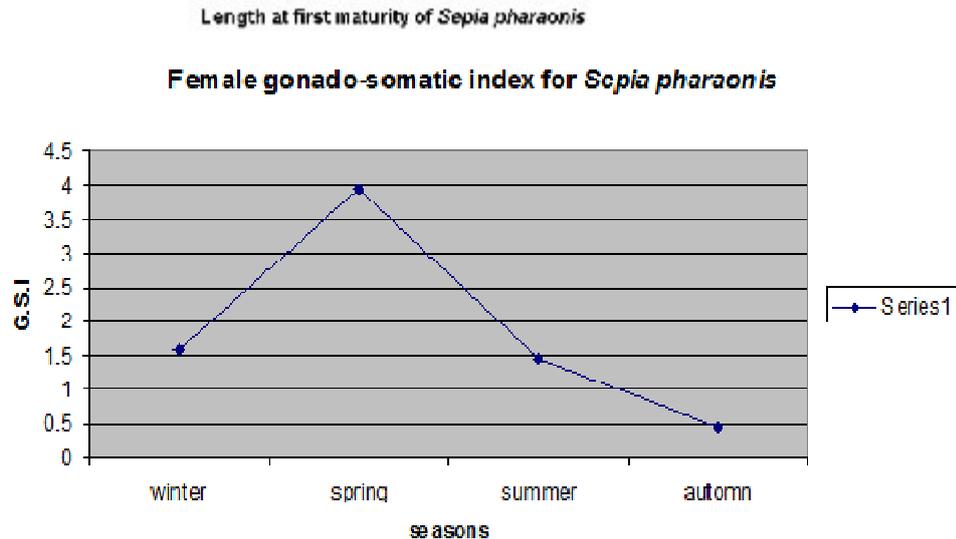
Table (1): illustrates the ratio of *Sepia pharaonis* males to females of the studied specimens. It appears that females outnumber males during all seasons.

Table 1: Seasonal ratio of male to female individuals of *Sepia pharaonis*.

Seasons	Total no	sex	no	%	ratios
spring	137	Male	16	11.679	1 : 5.587
		female	121	88.321	
summer	494	Male	75	15.182	1 : 2.861
		female	419	84.321	
Autumn	139	Male	36	25.899	1 : 2.636
		female	103	74.101	
winter	80	male	22	27.5	1 : 5.587
		Female	58	72.5	
All year	850	Male	149	17.529	1 : 4.7
		female	701	82.4711	

Length at first maturity

Size at first maturation is defined as the size at which 50 percent of the individuals are maturing or mature (Bakhayokho, 1983). In the present study, the size at first sexual maturity for males and females of *Sepia pharaonis* was estimated to Fig. (1).

Fig. 1: Length at first maturity of females and males of *Sepia pharaonis*.

Maturity index

Gonado Somatic Index (G.S.I). It is worth to note that the spawning season can be predicted from seasonal variations in gonadal size. The start of a gonadal increase in gonado-somatic index (GSI) is an indication of the onset of the breeding season. The Gonado-Somatic Index attains its maxima during breeding time. Gonado-somatic index minimal values were attained during autumn. The G.S.I for males and females clarified gradual increase starting in winter and peaked during spring (Fig. 2), indicating that spawning season of both sexes occurs during early spring, summer and extend to autumn.

