

Maturation, Fecundity and Seasonality of Reproduction of the Squid *Uroteuthis duvauceli* (Cephalopoda:Loliginidae) in the Suez Canal

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ABSTRACT

This study investigates for the first time the reproduction biology of the Squid *Uroteuthis duvauceli* in the Suez Canal, over 4 seasons. The sexual maturity was analyzed using the gonad condition in both sexes and by the color of the accessory nidamental glands in females. Spawning season was determined by calculating the gonadosomatic index (GSI) in both sexes as well as the nidamental gland-somatic index in females and spermatophoric complex index in males. Fecundity was estimated in 20 females that were in stage IV (spawning) and had dorsal mantle lengths (DML) ranging from 14.5-18.5 cm. The incidence of mating was indirectly detected by the presence of spermatophores in the seminal receptacle situated near the buccal membrane of the arm crown of females. The spawning season takes place in late spring and summer. Significant positive correlations were detected between fecundity and dorsal mantle length ($r^2=0.7753$), body weight ($r^2=0.4702$) and weight of ovary ($r^2=0.6665$). Most of spawning females (80.32%) were found mated and fully mature females (17.21%) were observed mating. No females at stage I were found to have been mated.

Key Words: Cephalopoda, Deversoir, Fecundity, *Loligo*, Reproduction, Spawning, Squid, *Uroteuthis duvauceli*.

INTRODUCTION

The cephalopods have a varied range of reproductive strategies, from typically semelparous (e.g. the coleoids) to fully iteroparous (e.g. *Nautilus sp.*). Semelparous females lay eggs in one single spawning bout or in several consecutive bouts, with no pause between each, after which they die. The animals with this reproductive pattern where gonad regeneration is absent are semelparous. Iteroparous species are those in which gonadal regeneration follows spawning making a new reproductive cycle. All loliginid squids have a strong preference for the benthic environment for feeding and spawning (Boyle and Rodhouse, 2005). They are all demersal spawners, in which the egg capsules are attached to any natural or artificial surfaces such as rocks, macroalgae, fishing traps etc.

Previous reproductive and fishery studies on *U. duvauceli* have been done in different regions such as Thailand (Supongpan *et al.*, 1992; Chotiyaputta, 1993; Sukramongkol *et al.*, 2007), India (Abdul Rahim and Chandran, 1988; Mohamed, 1993; Kaewnuratchadasorn *et al.*, 2003) and China (Sang, 2007). Very few recent studies have been done on northern Red Sea in Egypt (Kilada and Riad, 2010) and no studies have been done in Suez Canal; therefore, this paper presents the first detailed study of the reproductive biology of the Squid *U. duvauceli* in the Suez Canal region, based on specimens taken between 2010-2011.

This study deals with sex ratio, size at first maturation, spawning season, variation in maturation indices with maturity stage and fecundity.

MATERIALS AND METHODS

Collection and processing of specimens

Samples of *U. duvauceli* were collected monthly from the main fishing port in Deversoir, on the Suez Canal

from January 2010 to March 2011. A sub-sample of 5-10 animals (representing the full size range) was taken from each monthly sample for histological investigation. Small sections of testis and ovary were fixed for 24 hours in 10% formalin and then transferred to 70% ethyl alcohol for preservation. Clearing and paraffin embedding were performed using standard histological techniques; sections were cut at 6µm thickness and stained with hematoxylin (H) and eosin (E). Specimens were dissected by cutting along the ventral mantle to reveal the internal organs to determine the sex and the maturity stage. For each individual, sex was determined by checking the presence of the left arm IV hectocotylized (modified arm) typical for males (Richard, 1967). For some smaller individuals that could not be distinguished by sexual characteristics, they were then assigned as 'juvenile' where no maturity stage was applied. Four maturity stages for the female and five maturity stages for the male were determined using a modification of the scale proposed by Lipinski and Underhill (1995). Indices of reproductive status for males and females were calculated (after Durward *et al.*, 1979; Lipinski, 1979; Juanico, 1983; Ngoile, 1987; Joy, 1989) as follows:

For females:

Gonad somatic index: $GSI=100 \text{ OW} / \text{BW}$

Nidamental gland index: $NGI=100 \text{ NGW} / \text{BW}$

Nidamental gland length index: $NGLI=100 \text{ NGL} / \text{DML}$

Maturity coefficient: $MCO=100 (\text{OW} + \text{OV} + \text{W}) / \text{BW}$

Where: OW= ovary weight, BW= body weight, NGW= nidamental gland weight, NGL= nidamental gland length, DML=dorsal mantle length and OV W=oviductal complex weight.

