بيولوجية التكاثر للحبار (*Loligo forbesi*) في البحر المتوسط

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ملخص البحث

شملت هذه الدراسة بيولوجية التكاثرلذكور وإناث الحبار في البحر المتوسط حيث أثبتت الدراسة أن نسبة الاناث فاقت نسبة الذكور أثناء موسم التكاثر . كما تم تحديد مرحلتين للنضوج الجنسي للذكور ، وخمس مراحل للإناث من خلال الشكل الخارجي للمناسل إعتمادا على دراسة تغيرات مؤشر حالة المناسل ، وتغيرات مؤشر حالة الغدة الانثوية (النداميننتال) تم تحديد فصلي الربيع ، وبداية الصيف كموسم لوضع البيض .

Reproductive biology of the squid Loligo forbesi from the Mediterranean Sea

ABSTRACT

Reproductive biology of male and female were investigated from samples obtained from commercial catch caught by local fishermen using trawling net. The sample were seasonally collected (548).The results of sex ratio indicated that females outnumbered males during peak period of reproduction. Based upon morphological features of the gonads, two and five maturity stages for males and females were described, respectively. Depending on the proportion of each maturity stag, as well 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 early summer. The mean size at first maturity was 13 and 11.8cm. dorsal mantle length (ML) for males and females respectively.

INTRODCTION

Squid loliginidae play an important role in the food chain of the sea. They belong to the third trophic level in the food chain. They contain about 500 species of squids in different

seas and oceans (Standingar *et al*., 2013)[1]. Their size range from 1-2 inches of the tiny squid, *Idiosepius pygmaeus*, to 60 feet of the giant squid, *Architeuthis* sp. Squids are divided into two groups, the myopsid and the oegopsid (Standingar *et al*., 2013)[1]. The myopsid group inhabits the continental shelf regions and have covered eyes, however the oegopsids live in the open ocean. The major exploited family of myopsid squids is the Loligonidae, with major genera, *Loligo*, *Photololigo* and *Sepioteuthis*. Despite the abundance of loliginids in continental shelf habitats and their commercial and scientificed importance, loliginidae classification and phylogeny remain confusing (Vecchione *et al*., 1998)[2]. Regarding the distribution of *Loligo forbesi* extends throughout the Mediterranean Sea and Red Sea, Eastern Atlantic (excluding the Baltic Sea), Azores Islands and along West African coast south to the Canary Islands (Pierce *et al*., 1998)[3]. *L. forbesi* is commonly found in the following areas of the United Kingdom; the English Channel (Holme, 1974)[4], Irish Sea (Collins *et al*., 1995)[5], Rockall Bank and the Scottish west coast (Pierce *et al*., 1994a)[6] and in the Moray Firth (Young *et al*., 2006)[7]. Life history and biology of *Loligo forbesi* have been thoroughly investigated in various locations. Holme (1974)[4] reported on its life history in the English Channel. Most of previous study carried out investigation have focused on growth of *L. forbesi* using the Gonado-Somatic Index (GSI) (Boyle *et al* 1995)[8]. Information on maturation and spawing of *Loligo forbesi* will contribute to our knowledge of its general biology, population dynamics, and management of their stocks. In corrent study was designed to address the lack of information regarding the reproduction biology of *L. forbesi* in the Mediterranean Sea.

MATERIAL AND METHODS

Seasonal specimens of the squid *Loligo forbesi* were collected (548).The samples were caught by local fishermen using trawling net. All specimens were frozen and stored till

examined. After thawing at room temperature, the total body wet weight (To.Wt), gonad weight (G.Wt), nidamental gland weight (N.Wt) and dorsal mantle length(ML) were measured and recorded. The sex was determined by chekingthe left arm IV hectocotylize (modified arm) typical of males (Richard1967)[9]. Length at first maturity is determined according to Snedecor (1956)[10].Reproductive stats indices (Pierce *et al.*1994a)[6] were calculated for males and females in each season. The indices are Gonado-Somatic Index (GSI) for both sexes and the Nidamental -Somatic Index (NSI) for the females were calculated as following:

GSI= G.Wt×100 □ Total.Wt.