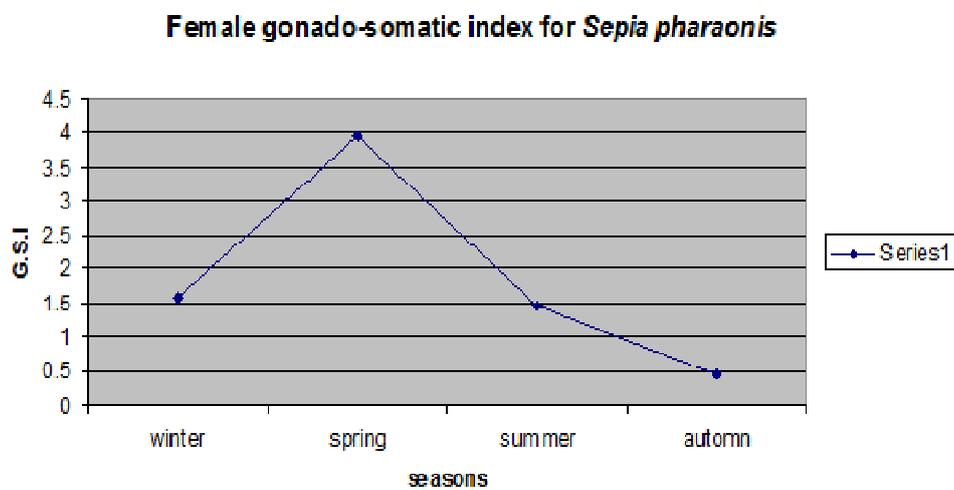
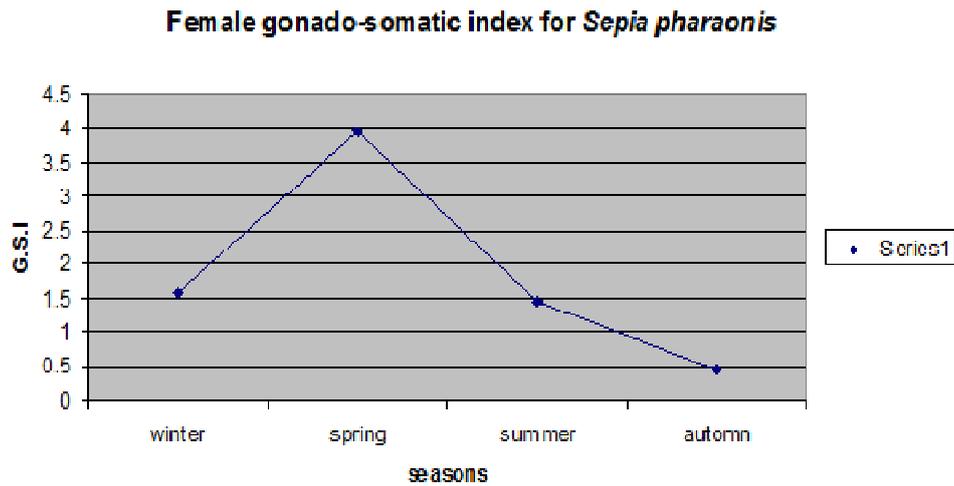


Fig. 2: Gonado Somatic Index for males and females of *Sepia pharaonis*.

Nidamental gland-Somatic Index N.S.I

It is to be noted that at the time of *Sepia pharaonis* spawning the females possess enlarged nidamental gland. The variations in the N.S.I could therefore, like those of the G.S.I, reflect the breeding cycle and spawning intensity.

The N.S.I of females was determined from fig (3) showing the seasonal N.S.I variation running more or less parallel to those of the G.S.I seasonal variations.

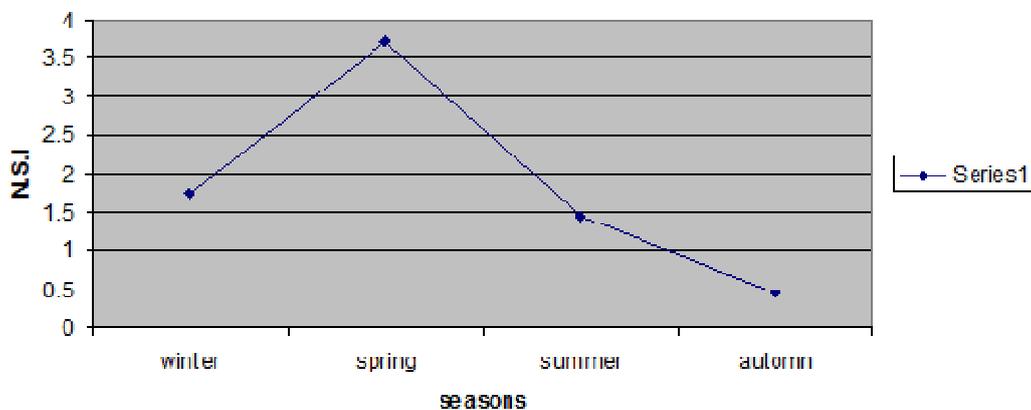


Fig. 3: Nidamental Somatic Index for females of *Sepia pharaonis*.

