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Some reproductive aspects of the Indian squid *Loligo duvauceli* in the Gulf of Suez, Egypt.

Ahmed G.A. Gewida[1,*]; Mohamed H. Yassien[2]; Mohsen S. Hussein[1] and Ahmed Mamoon[1]

[1] Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo 11884, Egypt.

[2] National Institute of Oceanography and Fisheries, Suez Branch, Egypt.

*Corresponding author: a.gweida@azhar.edu.eg

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ABSTRACT

A better knowledge of the reproductive biology of *Loligo duvauceli* squid, such as its sex ratio, sexual maturation, and reproductive seasonality is fundamental for establishing effective fishery management measures to ensure the fishery is sustainable. Samples of *Loligo duvauceli* were collected monthly for one fishing season during September 2014 to April 2015 at Attaka fishing harbor in the Gulf of Suez. A total of 759 individuals (434 males, 268 females, and 57 not recognized) in size range of 3.5 to 29.5 cm dorsal mantle length (DML) were used for this study. The sex ratio of males: females was 1.6:1.0. Females outnumbered males only in February. However, males were dominant in the rest of the fishing season. Four maturity stages for both sexes were recognized. Monthly variations in maturity stages and maturity indices showed that the spawning season extends from April to October (spring to early autumn) for females, while males from April to September (spring to late autumn). Length at first sexual maturity indicated that the females attain maturity at smaller DML than males. The mean length at maturity for this species was 14.8 and 17.5cm DML for females and males, respectively.

INTRODUCTION

The Gulf of Suez is considered as one of the major sources of fish production in the Egyptian sector of the Red Sea. Its importance as fish resource can be attributed to the shallowness and sandy bottom which make it suitable for trawling. It is characterized by the presence of a great diversity of highly economic fish and invertebrate species **(Sanders and Morgan, 1989).**

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 and Roper, 2010)**.

All cephalopods are dioecious. Sexual maturation involves the onset of the development of sexual organs from immature to mature individuals. Typically, a certain minimum size must be reached before maturation can proceed **(Jackson *et al.*, 1997)**. All loliginid squids have a strong preference for the benthic environment for feeding and spawning **(Boyle & Rodhouse, 2005 and Yassien *et al.*, 2016)**. They are all demersal spawners, where the egg capsules are attached to any natural or artificial surfaces such as rocks, macroalgae, fishing traps etc. Some species, such as *Loligo vulgaris*, directly insert their egg masses into sandy sea floors **(Sauer *et al.*, 1993)**.

Loligo duvauceli is one of the *loliginid* species that is abundant in the Egyptian Red Sea coast and the Gulf of Suez **(Riad, 2020)**. Along these coasts, the commercial catch of *L. duvauceli* is mainly landed as by-catch of multispecies demersal trawling fisheries (**Yassien *et al.*, 2016)**. Previous reproductive studies on *Loligo duvauceli* have been done in different regions such as India: **(Mohamed, 1993; Thomas & Kizhakudan, 2006; Mishra *et al.*, 2012)**, Thailand: (**Supongpan *et al.*, 1992; Chotiyaputta, 1996; Sukramongkol *et al.*, 2007)** and China: (**Sang, 2007; Wang *et al.*, 2015).** Recently, few studies have been done in Egypt: **Kilada & Riad (2010)** who inspected the seasonal reproduction biology of *Uroteuthis duvaucili* in the northern Red Sea. **Mohamed (2013)** studied maturation, fecundity and seasonality reproduction of *U. duvaucili* in the Suez Canal.

The main objective of the present study is to shed light on the reproductive aspects of *L. duvauceli* in the Gulf of Suez, including sex ratio, maturity stages and indices of reproductive parameters in addition to determining of the spawning season of this species.

MATERIALS AND METHODS

Sampling

Monthly sampling was done throughout the period of the study during one fishing season (from September 2014 to April 2015). A total of 759 samples (434 males, 268 females and 57 not recognized) ranged in size from 3.5 to 29.5 cm DML and 3.2 to 224.1g were collected from commercial stern trawlers at Attaka fishing port of the Gulf of Suez. The squid specimens were packed into an insulated icebox to keep them fresh during transporting them to the laboratory for identification, sex determination and subsequent biological analysis on reproductive organs.

Sex ratio

Each individual was dissected by cutting along the ventral mantle to reveal the internal organs and sex was determined by assessing its sexual organs visually. As for some smaller individuals that could not be distinguished by sexual characteristics, they were then assigned as ‘not recognized’ where no maturity stage was applied. Monthly sex ratios were calculated to determine the variations in sex ratios on a monthly basis. The following measurements were recorded to the nearest 0.01g using an electronic balance:

Spermatophoric organs weight (SOW): Weight of the spermatophoric complex and spermatophoric sac in males.

Testis weight (TW): Weight of the testis in g.

Nidamental glands weight (NGW): Weight of the nidamental glands and its accessory glands in females.

Oviductal gland weight (OGW): Weight of the oviductal gland in g.

Ovary weight (OW): Weight of the ovary in g.

Maturity stages

A macroscopic visual maturity stage scale (After **Lipinski and Underhill, 1995** with some modifications) was used to assess the gonads and their development. Four stages are defined for both males and females, stage I (immature), stage II (maturing), stage III (mature) and stage IV (spawning) as shown in figure (1).

For males, stages were defined based on the development of the spermatophoric complex, development of spermatophoric sac, size and appearance of the testis, and the presence or absence of spermatophores (the sperm packets). For females, the color of the accessory nidamental glands, size of the nidamental glands and ovary, and the presence or absence of fully mature oocytes in the proximal oviduct was used for the assessments (Figure 1).

Monthly variations in maturity stages

Monthly variations in maturity stages were used to determine the spawning season and the occurrence of mature males and females of *L. duvauceli* squid in the Gulf of Suez.