For males:

Gonad somatic index: $GSI=100 \text{ TW} / \text{BW}$

Spermatophoric complex index: $SCI=100 \text{ SCW} / \text{BW}$

Maturity coefficient: $MCO= 100(\text{TW} + \text{SCW}) / \text{BW}$

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Where: TW= testis weight and SCW= Spermatophoric complex weight.

Estimation of fecundity

Fecundity was defined as the total number of all maturing ova (carrying striation) and mature ova (large smooth ova) in the ovary, and the number of ova in the oviduct (proximal and distal parts). Fecundity was estimated in 20 females that were in stage IV (spawning) and had mantle lengths ranging from 14.5-18.5 cm DML.

Mating incidence

The incidence of mating was indirectly detected by the presence of spermatophores (more specifically the remains of the everted spermatophores, or spermatogonia) in the seminal receptacle situated near the buccal membrane of the arm crown of females (Lum-Kong, 1992; Lipinski and Underhill, 1995). Following mating, spermatophores can be stored by the females for a certain period until required.

RESULTS

Sex ratio

Sex ratio of *Uroteuthis duvauceli* in Suez Canal (Male: Female) was 1:0.81 for the whole year with males biased in the overall prevalence. There were seasonal variations in the sex ratios. Females notably outnumbered males in spring, especially in April. On the other hand, males were dominant in summer (particularly in June). Juveniles outnumbered females and males in autumn (particularly in October and November) (Fig. 1). Looking at the sex ratio with respect to length class (Fig. 2), juveniles were small in size and less than 12 cm DML and a dominance of females over males was noticed for smaller individuals 12–17.9cm DML, while males clearly dominated from 18cm DML onwards.

Oogenesis

Oogenesis began when germ cells differentiated and became primary oogonia. Primary oogonia enlarged to become secondary oogonia that in turn become oocytes. Oogenesis could be defined by four stages depending on the degree of follicular cell development in association with oocytes (Fig. 3).

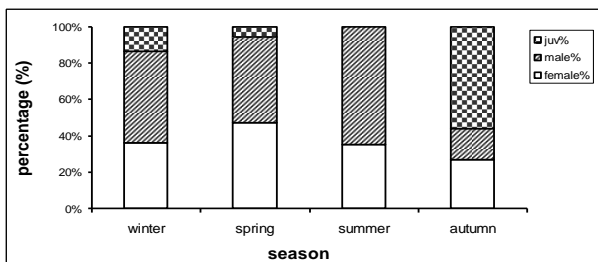


Figure (1): Seasonal variation in sex ratio of *U. duvauceli*.

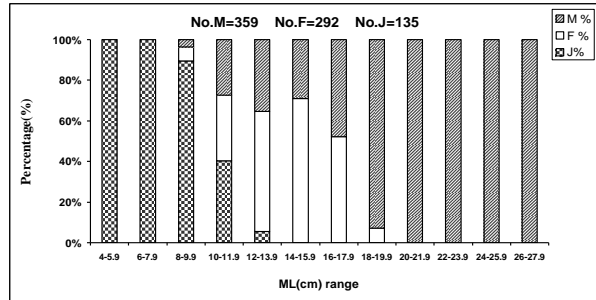


Figure (2): Sex ratio for *U. duvauceli* with respect to mantle length.