NSI=NG.Wt×100 □ Total.Wt. Where G.Wt is the gonad weigh, To.Wt is total body weight, N.Wt is the nidamental gland weight.

Maturity stages of the specimens were determined. The presence of spermatophoric sacs was detected in males, a simplified system of maturity stages was adopted based on modified Lipinski(1979)[11] scale which as following: I-immature, and II-mature, On the other hand, the white, orange,red, coral and rose color of the accessory nidamental gland in females indicated the different maturity stage (Riad1993)[12].Sex ratio,tabulation of the number of males and females,and immature individuals of each sex was determined.

RESULTS:

1-Gonado and nidamental gland-somatic index:

A.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 (G.S.I) is an indication of breeding season onset. The gonado – somatic index attains its maxima during breeding time. The gonado – somatic index of 310 males and 238 females *L. forbesi* were

calculated. Obviously, the seasonal variation trend in G.S.I was found to be more or less like the same for males and females belonging to this species (Fig.1 a.b).

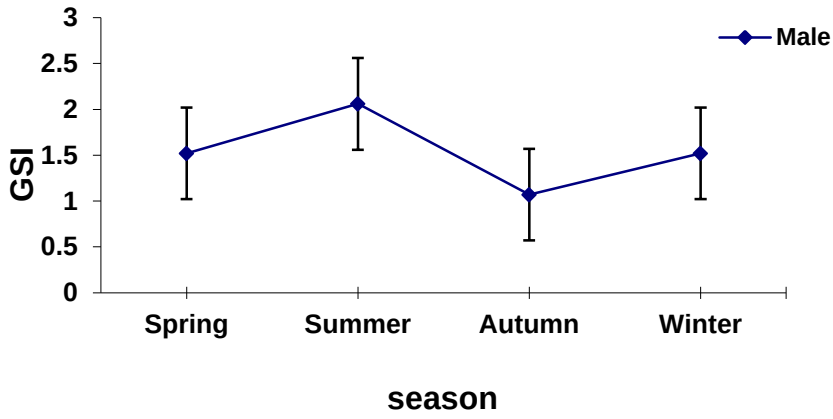

Fig.1.a: Seasonal Gonado somatic index GSI. for males *L.forbesi*.

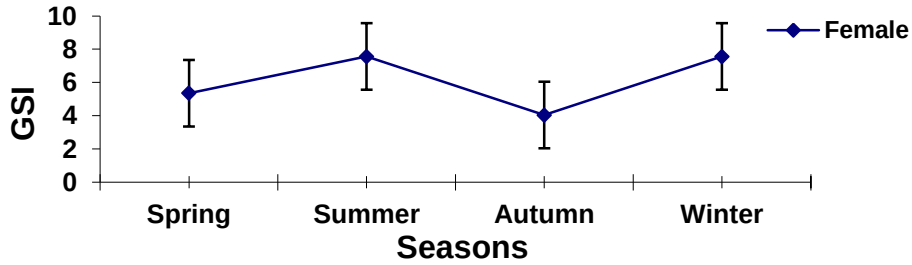

Fig.1,b: Seasonal Gonado somatic index GSI. for females *L.forbesi*.

Gonado somatic index minimal values were attained during autumn. The G.S.I for males and females clarified agradual increase starting in winter, attaining its highest values with a peak during summer (Fig.1 a,b). Thus, we can deduct that spawning peak of both sexes occurs during spring and early summer.