Length at first sexual maturity (Lm)

The length at first sexual maturity (Lm); length at which 50% of squid reach their sexual maturity **(Bakhayokho, 1983)** was estimated by fitting the percentage of maturity against mid lengths. Lm was estimated as the point on X-axis corresponding to 50% point on Y-axis.

Indices of reproductive parameters

The reproductive parameters indices were calculated as follows:

For females

Gonadosomatic index (GSI) = 100 * OW / BW

Nidamental gland index (NGI) = 100 * NGW/ BW

Oviductal gland index (OGI) = 100 * OGW / BW

Maturity coefficient (MC) = 100 * RSW / BW

Where:

BW = Total body weight, OW = Ovary weight, NGW =Nidamental gland weight, OGW = Oviductal gland weight, RSW = Reproductive system weight (NGW + OGW + OW)

For males

Gonadosomatic index (GSI) = 100 * TW / BW

Spermatophoric organs index (SOI) = 100 * SOW / BW

Maturity coefficient (MC) = 100 * RSW / BW

Where:

BW = Total body weight, TW = Testis weight, SOW = Spermatophoric organs (complex & Sac) weight, RSW = Reproductive system weight (SOW + TW)

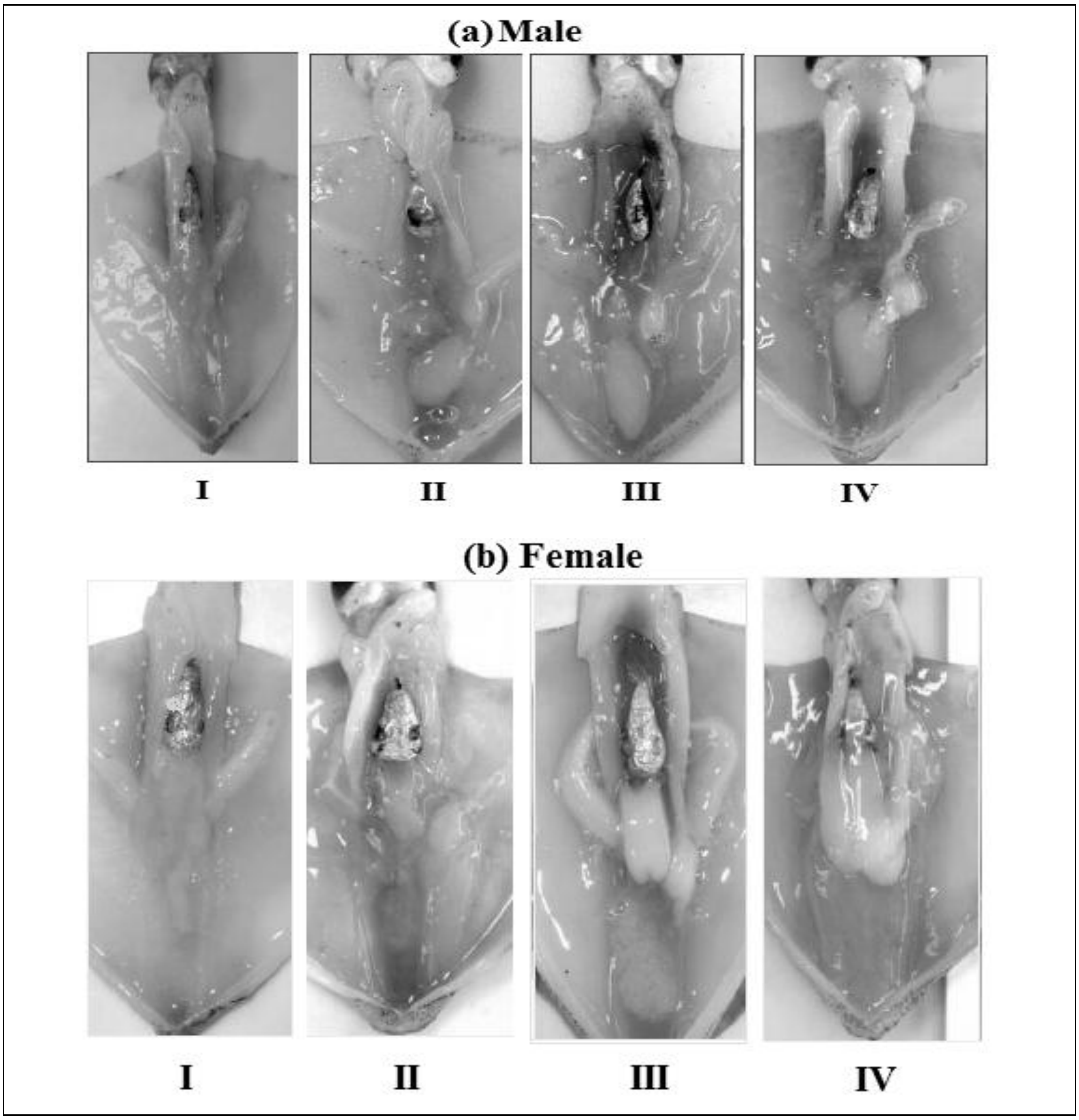

Figure 1. Different visual maturity stages of (a) male and (b) female of *L. duvauceli* (I): immature, (II): maturing, (III): mature, (IV): spawning. (After Lipinski and Underhill 1995)

RESULTS

Sex ratio

In total sample (702 specimens) of *L. duvauceli* the sex ratio (M: F) was 1.6:1.0. Males resembled 61.82%, while female formed 38.18% of the examined samples during the study period. Females outnumbered males only in February; however, males were dominant in the rest of the months (Figure 2). As to the seasonal variation of sex ratio in the present study, it was found that males were predominant over females in all seasons (Figure 3).