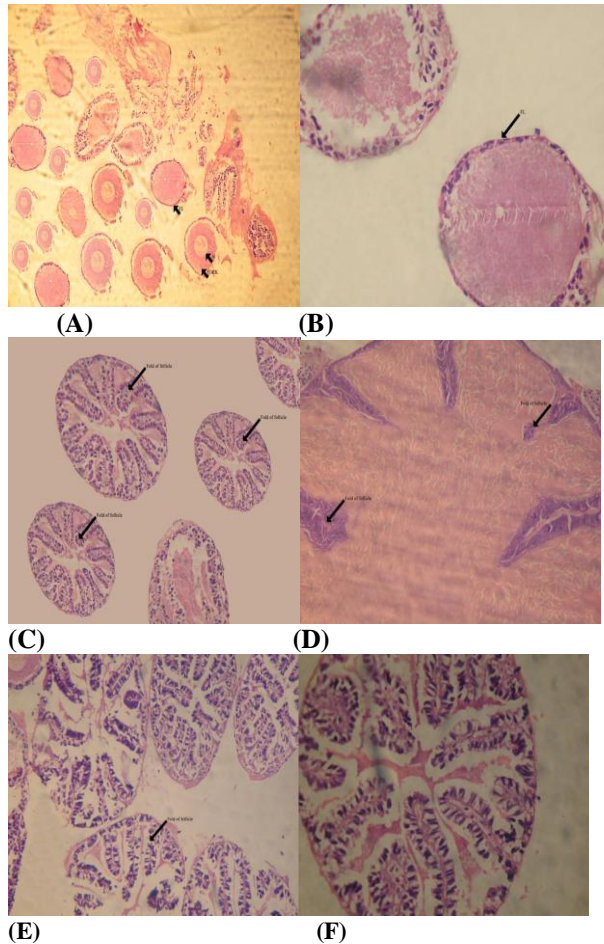


Figure (3): Microscopic stages of oocyte development for *U. duvauceli*: (A) Stage I, immature; (B) magnification of an oocyte showing squamous follicular cells (C) Stage II, maturing; (D) Stage III, fully mature, (E) magnification of an oocyte showing folds of follicle and (F) Stage IV, spawning. FL: follicle cells, N: nucleus.

Spermatogenesis

The testis of mature male squid is formed of seminiferous tubules that contain different stages of spermatogenesis. From the periphery to the centre of each tubule are found: spermatogonia, spermatocytes, spermatids and spermatozoa respectively. These different germ cells are derived from spermatogonial differentiation. Spermatogenesis can be divided into 5.

stages according to the degree of development within the seminiferous tubules (Fig. 4).

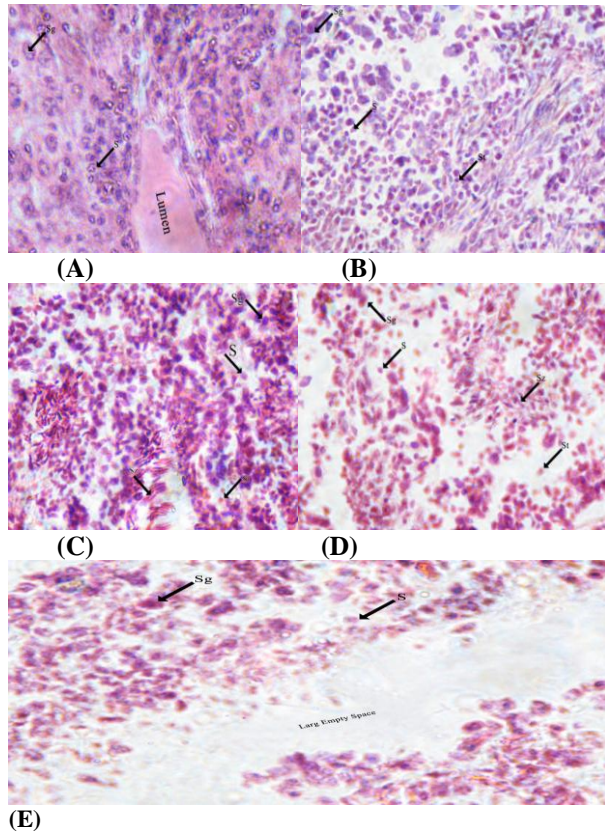


Figure (4): Microscopic stages of spermatid development for *U. duvauceli*: (A) Stage I, immature; (B) Stage II, maturing; (C) Stage III, fully mature; (D) Stage IV, spawning and (E) Stage V, spent. Sg: spermatogonia; S: Spermatocyte; St: spermatid; Sz: spermatozoa.