B. Nidamental gland-somatic index:

Female squid have nidamental glands whose function is to secrete a substance for egg protection. Spawning is accompanied by emission of this substance, which envelops the egg which is then being attach to a substrate (Bakhayokho1983), At the time of spawning females have enlarged nidamental glands.The of 238 nidamental gland-somatic index female *L. forbesi* were determined from (Fig. 2)

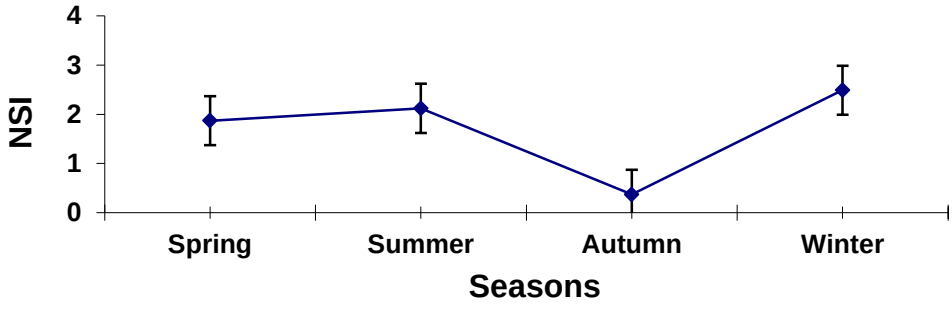

Fig. (2) Seasonal nidamental gland- somatic index(N.S.I) 0f *L. forbesi*

,the seasonal N.S.I variations run more or less parallel to those of the G.S.I seasonal variations(Fig. 2). It was found minimal in autumn and gradually increase attaining its highest values in summer. This confirms the previous conclusion from G.S.I study that spawning occurs during spring and early summer.

2. Maturity stage:

In order to define different maturity stages in males we have to detect the presence of spermatophores in the Needham's sac. On the other hand egg size and color of accessory nidamental gland are good tools for the females Present results obtained classified maturity as two stages for males (immature and mature), five stages for females (immature, maturing, mature, ripe and spent). Stage I-immature, testes can be easily recognized externally, there are

no spermatophores in Needham's sac. In females the eggs are small and accessory nidamental gland is white in color. Stage II – maturing**,** In females the eggs are medium sized and the accessory nidamental gland is orange in color. Stage III – mature Needham's sac can be easily recognized filled with spermatophores. For females the eggs are large sized and the accessory nidamental gland is red in color. Stage IV – ripe in females the eggs are large and the color of the accessory nidamental gland is coral. Stage V – spent, in females no ripe eggs remain and the accessory nidamental gland is rose in color.

Seasonal variations

Seasonal variations of maturity stages during the period between spring for males and females *L. forbesi* and are graphically represented in Figs.(3 and 4), respectively. It is clear that mature males appeared throughout the year, while ripe females appeared in summer and winter. Immature males and females first appeared in the catch during summer and became dominant in autumn. Therefore, *L. forbesi* spawning season (as indicated by the significant higher frequency of mature males and ripe females) occurs during spring and early summer.