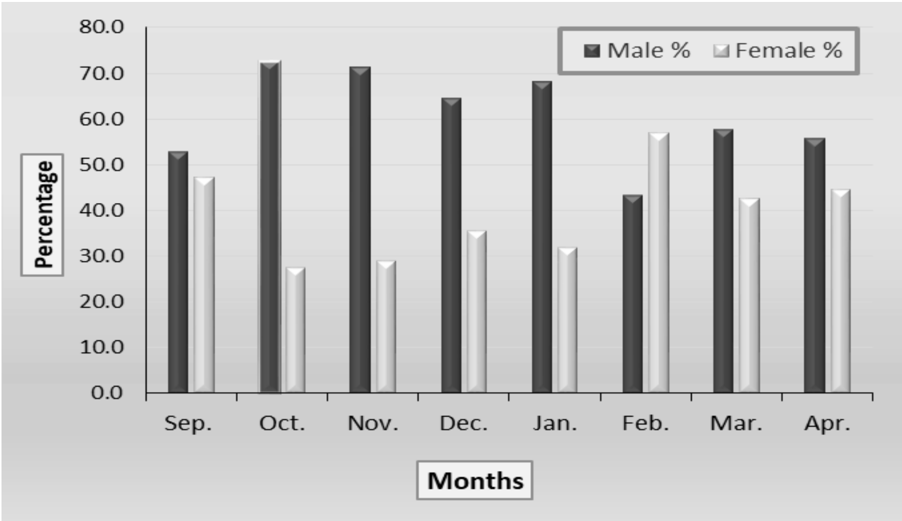

Figure 2. Monthly variations in sex ratio of *L. duvauceli* collected from the Gulf of Suez.

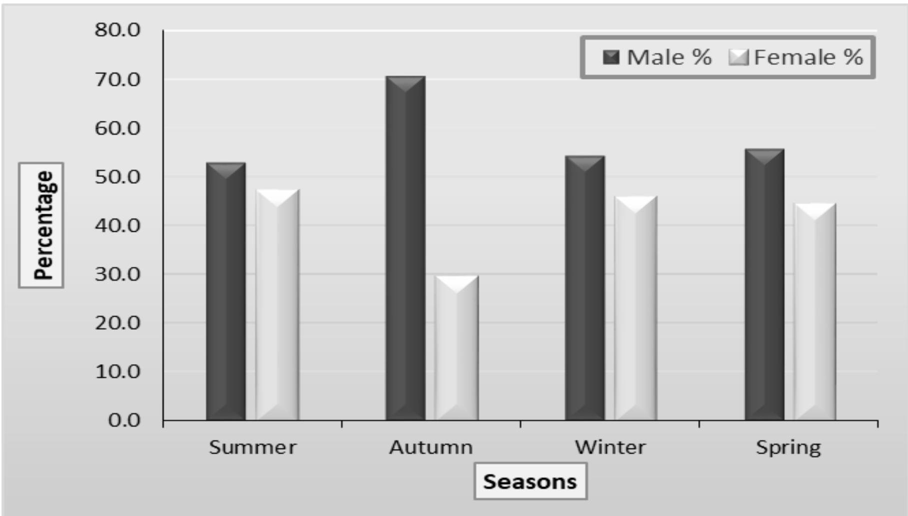

Figure 3. Seasonal variations in sex ratio of *L. duvauceli* from the Gulf of Suez.

The sex ratio in relation to dorsal mantle length (DML) classes for 759 specimens of *L. duvauceli* (Figure 4). The not recognized specimens (7.51%) were smaller in size and less than 11.5 cm DML and a presence of female (35.31%) was noticed for individuals from 7.5 to 21.5 cm DML, while males (57.18%) clearly dominated from 7.5 cm DML onwards.

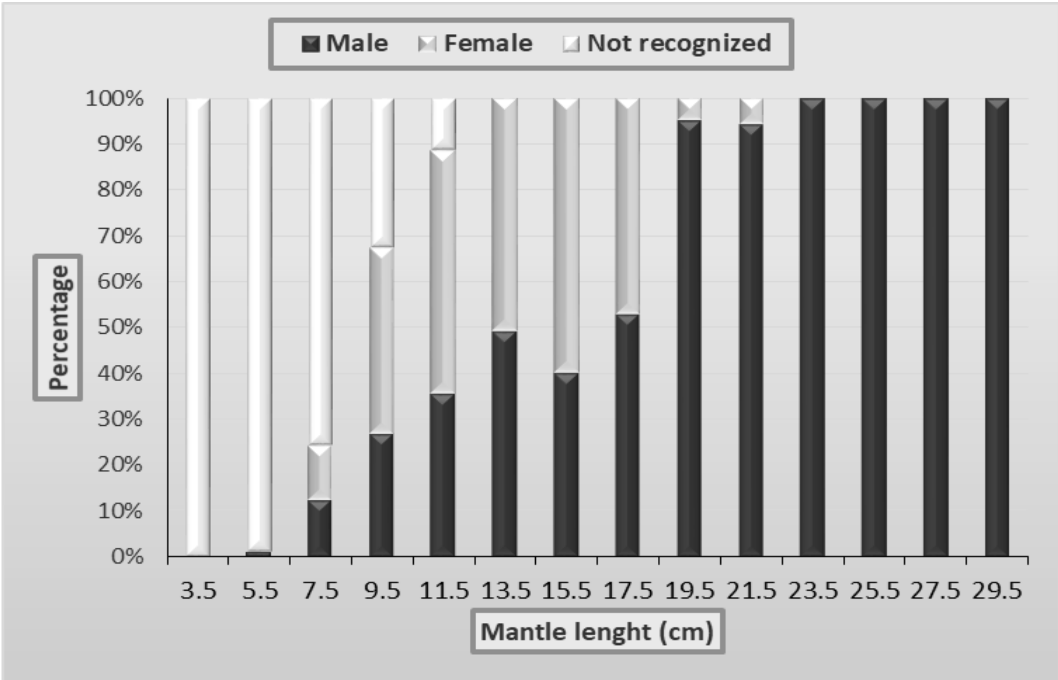

Figure 4. Sex ratio for *L. duvauceli* in relation to dorsal mantle length from the Gulf of Suez.