Size at first maturation

Size at first maturation is defined as the size at which 50 percent of the individuals are maturing or mature (Bakhayokho, 1983). Figure (5) illustrates the relationship between the proportion of maturing and mature animals (stages II, III, and IV inclusive) and the dorsal mantle lengths (DML) of *Uroteuthis duvauceli*.

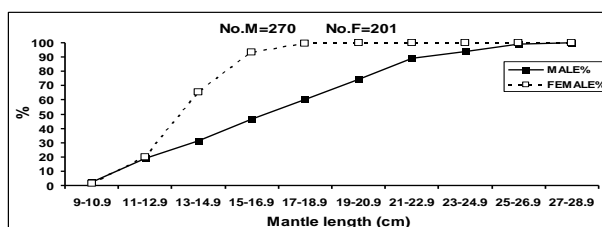


Figure (5): Percentage of maturing and mature (Stage II to IV) female and male *Uroteuthis duvauceli* for each mantle length class.

This figure indicates that the females of *U. duvauceli* became mature at smaller DML and attained a smaller adult size than did the males. The mean size at maturity for this species was 13-14.9cm and 15-16.9cm DML for

females and males, respectively. The size range of mature animals was wide, 11- 19.5 cm DML in females; 11-27 cm DML in males.

Maturity indices

Monthly variation in maturity indices

For *U. duvauceli* monthly variation in maturity indices (GSI, NGI, SCI and MCO) was pronounced for both sexes (Fig. 6). Female maturity indices increased in March and reached a peak from April to September, this coincided with peak percentage of specimens in spawning condition. All maturation indices declined gradually starting from October and reached their lowest values in November and December. Male maturity coefficient (MCO) showed the clearest pattern, increasing gradually in January and reached its highest peak in August.

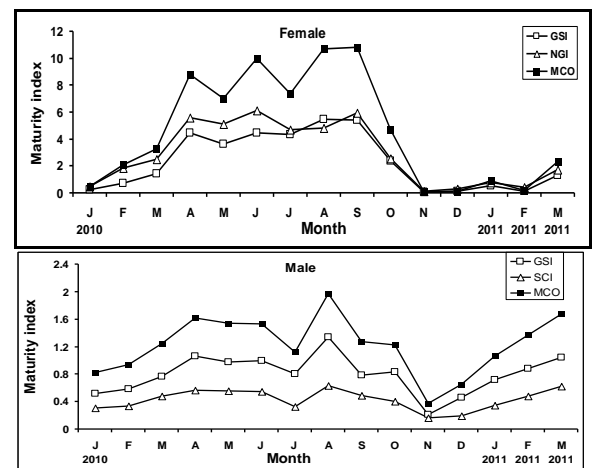


Figure (6): Variation in monthly means of gonad somatic index (GSI), nidamental gland index (NGI), spermatophoric complex index (SCI), and maturity coefficient (MCO) for female and male *Uroteuthis duvauceli*.

Incidence of Mating

Out of the 292 females examined for mating (spermatophores in the buccal membrane), 122 females (41.78%) of all individuals had been mated.

While no females at stage I were found mated, there were 80.32% of spawning females (stage IV) mated and 17.21% of the fully mature females (stage III) were observed mating. Only 2.46% of maturing females (stage II) had mated (Fig. 7).

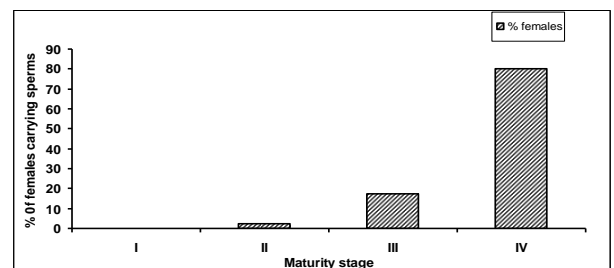


Figure (7): The relation between maturity stages and percentage of female *Uroteuthis duvauceli* carrying sperms in their seminal receptacles.