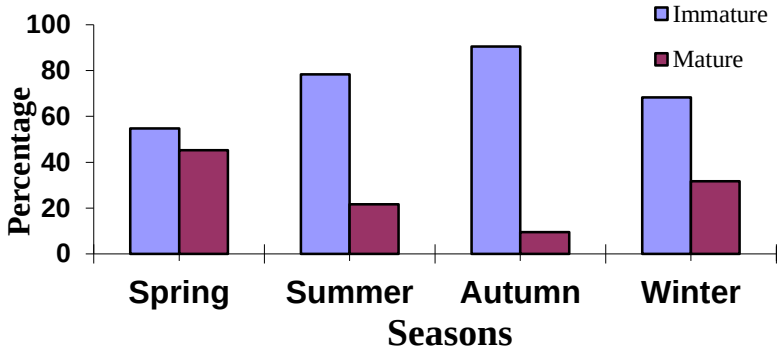

Fig. (3) Seasonal distribution of maturity stages of males *L. forbesi*

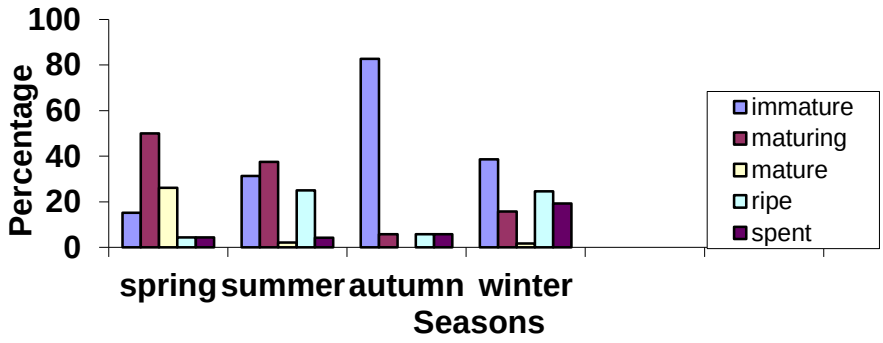

Fig. (4) Seasonal distribution of maturity stages of female *L. forbesi*

3. Size at first maturity:

Knowledge about size of first sexual maturity has its practical application in determination the minimum legal size that may be needed to secure a spawning part of a fish population. In the present work, maturity was studied throughout the year for *L. forbesi.* The percentages of mature *L. forbesi* at 1 cm length intervals for both sexes are given in (fig.5). Present result obtained clarify that all individuals less than 9 cm and 10 cm mantle lengths are immature for males and females *L. forbesi*, respectively. The majority of individuals longer than 12 cm and 11 cm mantle length are mature for males and females *L. forbesi*, respectively.

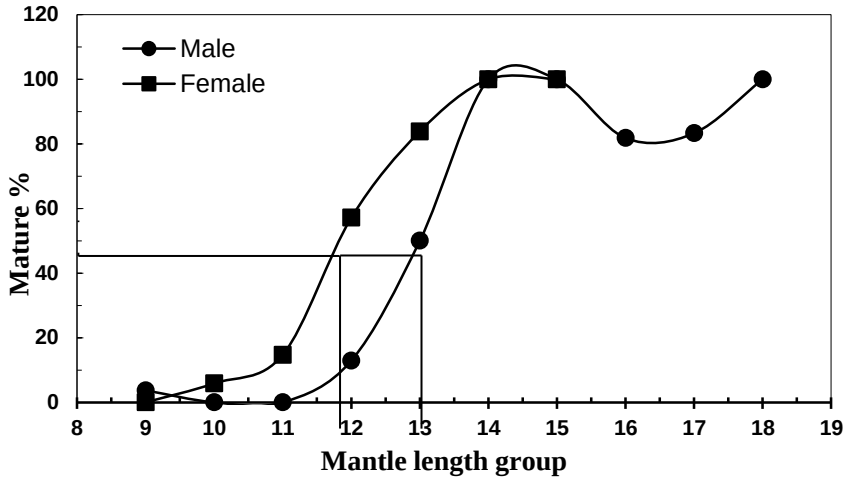

Fig. 5 Relation between mantle length group and mature percentage of males and females *L. forbesi*

4. Sex Ratio:

Table (1) illustrates the ratio of *L. forbesi* males to females of the studied specimens. It appears that males outnumber females throughout all seasons.Table (2) exhibitsthe the ratio of immature to mature *L. forbesi* individuals for both sexes according to seasons. It is evident that, for males, immature outnumber mature throughout all seasons with high percentage in autumn. Mature females show a higher representation than immature ones throughout spring and summer, while immature females were higher than mature ones throughout autumn and winter. This confirms that spawning season occurs during spring and early summer.