Maturity stages

Four maturity stages (according to **Lipinski and Underhill (1995)** with some modifications) for both males and females were recorded throughout the sampling period and classified as follows:

Stage I (immature)

For males: Spermatophoric complex is visible and testes are small, usually milky white. For females: Nidamental glands are easily visible and ovary does not preserve well.

Stage II (maturing)

For males: Separate parts of the spermatophoric complex are visible. Testes are small and without its normal structure. For females: The nidamental glands do not obscure the underlying viscera, semi-translucent and white. Accessory nidamental glands are visible and orange in color. Ovary is semi-transparent, rather flat, and irregularly segmented.

Stage III (mature)

For males: The spermatophoric complex is enlarged and opaque, and it has a white band of tentative spermatophores visible inside, spermatophores are present in the Needham's sac. Testis is enlarged and opaque. For females: The nidamental glands are enlarged, partially obscuring the underlying viscera. The accessory nidamental glands are in the lateral position and they are covered densely with red dots and/or patches. There are no eggs at this stage in the oviduct. The ovary is rather compact and semi-opaque. The clusters of oocytes are large, and single oocytes are visible.

Stage IV (spawning)

For males: The spermatophoric complex is large and opaque, and spermatophores are visible inside. The testis is large and opaque, and the structure is clear on all surfaces.
For females: The nidamental glands are large, covering large parts of the viscera. Red patches cover the accessory nidamental glands. The ovary is also enlarged and extended forwards. The mature oocytes tend to be placed proximally and the immature oocytes are distal.

Monthly variations in maturity stages

To determine the spawning season, the occurrence of mature males and females throughout the period of study was examined. For males, maximum numbers of immature individuals (stage I) occurred in October to March (Figure 5). Maximum numbers of stage II (maturing) occurred in September and April. Stage III (mature) appeared slightly in September, October, November and April, while spawning (stage IV) occurred only in September (Figure 5).

For females, maximum numbers of immature individuals occurred in all fishing months except October was depressed (Figure 6). Stage II (maturing) appeared in all fishing months. Mature stage (III) occurred slightly in all fishing months except March it was absent, but it was increased in October. Stage IV (spawning) was found only in October and April (Figure 6).

Generally, may be long spawning season of *L. duvauceli* from the Gulf of Suez during fishing season with peaks from April to October (spring to early autumn) for females, while males from April to September (spring and summer).

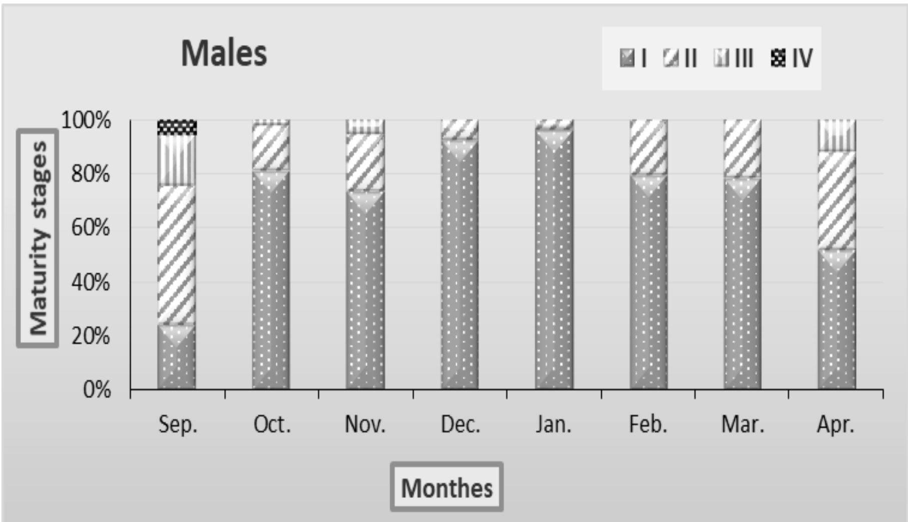

Figure 5. Monthly distribution of different maturity stages of males *L. duvauceli* from the Gulf of Suez.

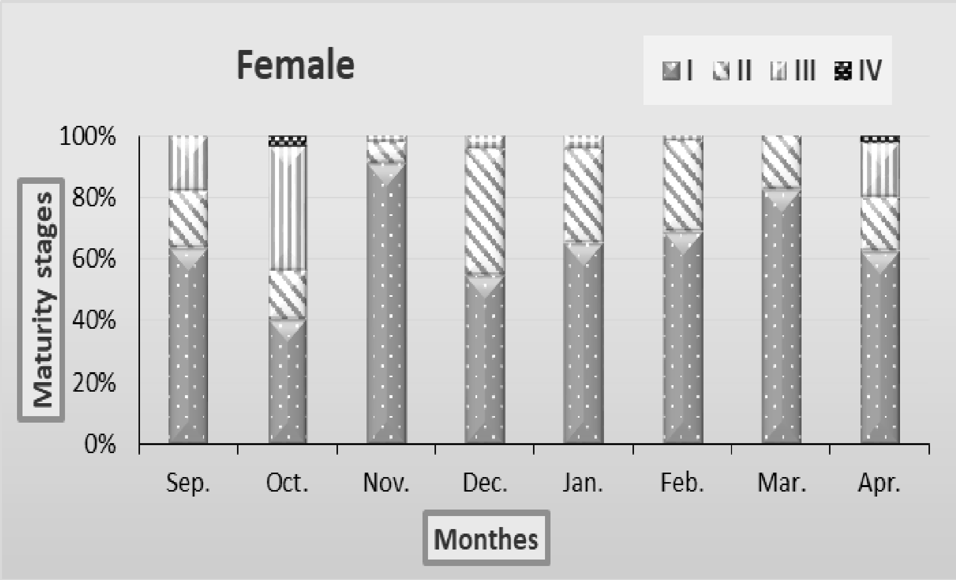

Figure 6. Monthly distribution of different maturity stages of females *L. duvauceli* from the Gulf of Suez.