In terms of the percentage of mated females by month (Fig. 8) peaks were found from April to July 2010; with the highest peak in April 2010. This coincided with the high percentage of mature females. Minimum numbers of mated females were detected in January and September 2010 and no mating took place in November, December 2010 and February 2011.

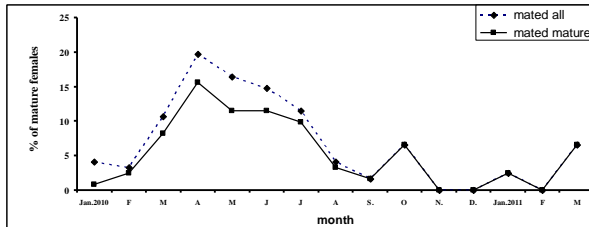


Figure (8): Monthly percentage of all mated and mature mated females of *U. duvauceli* from Suez Canal, January 2010 to March 2011.

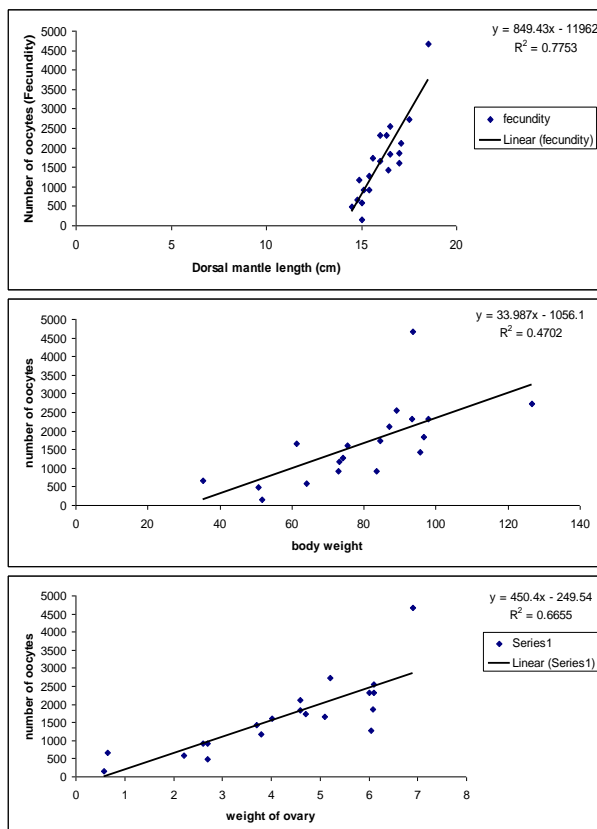


Figure (9): Relationship between fecundity (dorsal mantle length, ovary and ovary weight) of *U. duvauceli*

Ova and Fecundity

The morphology and size of the ova and the morphology and the relative size of the gonads aid in estimating of female maturity (Voss, 1983). Mangold-Wirz (1963) and Boyle and Knobloch (1983) observed in several octopod species that the stage of egg maturation is indicated by the appearance of striations on the surface of the ova.

To obtain an estimate of fecundity, the number of maturing and mature ova was counted from females at

stage IV (spawning). It was assumed that the immature ova would not be spawned that season. The total number of ova in spawning female (stage IV) ranged from 480 ova in a female measured 14.5cm DML and an ovary weight of 2.69 g to 4671 ova in a female 18.5 cm DML and an ovary weight of 6.9g. There was a tendency for the larger females to be more fecund than the smaller ones.

Fecundity (as number of oocytes) was plotted against (a) dorsal mantle length, (b) body weight and (c) ovary weight (Fig. 9). Significant positive correlations were detected ($r^2 = 0.7753, 0.4702$ and 0.6665 respectively) between fecundity and (a), (b) and (c).

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. It is possible that differences between male and female abundance (sex ratio) of *U. duvauceli* in the Suez Canal could be due to catch bias rather than to population structure. Nevertheless, the pattern observed when examining the sex ratio with respect to size is quite interesting.