Maturity stages

In order to define different maturity stages in males we have to detect the presence of spermatophores in Needham's sac and accessory nidamental gland color and egg size in females. The present results obtained classified maturity as five stages for males (immature, maturing, mature ripe and spent) and four stages for females (immature, maturing, mature, and ripe) as shown in Table (2).

Table 2: Sexual maturity stages of *Sepia pharaonis*

Stage	Female	Male
Stage I immature	<ul style="list-style-type: none"> • Ovary very small , white color • Egg cluster very small , occupy the posterior part of mantle as a patch, no reticulated eggs appear , diameter <1 • Nidamental gland very small, accessory nidamnetal gland with translucent, white to light creamy color (Fig. 4). 	<ul style="list-style-type: none"> • Testis small • Needham's sac small and transparent in color, with no visible spermatophore, white fluid only
Stage II maturing	<ul style="list-style-type: none"> • Ovary occupies half of posterior body cavity , gonad creamy in color • Egg cluster , occupies the posterior part of the mantle as a large patch with few visible reticulated eggs, diameter 1-3, 4 mm • Nidamnetal gland large, slightly oval in shape, accessory nidamnetal gland with creamy to yellow color (Fig. 5). 	<ul style="list-style-type: none"> • Testis large and thicker than in immature stage • Needham's sac slightly large, slightly transparent to white, with few visible short Spermatophore
Stage III mature	<ul style="list-style-type: none"> • Ovary very prominent occupies entirely the posterior part of mantle cavity • Large egg cluster occupies the posterior part , with many reticulated eggs, diameter 1-5 mm • Large nidamnetal gland and thick, oval, accessory nidamnetal gland yellowish to orange in color (Fig. 6). 	<ul style="list-style-type: none"> • Testis large and fully developed • Needham's sac fully packed with developed spermat-ophores, with tall spermatophore
Stage VI ripe	<ul style="list-style-type: none"> • Ovary very prominent occupies all the posterior part of mantle cavity with heavy gelatinous secretion • egg cluster very large with heavy gelatin with fully developed eggs (smooth or reticulated), diameter 2-8mm • Nidametal gland enlarged, very thick, accessory nidamnetal gland with reddish orange to dark red with visible red (light, dark) dots or stripes (Fig. 7) 	<ul style="list-style-type: none"> • Testis fully developed • Needham's sac flaccid with degenerating spermatophores in mantle cavity
Stage V spent		As the immature stage but large gonad and large mantle length, nedham's sac empty only contain white fluid no spermatophore available



Fig. 4: Stage I immature, white to light creamy accessory nidamental gland.



Fig. 5: Stage II maturing, creamy to yellow accessory nidamental gland.



Fig. 6: Stage III mature, yellow to orange accessory nidamental gland.



Fig. 7: Stage VI ripe, reddish orange to dark red accessory nidamental gland.

Fecundity

The minimum fecundity range in the present study has been determined by counting the eggs in the egg clusters (Fig. 8) and it was 102 for 9.5 cm mantle length and the maximum was 616 for 12.9 cm mantle length.