Length at first sexual maturity (L_m)

Length at which 50% of specimens reach their sexual maturity was determined by the relationship between the proportion of maturing, mature and spawning animals and the dorsal mantle length (DML) of *L. duvauceli* (Figure 7). This figure is based on 204 samples of *L. duvauceli* collected throughout the sampling period. The data indicated that the females of *L. duvauceli* mature at smaller DML and attain a smaller adult length than do the males. The mean length at maturity for this species was 14.8 and 17.5cm DML for females and males, respectively. The length range of mature animals was wide, 11- 21 cm DML in females and 11- 27 cm DML in males.

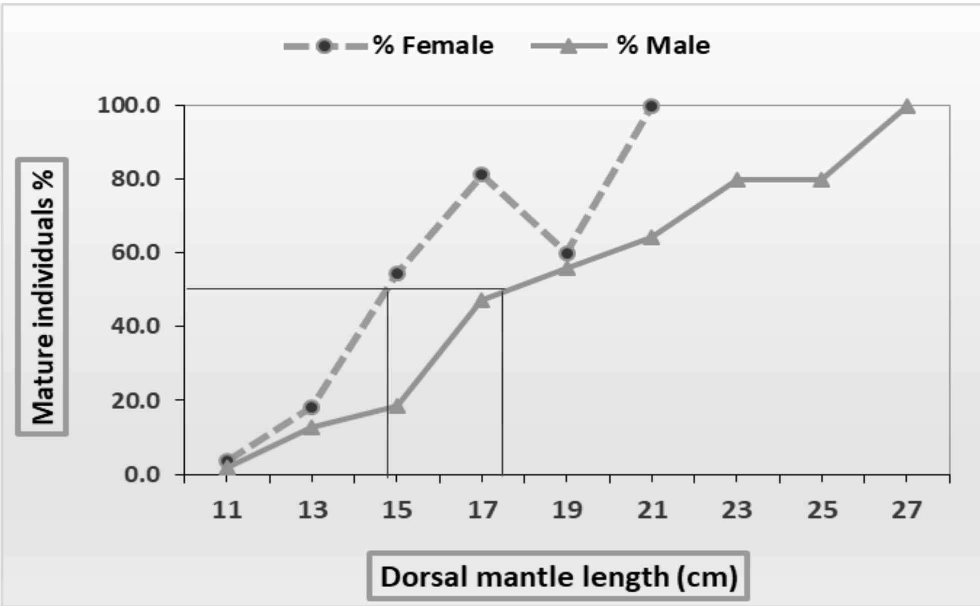

Figure 7. Percentage of maturing, mature and spawning of females and males *L. duvauceli* for each dorsal mantle length class from the Gulf of Suez.

Indices of reproductive parameters

In males: The variation in maturity indices; gonadosomatic index (GSI), spermatophoric organs index (SOI) and maturity coefficient (MC) for males of *L. duvauceli* were estimated during the four seasons (Figure 8). The gonadosomatic index ranged from 0.52 to 1.02, with an average of 0.76. The spermatophoric organs index (SOI) varied from 0.31 to 0.44, with an average of 0.39. The maturity coefficients (MC) ranged between 0.84 and 1.46 with an average 1.15. Generally, males had two peaks (summer and spring) in all studied maturity indices in the year around spawning period (Figure 8).

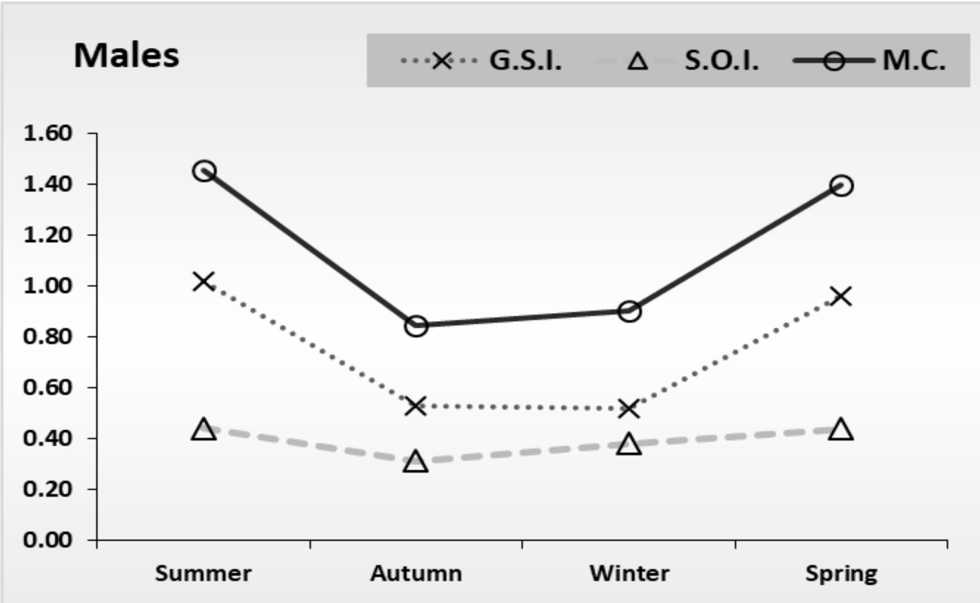

Figure 8. Seasonal variations in maturity indices for male *L. duvauceli* from the Gulf of Suez.

The relationship between testis weight (TW) and total body weight (TBW) was linear regression (Figure 9 A) giving the equation: TW = 0.011 TBW – 0.187 (R^2 = 0.690). While the relationship between testis weight (TW) and dorsal mantle length (DML) with power regression equation (Figure 9 B): TW = 1^{-4} $DML^{3.11}$ (R^2 = 0.805).

The relationship between spermatophoric organs weight (SOW) and total body weight (TBW) was linear regression (Figure 10 A) giving the equation: SOW = 0.0064 TBW – 0.1045 (R^2 = 0.747). While the relationship between spermatophoric organs weight (SOW) and dorsal mantle length (ML) with power regression equation (Figure 10 B): SOW = 0.0001 $ML^{2.857}$ (R^2 =0.769).