Table (1): Seasonal ratio of males to females individuals of *L. forbesi.*

Season	Total No.	Sex	Number	%	Ratios
Spring	99	Males Females	53 46	53.54 46.46	1.2 : 1
Summer	108	Males Females	60 48	55.56 44.44	1.3 : 1

Autumn	**224**	**Males Females**	**137 87**	**61.16 38.84**	**1.6 : 1**
Winter	**117**	**Males Females**	**60 57**	**51.28 48.72**	**1.1 : 1**
All year	**548**	**Males Females**	**310 238**	**56.57 43.43**	**1.3 : 1**

Table (2) : Seasonal ratio of immature to mature males and females of *L.forbesi*.

Season	**Males Total Number**	**Maturity**	**Number**	**Ratios**	**Females Total Number**	**Maturity**	**Number**	**Ratios**
Sprin g	**53**	**Immature Mature**	**29 24**	1.2 : 1	46	**Immature Mature**	9 37	1 : 4.1
Sum mer	**60**	**Immature Mature**	**47 13**	3.6 : 1	48	**Immature Mature**	17 31	1 : 1.8
Autu mn	**137**	**Immature Mature**	**124 13**	9.5 : 1	87	**Immature Mature**	77 10	7.7 : 1
Winte r	**60**	**Immature Mature**	**41 19**	2.2 : 1	57	**Immature Mature**	33 24	1.4 : 1
All year	310	**Immature Mature**	241 69	3.5 : 1	238	**Immature Mature**	136 102	1.3 : 1

DISCUSSION

The maturity index spawning seasons has been described for many loliginid squids i.e. *L. forbesi* and *L. vulgaris* (Moreno *et al*., 1994)[13], *L. gahi* (Hatfield, 2000)[14] . It is worth to note that for most species, a single peak spawning episode occurs each year although two spawning peaks are suggested for *L. gahi* in Falkland Island waters (Hatfield, 2000)[14] and for *L. pealei* in the North West Atlantic (Brodziak, 1998)[15]. Female cephalopods have nidamental glands whose function is to secrete a substance for protection of the egg. Spawning is accompanied by emission of this substance, which envelops the egg, which is then attached to a support (Bakhayokho,1983)[16]. In the present study, the cycles of gonadosomatic index (G.S.I) and nidamental gland somatic index (N.S.I), seasonal variation in the maturity stages and sex ratio show that spawning season for males and females *L. forbesi* occurs during spring and early summer. This spawning cycle is analogous to the cycle observed by other authors working in different regions of the world. Gabr and Riad (2008)[17] for Suez Gulf, Red Sea, Egypt *L. forbesi* and Kilada and Riad (2008)[18] for the Suez Gulf, Egypt for the same species. Their results are in good accordance with that of the present work, which referred that Suez Gulf and Mediterranean *L. forbesi*s spawn during spring and early summer. Pierce *et al*. (1994b)[19] and Collins *et al*. (1995)[5] observed that spawning of the same species in the British waters extended over long period, peaking during winter and spring. In the Irish and Celtic seas spawning of *L. forbesi* occurs primarily during winter, with a peak in December. In the Scottish waters peak spawning of *L. forbesi* was slightly later (Lum-Kong *et al.*, 1992)[20]. In the Faroe Bank *L. forbesi* is reported to spawn mainly in April and May, (Gaard, 1987)[21]. The Rockall Bank squid was suggested by Pierce *et al.* (1994a)[6] to be an additional offshore population of *L. forbesi* which recruits in spring, appearing in summer on the Rockall

Bank and probably spawning occurs during summer. Pierce *et al.* (1994b)[19] recorded mature animals on the Rockall Bank in autumn, but it is not quit sure if spawning occurs there or not. Guerra and Rocha (1994)[22] observed that, breeding season of *L. forbesi* from Galician waters (North-west Spain) extends during winter and spring, with intensive spawning during winter.