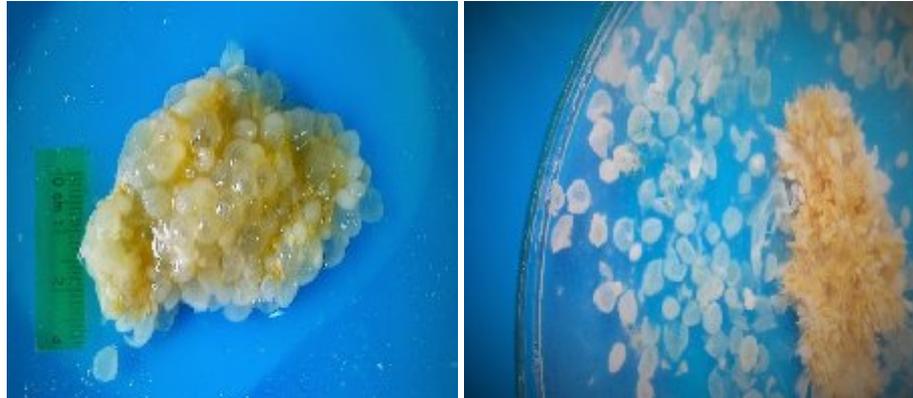


Fig. 8: Egg cluster of *Sepia pharaonis*.

DISCUSSION

Length at first maturity

The present work investigation revealed that the length at first maturity for males and females *Sepia pharaonis* was estimated to be 7 cm ML and 8.6 cm ML, respectively.

Data observed from other authors revealed that the length at first maturity for *Sepia pharaonis*, markedly vary at different locations. Sanders and Bouhlel (1981) found that at Yemeni waters the length at first maturity for the species was 20 cm. Bouhlel and Musaibli (1985) reported the length at first maturity for the same species to be 25 cm. On the other hand, results obtained for female *Sepia pharaonis* from Indian waters is 16 cm, (Silas *et al.*, 1986). Gabr (1998) estimated the first maturity at 6.1 cm and 12.2 cm for males and females, respectively, for the same species collected from the Suez Canal, Egypt. Mehanna *et al.* (2009) determined the length at first maturity and found it to be 8.5 cm in *S. pharaonis* from Suez Gulf. Al-Marzouki *et al.* (2009) studied *Sepia pharaonis* from the Arabian Sea off Oman, and estimated that the length at first maturity for females was 20.2 cm ML, and 19 cm for males. Mehanna *et al.* (2014) found that the length at first maturity for *S. pharaonis* from omani coast Arabian sea, was 19.6 cm.

This difference may be due to the length range of samples used in each study. As *S. pharaonis* is suspected to be a complex of three to five species or Subspecies (Norman, 2000; Anderson *et al.*, 2007), this may be the reason for the differences encountered in length at first maturity and spawning in different regions.

Maturity stages

In the present study, five stages were recognized for males (immature, maturing, mature, ripe and spent) according to the presence of spermatophore in the Nedham's sac. Four stages for females (immature, maturing, mature, ripe) for *Sepia pharaonis* were defined according to the gross morphology of the gonad and the color of the accessory nidamental glands for females. Gabr *et al.* (1998) recorded 4 stages of sexual maturity for both species *Sepia pharaonis* and *Sepia dollfusi* according to the gross morphology of the gonads and by the microscopic development in the accessory gland.

It's worth to note that the above mentioned results regarding maturity stages obtained by Gabr *et al.* (1998) slightly differs from that obtained during the present study. Females of the present work exhibited marked red color of the accessory nidamnetal gland in ripe stage which is not mentioned by Gabr *et al.* (1998) at the same stage. Besides, five stages of male maturity was assigned during the present work in contrast to only four found by Gabr *et al.* (1998)

Maturity index and spawning season

In the present study N.S.I values were found minimal in autumn and gradually increase attaining its highest value in spring which confirms the previous conclusion from the G.S.I study that spawning season occurs during early spring, summer and extends to autumn. The spawning season of *Sepia pharaonis* in the present study extend from early spring, summer and extend to autumn with a peak at spring.