The relationship between gonad weight (GW) and total body weight (TBW) was linear regression giving the equation (Figure 11 A): GW = 0.0164 BW – 0.264 (R^2 = 0.707). While the relationship between gonad weight (GW) and dorsal mantle length (DML) (Figure 11 B) with power regression equation: GW = 0.0001 $DML^{3.189}$ (R^2 = 0.736).

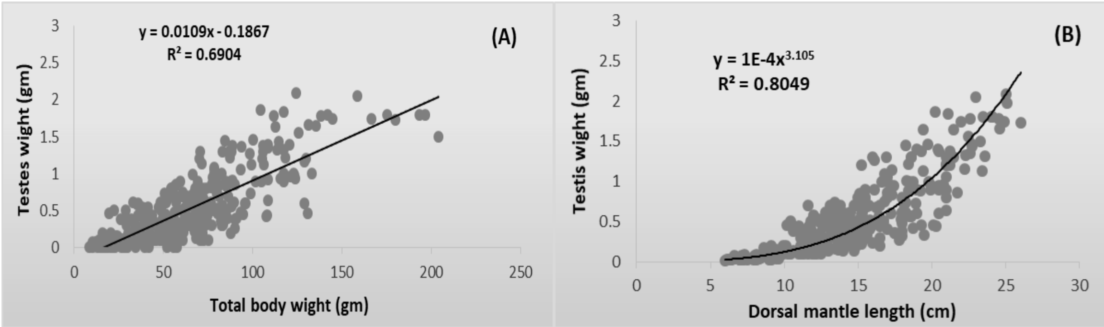

Figure (9): Relationships between testis weight and Total body weight (A) & dorsal mantle length (B) for males *L. duvauceli* from the Gulf of Suez.

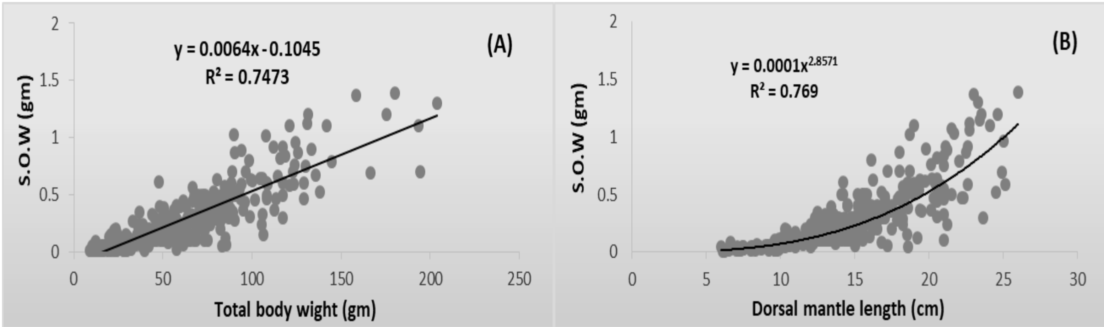

Figure (10): Relationship between spermatophoric organs weight and total body weight (A) & dorsal mantle length (B) for males *L. duvauceli* from the Gulf of Suez.

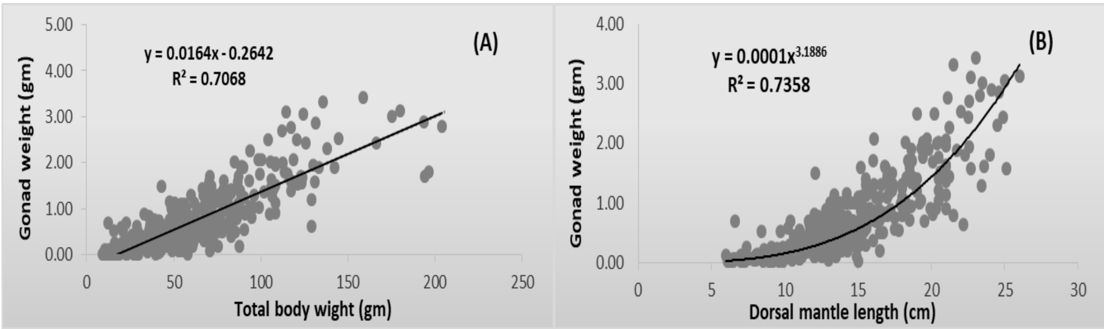

Figure (11): The relationship between gonad weight and total body weight (A) & dorsal mantle length (B) for males *L. duvauceli* from the Gulf of Suez.

In females: The variation in maturity indices; nidamental gland index (NGI), oviductal gland index (OGI) and gonadosomatic index (GSI) as well as maturity coefficients (MC) for females *L. duvauceli* were estimated during the four seasons (Figure 12).

Nidamental gland index (NGI) ranged from 1.20 – 2.54, with an average of 2.09 and its graph (Figure 12) shows that females had one peak at the winter in the fishing season around spawning period. The oviductal gland index (OGI) varied from 0.97 to 2.14, averaged 1.66 and it had one peak also at the winter. The gonadosomatic index (GSI) ranged from 1.33 to 2.14, averaged 1.83 and it had two peaks (autumn and spring). The maturity coefficients (MC) varied between 2.83 and 4.17 with an average of 3.50 with a peak at the spring (Figure 12).