In *L. forbesi*, seasonal spawning peaks in winter or spring were reported in the British waters (Boyle and Ngoile, 1993a)[23] and off the Faroes (Gaard, 1987)[21] never the less spawning occurs at other times throughout the year. Memard (2013)[24] suggested a summer spawning for *L. forbesi* in the English Channel. In the Azores islands, the highest values of the gonado- somatic index (G.S.I) were recorded during winter and spring (Porteiro and Martins, 1994)[25]. Lum-Kong *et al.* (1992)[20] recorded spawning females in the Scottish waters during winter and spring, with a peak spawning occurring in spring. Eggs have also been found in summer, which suggests that there is an extended spawning season in the Scottish waters. Moreno *et al.* (1994)[13] indicated that off the Portuguese coast, spawning takes place in autumn and winter. Pierce *et al.* (1994a)[6] stated that, the breeding season for *L. forbesi* in the Scottish waters extends during winter and spring like the same as recorded by Ngoile (1987)[26] in their studies on the Scottish population. Pierce *et al.* (1994b)[19] advocated that, recruitment of both sexes apparently occurred primarily in spring, summer and early autumn; with some small and immature animals existing all the year round. On the other hand, Pierce *et al.* (2004a)[6] stated that, breeding season for *L. forbesi* in the Scottish waters extends during winter and summer. In the Azores, Martins (1982)[27] found mature *L. forbesi* represented in samples from the fishery catch throughout the year, but with a highest degree of maturity in spring. It is likely that spawning areas exist in the north-western part of the North Sea (Oesterwind *et al.*, 2010)[28]. *L.*

forbesi spawning season is considered to be intermittent and terminal, meaning several batches of eggs are laid and death occurs at the end of the spawning season after all of the eggs are laid (Roch and Guerra, 1996)[29]. Changes in the accessory organs can be used to identify trends in maturation and growth of *L. forbesi* in the Scottish waters (Smith *et al.,* 2005)[30]. The exploited population of *L. forbesi* in Galician waters was composed of two groups: one formed by individuals hatched in winter- spring and another by specimens hatched in summer-autumn. These two groups were also observed in *L. vulgaris* (Rocha and Guerra, 1999)[31]. This obviously indicates that the spawning season and the reproductive peak for *L. forbesi* are remarkably flexible and probably this may be attributed to environmental conditions prevailing. Mangold- Wirz (1963)[32] considered temperature and light as the most important external factors that influence sexual maturation in cephalopods.

Maturity stages, for females sexual maturity stages and two for males have been defined. In the present study *L. forbesi* sexual maturity Scales have already been described by several authors. Results of the maturity process and spawning season of *L. forbesi* obtained in the present study were similar to those found by Howaida and Riad (2008)[33] who stated that three maturity stages for *L. forbesi* in the Suez Gulf, Red Sea, Egypt are recognizable and were encountered in both sexes. They added that seasonal variation in the maturity stages and maturity indices show that the mature animals of both *L. forbesi* sexes can be found throughout most of the year with spawning peak for both sexes occurring during spring and early summer. Three maturity indices for males and females *L. forbesi* in the Azores were applied. Both sexes showed highest maturity values in winter and spring, lowest in autumn, but mature squids were found in all monthly samples (Porteiro and Martins, 1994)[25]. For all maturity classes, higher abundance in