Voss and Williamson (1971) have been reported between March and May in Hong Kong, spawning in *Sepia pharaonis* is restricted to March-May period in Hong Kong waters, and to August - October in the Red Sea (Sanders 1981). A restricted spawning in *S. pharaonis* between August and October in the neighbouring Yemeni waters (Sanders and Bouhleh, 1981). Silas *et al.* (1986) investigated that spawning occurs from October to April in general on both coasts of India, sometimes extending up to August. Nair *et al.* (1993) observed in both coasts of India that Cuttlefishes of both sexes' with mature gonads were noted in several months indicating that spawning is not restricted to any season but extended over a period of time. According to Gabr *et al.* (1998), the spawning season of *Sepia pharaonis* in the Suez Canal, Egypt takes place from March to June. Abdussamad (2004) stated that the spawning season of *Sepia pharaonis* from Kakinada along the east coast of India was found to be during August/march (summer/spring) with two peaks during November and December (winter). AL Marzouqi *et al.* (2009) stated that in the Arabian Sea off Oman, the spawning of *Sepia pharaonis* during August-December (summer-winter) and April-May (spring). Sundaram (2014) stated that the spawning season for *Sepia pharaonis* from north west coast of India takes place from February to may (winter-spring). Ghazvineh *et al.* (2012) recorded that the spawning season of *Sepia pharaonis* from the Persian Gulf covers the whole months with two maximum peaks in May and June (spring-summer). Sasikumar *et al.*, (2013) recorded that the peak of spawning of *Sepia pharaonis* in the Indian waters (Eastern Arabian Sea) is reported in October/November (Autumn). Sasikumar *et al.* (2013) stated that the spawning season of *Sepia pharaonis* from the Eastern Arabian sea is reported during October/November (Autumn) and in February/March (winter). Thus it is clear from the above mentioned results that there is marked variations in the spawning season of *Sepia pharaonis* regarding different localities, for the same species. Differences were seen between spawning seasons in various ares because of various ecological conditions and effective factor on spawning as food availability (Ghazvineh *et al.*, 2012).

However, there is elasticity in the spawning season of *S. pharaonis* from different regions (Norman, 2000). This indicates that the spawning season and the reproductive peak of *S. pharaonis* are remarkably flexible and probably this may be attributed to environmental conditions prevailing as Mangold-Wirz (1963) considered temperature and light as the most important external factors that influence sexual maturation in cephalopods.

Sex ratio

In the present study females *Sepia pharaonis* were dominant with a sex ratio of 4.7:1. In spring, out of a total 137, 16 were males (11.679 %) and 121 were females (88.321 %). In summer, out of a total 494, 75 were males (15.182 %) and 419 were

females (84.321 %). In autumn, out of a total 139 collected animals, 36 were males (25.899 %) and 103 were females (74.101 %). In winter, out of a total 80, 22 were males (27.5 %) and 58 were females (72.5 %).

Silas *et al.* (1986) Reported that sex-ratio in *S. pharaonis* is highly variable, There was a great numerical difference in the sex-ratio during most part of the year and females dominated over males in the trawl catches from the Bay of Bengal and in the hook and line fishery in the Arabian Sea. Ayoama and Nguyen (1989) found that the sex ratio of the same sp with mantle length below 30 cm 1:1 in Yemeni waters, while all those larger than 30 cm are males and the largest one is about 43 cm, reasons for the absence of large females may due to different growth rate and shorter life span. According to Nair *et al.* (1993) during certain months, only males were landed by the trawlers off Visakhapatnam from the Bay of Bengal from Indian waters. Persian and Vallinassab (1994) found that the proportion of males to females were 70:30 in waters of Sistan and Baluchistan (Iran). Sundaram (2014) recorded that the females were dominant with sex ratio 1.24:1 for *Sepia pharaonis* from North West of India. Ghzvineh *et al.* (2012) noted that that 70% of population was males and 30% was female.