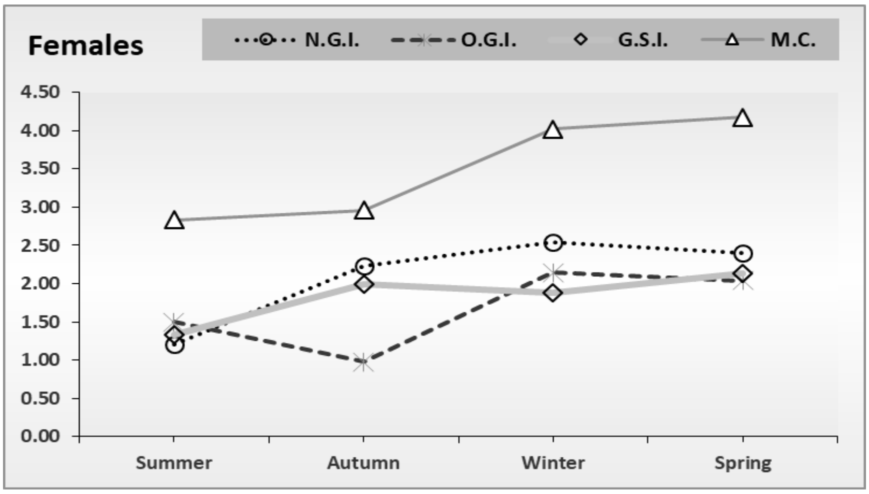

Figure 12. Seasonal variations in maturity indices for females *L. duvauceli* from the Gulf of Suez.

The relationship between nidamental gland weight and dorsal mantle length (Figure 13 A) with power regression equation: NGW = 3^{-8} $DML^{6.797}$ (R^2= 0.740). While the relationship between nidamental gland weight and total body weight was linear regression (Figure 13 B) giving the equation: NGW = 0.061 TBW – 1.414 (R^2= 0.694). The relationship between ovary weight and dorsal mantle length (Figure 14 A) with power regression equation: OW = 1^{-8} $DML^{7.061}$ (R^2= 0.755). While the relationship between ovary weight and total body weight was linear regression (Figure 14 B) giving the equation: OW = 0.057 BW – 1.257 (R^2 = 0.731).

The relationship between reproductive system weight and dorsal mantle length (Figure 15 A) with power regression equation: OW = 1^{-6} $ML^{5.743}$ (R^2= 0.747). While the relationship between reproductive system weight and total body weight was regression linear (Figure 15 B) giving the equation: OW = 0.159 BW – 3.811, R^2 = 0.739.

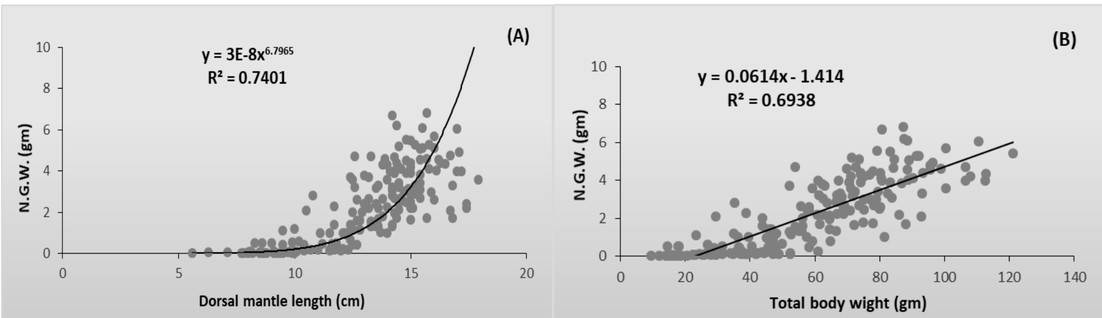

Figure 13. The relationship between nidamental gland weight (NGW) and dorsal mantle length (A) & total body weight (B) for females *L. duvauceli* from the Gulf of Suez.

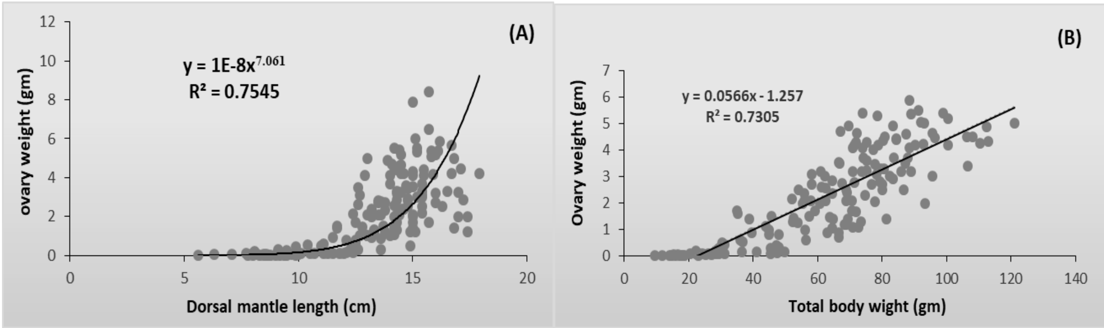

Figure 14. Relationship between ovary weight and dorsal mantle length (A) & body weight (B) for females *L. duvauceli* from the Gulf of Suez.

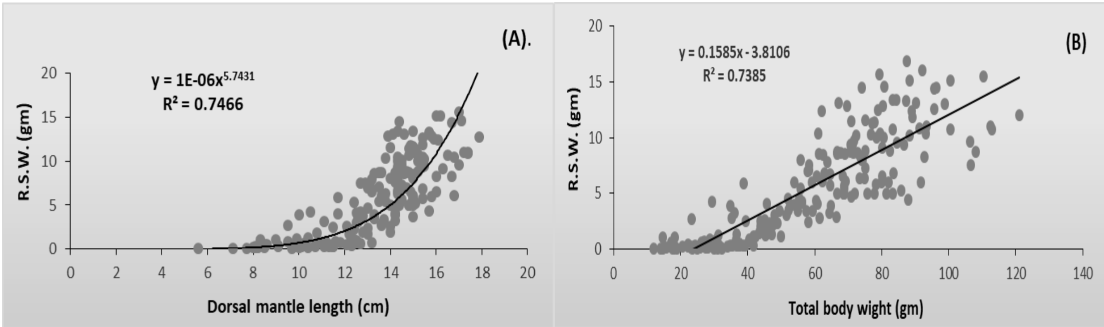

Figure 15. Relationship between reproductive system weight (RSW) and dorsal mantle length (A) & total body weight (B) for females *L. duvauceli* from the Gulf of Suez.