winter and spring was associated with deeper water while higher abundance in summer and autumn was associated with shallower water, consistent with seasonal onshore- off shore migrations but suggesting that most spawning may take place in deeper waters. In the Scottish waters for example, there is a seasonal effect on maturity with animals having a higher probability of being mature in winter and spring than in summer and autumn (Pierce *et al.*, 2005)[34]. Mature *L. forbesi* in the Scottish waters were found in commercial samples throughout early and late parts of the year. Immature squid exists in the commercial samples, although with varying abundance, in all months for which there are data except March when the entire sample was mature. The proportion of maturing and mature males in the samples increased from July (summer) onwards, with the highest proportion of mature males in March (spring) (Lum- Kong *et al.*, 1992)[20]. The earlier appearance of mature males *L. forbesi* in the Scottish waters in winter samples suggests that males mature earlier than females (Howard *et al.*, 1987)[35] and also for other loligindes, including *Sepioteuthis arctipinnis* (Rao, 1954)[36] and *Alloteuthis subulata* (Rodhouse *et al.*, 1988)[37]. In the Azores mature *L. forbesi* are present in catches all year round (Martins, 1982[27]; Martins and Porteiro, 1988)[38]. Forsy and Hanlon (1989)[39] suggested that, small immature squid are apparently represented in the population in most months, suggesting that recruitment occurs throughout the year, as might be expected, giving an extended spawning season showing the effects of seasonal differences in water temperature and food abundance on growth rates of hatchlings. Some cephalopod species clearly exhibit decreasing somatic growth rates with increasing sexual maturation (Arkhipkin *et al.*, 1998)[40]. Maturation in both sexes of *L. forbesi* in Galician waters shows a clearly defined annual cycle (Guerra *et al.*,1992)[41]. Mature female *L. forbesi* were caught from February to May. According to Guerra and Rocha (1994)[22],

maturation was apparent from November to April, as indicated by the increasing proportion of maturing (stage III) and mature (stages IV and V) squid. Mature *Loligo forbesi* males exist from February to July and male maturity increased from September to April. Boyle *et al,* (1995)[8] stated that, maturity can be categorized on a scale as I (immature) to V (mature) for males and females *L. forbesi* in the Scottish waters. In the Irish waters the main recruitment of *Loligo forbesi* occurs in the summer (Collins *et al.*, 1995)[5], at a similar time and size to that described by Holme (1974)[24] for the English Channel. The second period of recruitment in December may be equivalent to the weak November recruitment reported by Boyle and Ngoile (1993)[42] in the Scottish waters. It is worth to note that, Boyle and Ngoile (1993)[42] and Pierce *et al.* (1994b)[9] referred an April recruitment in the Scottish waters, which probably is contributed to the Rockall Bank fishery, but there was no evidence of recruitment at this time in the Irish or Celtic Seas. Three maturity indices for females and males *L. forbesi* in the Azores were applied. High percentages of mature squid (stages IV-V) represent 87.4 % of the females and 81 % of the males. Stage IV males were the least frequently encountered. Spent females were not found but spent males were grouped with stage V squid as they still had functional spermatophores (Porteiro and Martins, 1994)[25]. Mature and immature-maturing squid exist simultaneously on the same grounds and males reach maturity before females, as pointed by Collins *et al.* (1993)[43]. It is known that fertilization is close to 100 % (Hanlon *et al.*, 1989)[44] but very little is known about the mating strategy of this species. The presence of mature spermatophores in the penis, while the Needham's sac contains very few, can be an argument for this assumption. Low numbers of stage IV males have also been observed for other areas Portuguese (Moreno *et al.*, 1994)[13] and the use of stages IV and V combined was adopted by Guerra and Rocha (1994)[22].