These difference in sex ratio from different location may due to sampling method, The reasons for dominance of females over males in the present work study cannot be explained as the data obtained was only restricted for one year. Further studies for the Egyptian waters have be carried out in order to understand Whether or not there is sex segregation in different seasons and sizes of the cuttlefish that was targeted by specific gear

Fecundity

In the present study, ovum sizes vary from less than 1 mm to about 40 mm. The minimum range fecundity in the present study was (102) for 9.5cm mantle length and the maximum was (616) for 12.9 cm mantle length for *Sepia pharaonis*.

According to Gabr *et al.* (1998) *Sepia pharaonis* from the Suez Canal exhibited relatively more fecundity than other Cuttlefishes. *Sepia pharaonis* is relatively more fecund than *S. dollfusi*. For example, the number of ova in *S. pharaonis* of 140 mm ML was 730 compared to 273 ova in a female *S. dollfusi* of a similar size.

Boletzky, (1983) Compared *S. pharaonis* with the common Mediterranean cuttlefish *Sepia officinalis* as they share a similar size and life history. He found a maximum fecundity of *S. pharaonis* estimated at 517-1525 ova for females of 110-240 mm mantle length, respectively, and appears comparable to various fecundity estimates in *S. officinalis*. Mangold-Wirz (1963) reported that a single female of similar size may lay \approx 200- 550. Richard (1971) indicated a similar fecundity value (150-500) for *S. officinalis hierredda* from the English Channel. Persian and Vallinassab (1994) recorded 500-700 ova for the same sp from Iranian water. Ghzvineh *et al.* (2012) found that Total number of ova in spawning female of *Sepia pharaonis* in Persian Gulf ranged from 53 in mantle length 19.8 cm and body weight 815gm to 1589 in a female with 25.4 cm mantle length and body weight 1660gm. Chembian and Mathew (2011) reported that The total number of maturing and matured ova in the pre-spawning and spawning female *S. pharaonis* (stage III and IV) ranged from 146 in a female of 161 mm ML to 1400 in a female of 259 mm ML. In spite of these enormous size differences depending on the species, the basic pattern of embryogenesis is the same in all cephalopods (Iglesias *et al.*, 2014).

RECOMENDATIONA

It's worth to note that this is the first study regarding *Sepia pharaonis* that dwells the Egyptian Red Sea waters, in order to clarify its biological status. Hence it's essential to carry out further additional investigations, so as to evaluate the following:

- 1) Percentage of the species catch compared to the total cephalopod catch, in order to be aware with its stock assessment.
- 2) To find out whether or not the species has migrated via Suez Canal to the Mediterranean water similar to *Sepia dollfusi* (Riad, 2015), as they were frequently encountered altogether in the same catch.
- 3) Evaluating the species nutritional value.
- 4) Prevention of fishing operations during the spawning season which mainly occurred during spring and summer.

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ARABIC SUMMARY

بيولوجية التكاثر للنوع *Sepia pharaonis* (Cephalopoda: Sepioidea) (1831 Ehrenberg) من خليج السويس (البحر الأحمر) - مصر

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تشمل هذه الدراسة بيولوجية التكاثر لذكور وإناث الحيوان الرأسقدمي نوع *Sepia pharaonis* من مياه خليج السويس المصرية. إتضح من الدراسة أن متوسط الطول في بداية النضوج الجنسي للذكور ٧ سم و الإناث ٨.٦ سم، وأن عدد الأناث يفوق عدد الذكور في الأربع مواسم. تم تحديد ٤ مراحل للنضوج الجنسي للذكور اعتماداً على وجود الكيسولات المحتوية على الحيوانات المنوية (spermatophores) و خمس مراحل للإناث من خلال حجم البيض، لون الغدة الأنثوية الإضافية و طول الغدة الأنثوية. اعتماداً على دراسة تغيرات مؤشر حالة المناسل و مؤشر حالة الغدة الأنثوية وجد أن موسم وضع البيض يكون في بداية فصل الربيع و فصل الصيف و تمتد للخريف. لذلك فإن الدراسة تقترح عدم صيد هذا النوع خلال هذه الفترة.