DISCUSSION

The differential sex ratio of squids is more or less, similar to that known from previous observations. Generally, the ideal 1:1 male: female ratio rules do not exist in nature as seen from many earlier findings. This is more or less similar to the results on the same species in Andaman Sea of Thailand; the number of males was greater than females with a mean ± SD of 1.1 ± 1.0 except in June and late July **(Sukramongkol *et al.*, 2007)**. Also, in Hong Kong water, from Sai Kung trawl samples, the overall sex ratio (males: females) was 1.0 :0.91 **(Sang, 2007)**. **Kilada & Riad (2010)** revealed that both sexes of *U. duvauceli* in Northern Red Sea, Egypt were present in almost equal ratios in the summer, fall, and winter seasons, although males outnumbered females in spring and the ratio was 1.6:1.0 in favor of the males, which is very close to the sex ratio in this study. Recently, **Mohamed (2013)** showed that sex ratio of *Uroteuthis duvauceli* in Suez Canal (males: female) was 1.0 :0.8 for the whole year with males biased in the overall prevalence. Females significantly outnumbered males in April and October 2010 only, where males were dominant in February, May, June and July 2010. However, this ratio is different from the sex ratio in Saurashtra region, Gujarat, India where the overall ratio was 1.0 :1.3 in favor of females **(Thomas & Kizhakudan, 2006)**.

Males and females in relation to dorsal mantle length results agree with several studies on the same species such as **Supongpan *et al.* (1992)** in west coast of Gulf of Thailand, the maximum sizes of males and females were 26 cm and 18 cm, respectively. Also, in east coast of India, 26 cm for males and 21.5 cm for females **(Meiyappan *et al.* 1993)**. Moreover, in Saurashtra region, Gujarat, male and female specimens recorded measured 28.5 cm and 27.5 cm DML respectively **(Thomas & Kizhakudan, 2006)**. Also, in Northern Red Sea, Egypt the maximum length recorded for females was 18.5cm and 23.5cm for males **(Kilada & Riad, 2010)**. Recently, *U. duvauceli* caught from Suez Canal were medium in size, where largest sizes recorded for males and females 27 and 19.5 cm respectively **(Mohamed, 2013)**. Unless *U. duvauceli* in Hong Kong, females larger than males, 10.5cm and 8.5cm respectively **(Sang, 2007)**. This may be related to geographical and latitudinal differences. Where, Hong Kong is located in the subtropics, in comparison to the tropical regions of India and Thailand.

Results of spawning season of *L. duvauceli* in the present study may be indicated that it was from April to October for females, while males from April to September. Many studies discussed the spawning season of *L. duvauceli* from different regions. **Rao (1988)** observed that from December to May can be generally considered as the major spawning season for *L. duvauceli* from Mangalore, India. **Meiyappan & Srinath (1989)** showed that this species in Cochin, Indian water seems to spawn almost throughout the year without any clear-cut seasons. While **Supongpan *et al.* (1992)** in west coast of the Gulf of Thailand found that there are two spawning periods from December to March and from July to October. **Mohamed (1993)** observed that the peak spawning season of *L. duvauceli* in Mangalore and Malpe was in September and October. In Saurashtra region, Gujarat, **Thomas & Kizhakudan (2006)** showed that the spawning season was found to occur throughout the year with peak during post monsoon period in December. **Sang (2007)** found that *U. duvauceli* spawns throughout the year with the peak in summer season (June - August) and with a minor peak in winter (January - March) in Hong Kong water. Also, in Northern Red Sea, Egypt **(Kilada & Riad, 2010)** showed that the spawning season was in winter and early spring. Recently, **(Mohamed, 2013)** detected that the peak spawning of females *U. duvauceli,* from Suez Canal occurs during spring to early autumn (April – September), while the peak spawning for males was in summer (June to August).

Both males and females found in Gulf of Suez attained first maturity at a larger length. This may suggest that animals that mature at smaller length have slower growth rates than those maturing at larger length, both being of equivalent ages. Length at first maturity of the same species in Mangalore, India, was 12.4cm for male and 10.8cm for female **(Rao, 1988)**. In Cochin, India, they were generally small 12.5cm and 12.9cm for males and females, respectively **(Meiyappan & Srinath, 1989)**. In Hong Kong, length at 50% maturity of males was 7.1cm DML from both Sai Kung and Lamma Island samples, while that of females was 10cm and 9.6cm, respectively **(Sang, 2007).** In Thailand, DML at 50% maturity of males and females were 7.89 and 9.41 cm for the Gulf of Thailand and 10.69 and 9.15 cm for the Andaman Sea **(Srichanngam, 2010)**. Length at first maturity in this study was greater than that in many of the other regions. This may be due to the influence of environmental conditions such as temperature. In the present study, it was suggested that the stages of maturity were related more to size (length) than age. The size is closely related to external factors such as temperature and food availability, which agrees with **(Jackson & Moltschaniwskyj, 2001; Forsythe, 2004 and Yassien *et al.*, 2016)**.

Monthly variations in maturity stages and Seasonal variations in maturity indices showed that the spawning season of *L. duvauceli* for females is may be from spring to early autumn, while that of males is may be in spring and summer. Mature animals of both sexes can be found throughout most of the year. The comparison of the sexual cycle of *L. duvauceli* with data from different regions showed similarities as well as differences. This indicated that spawning season and the reproductive peaks for *L. duvauceli* are very flexible and probably closely linked to environmental conditions.

CONCLUSION

The present study is important as it gives clear information of the current reproductive aspects of the Indian squid *L. duvauceli* in the Gulf of Suez, to improve the current situation and sustain the fishery stock of this species in the Gulf of Suez, and it recommends stopping fishing during the spawning season (spring to early autumn).

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