Size at first maturity, the present study revealed that the males *L.forbesi* grew to a large size than females; the maximum revealed that the size for males was 25 cm. mantle length while for females 17cm. mantle length. Male loliginids typically exhibit greater range of size than females (Hatfield *et al.,* 2000)[14]. Snedcor (1956)[10] stated that the length, at which 50% of the examined animals acquire a certain character, could be taken as the length representing the onset of that character. Accordingly it can be concluded that female *L. forbesi* attain their first sexual maturity at 11.8 cm mantle length, while the male at 13 cm mantle length. Howaida and Riad (2008)[33] estimated first maturation at 10.6 cm. and 10.4 cm mantle length for males and females *L. forbesi,* respectively for individuals dwelling the Suez Bay, Red Sea, Egypt. Collins *et al.* (1995)[5] recorded first maturation at 12 cm and 15 cm. mantle length for male and female *L. forbesi* , respectively catches off the east coast of Ireland. The smallest mature male and female *L. forbesi* in Galician waters was 12.2 cm and 16 cm mantle length, respectively from (Guerra and Rocha, 1994)[22]; their higher value may be a result of selectively sampling larger animals of commercial catches. According to Thomas *et al.* (1999)[45], length at first maturity for *L. forbesi* does not show significant different between northern fisheries (Scotland and the English Channel) and southern fisheries (Portugal and Azores) which are significantly higher in the south than in the north. Pierce *et al.* (2005)[34] recorded that, the size at first sexual maturity for males and females *L. forbesi* from the Scottish waters was 10 cm. and 11 cm mantle length, respectively. Maturity of Veined Squid occurs at a range of sizes, with males typically growing to a larger size than females (Hastie *et al.*, 2009)[46]. Maturity is typically reached by 20 cm mantle length (Challier *et al.*, 2015)[37]. Most studeis targed many loliginid squid, there are at least two different sizes at maturity classes in male *L.*

forbesi throughout its range (Moreno *et al.,* 1994)[13]. Lum-Kong *et al.* (1992)[20] stated that, the minimum size at maturity was 14 cm for males *L. forbesi* in Scottish waters, while the minimum size at maturity for females was 15 cm.

The sex ratio in the present work, males of *L. forbesi* in the samples constituted almost 56.57 % of the specimens collected and predominated through all seasons. This value varies in different authors findings. *L. forbesi* males in the Azores waters constitute 59 % (Martins, 1982)[27]. In Scotland waters Pierce *et al.,* (1994a)[6] suggested the predominance of females during periods of breeding activity. Females *L. forbesi* in the Azores waters were dominating in winter, without showing a significant variation from the ratio 1:1. However in spring, males increased in number and in autumn, they were significantly more abundant than females (Porteiro and Martins, 1994)[25]. Males *L. forbesi* in Galician waters were significantly more abundant than females during spring and summer, females only outnumbered males in November (Guerra and Rocha, 1994)[22]. Pierce *et al.* (1994b)[19] stated that, females *L. forbesi* in the Scottish waters exhibit predominance in the majority of samples and were consistently more numerous during autumn, winter and early spring, with the proportion of females being highest at the peak of the breeding season (late winter and early spring). Conversely, males tended to be more abundant in samples containing the most small and immature animals during spring and summer. Thus it could be concluded that males both recruit to the fished population and disappear from the fished population earlier than females. Poteiro and Martins (1994)[25] concluded that, the predominance of immature- maturing *L. forbesi* in the Azores during autumn might suggest the existence of an optimum spawning peak. It is worth to note that in the Egyptian waters (Suez Gulf, Red Sea) males outnumbered females in all seasons except summer (Howaida and Riad, 2008)[33]. They

explained that the difference between males and females abundance could be due to catch bias rather than to population structure, where the small sample sizes need to be taken into consideration. Pierce *et al.* (1994b)[19] investigating *L. forbesi* in the Scottish waters, and Guerra and Rocha (1994)[27] studying *L. vulgaris* in the Spanish waters, found that the sex ratio is related to the type of gear, while equal sex ratios were apparent throughout the year except in the peak- spawning period, when females were more dominat than males. Augustyn *et al.* (1994)[48] have previously reported a biased ratio in favor of males for chokka on the spawning grounds. This has been reported for other *Loligo* species i.e. *Loligo vulgaris* from the Algarve region in southern Portugal (Coelho *et al.*, 1994)[49] but these estimates were based on jig caught samples and showed ratios that could reflect more attacks on the jigs by males than females rather than greater males abundance (Logan *et al.*, 2013)[50]. In the present study, *L. forbesi* were only encountered as a bycatch of the trawling techniques.

RECOMMENDATION

Carrying more studies on mathentical modls to study the impact of multivariables (ecological and biological) on *L.forbesi.*

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