The preponderance of males over females at larger sizes was expected, considering that males grow to sizes exceeding these of the females. On the other hand, at smaller sizes females override males as males has higher growth rate than females (Supongpan and Natsukari, 1996). *U. duvauceli* caught from Suez Canal were medium in size, where largest sizes recorded for males and females were 27 and 19.5 cm respectively. Variability in size at maturity appears to be a common phenomenon in cephalopods. Both males and females found in Suez Canal attained first maturity at a larger size.

This may suggest that animals mature at small sizes have slower growth rates than those maturing at large sizes, both being of equivalent ages. Size at first maturation in this study is greater than that in many of the other regions; this may be due to the influence of environmental conditions such as temperature. The maturity scale proposed in this work shows reliable correlation with the maturity indices and histological results for all stages. This scale is relatively easily applied without ambiguity. Curiously, no spent females (stage IV) were ever found in *Uroteuthis duvauceli* in this study.

These females may eventually move off the fishing ground, where they may be preyed upon due to their weakened condition, or they might return to the spawning ground in the next season. One of the possible reasons contributing to different spawning patterns sometimes seen in the same species of loliginid squid is a latitudinal difference. This indicates that latitudinal differences in terms of sea temperature would affect spawning strategies, where those species inhabiting tropical regions may experience higher growth rates,

shorter life spans and more rapid population turnover (Jackson, 2004). Mating and egg laying have not been observed directly for *U. duvauceli* in this study. It can be inferred that mating takes place from approximately March until July with highest peak in April. The presence of spermatophores in the seminal receptacle near the buccal membranes of some loliginidae is typically used as proof of mating activity (Boyle and Rodhouse, 2005). Egg hatchling is suspected to take place in Autumn season, where juveniles were totally absent in Summer season and appeared extensively in Autumn. In general, fecundity is low in cephalopods because of the absence of a larval stage and the hatchlings are virtually miniature adults. There were significant positive correlations between the number of oocytes and (1) dorsal mantle length (DML), (2) body weight and (3) ovary weight.

After all it can be assumed that the behaviour, as well as the structure of the squids are greatly responsible of the shortening of its life span (about one year). These animals are more or less aggressive against many of the strong animals, including vertebrates such as fishes and some of them may be larger in size than the squids. Such enemies will get squids exerting much effort during most of their life. This very highly energetic life will lead to their short life span. During their short life the animal must develop, mature, copulate and give birth to new generation. These generations are suspected to be vigorous in the populations.

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النضوج الجنسي، وكمية البيض، والتناسل الموسمي للكالمارى *بيروتيثيس دوفوسيلي* (رأسقدميات – لوليجينيدي) في قناة السويس

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الملخص العربي

تهتم هذه الدراسة لأول مرة ببيولوجية التكاثر عن الكالمارى *بيروتيثيس دوفوسيلي* في قناة السويس في اربعة مواسم متصلة. وقد تم تحليل النضوج الجنسي باستخدام حالة المناسل في الجنسين وبلون غدد العشيتين (نيدامنتال) في الأنثى. ولقد تحدد موسم التناسل بعد حساب المعامل الجسدى المنسل في كلا الجنسين وكذلك معامل الغدتين العشيتين الجسدى في الأنثى ومركب معامل حامل المنى في الذكر. وتم تقدير كمية انتاج البيض في الأنثى في طور رفق (الفقس) وفيها تراوح طول البرنس ما بين 14,5 و 18,5 سم. ولقد لوحظ الجماع بطريقة غير مباشرة وذلك عن طريق تواجد حاملات المنى في المستقبل المنوى الذى يوجد قرب الغشاء الحنكى لتاج الأذرع في الأنثى. ولقد تبين ان موسم التناسل يقع ما بين أواخر الربيع والصيف، ولقد ظهرت علاقات ايجابية واضحة بين كمية البيض وطول البرنس ظهريا ووزن الجسم، ووزن المبيض. ولقد وجدت معظم الإناث في حالة وضع بيض (80,3%) والتي في حالة نضوج جنسى كانت قد قامت بعملية التزاوج، بينما اناث الطور الأول لم توجد في حالة تزاوج.