FLEXIBLE REPRODUCTIVE PATTERNS IN THE SQUIDS
LOLIGO VULGARIS AND *LOLIGO FORBESI*

by

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ABSTRACT

The reproductive pattern of *Loligo vulgaris* and *Loligo forbesi* was studied on the basis of gonadal maturation, mating and spawning in males and females of both species present off the northwest coast of Spain (Galicia), between February, 1991 and February, 1993. Males and females of both species appear to present an extended mating period, during which somatic growth occurs. The size of spermatophores in a given mature male appears to increase with the growth of the squid. Several signs indicate that both female *L. vulgaris* and *L. forbesi* experience a partial ovulation at the time of spawning, and spawning occurs intermittently involving new oocyte maturation between spawning events, known as fractioned final spawning. *L. vulgaris* and *L. forbesi*, therefore, may be considered as multiple spawners.

INTRODUCTION

Until recently, it was generally considered that female cephalopods lay their eggs in one single spawning or in various consecutive ones, with no pause between each, after which they would die by extenuation (McGowan 1954, Mangold 1987, Harman et al. 1989, Mangold et al. 1993). The animals with this reproductive pattern where gonad regeneration is absent are known as semelpareous. The iteropareous species, on the contrary,arethosewhere a gonadal regeneration following spawning occurs, making new reproductive cycles possible.

As knowledge has grown regarding the biology of this group, it has become

increasingly difficult to support the notion of one sole reproductive pattern in the case of cephalopods (Mangold et al. 1993). A case in point is the *Nautilus*, with a life span of 20 years, which must be considered as an iteropareous species since the females spawn once a year, surviving the spawn, feeding, growing and regenerating their gonads for a further reproductive event the following year (Ward 1987). With the exception of this particular case, the rest of the cephalopods may be considered as semelpareous, although there are significant·differences between the maturation processes and spawning, from one species to another (Mangold 1987, Mangold et al. 1993). According to these differences in cephalopods, several reproductive strategies may be defined with regard to the oocyte maturation process and to the type of spawning process in each species (Mangold et al 1993, González 1994, Rocha 1994).

One of the most striking of these strategies is the so-called "partial ovulation" followed by a "fractioned final spawning" (González 1994, Rocha 1994). Partial ovulation in cephalopod ovaries implies the presence of oocytes at various stages of development, thus enabling the continued production of ovules once spawning has commenced. This phenomenon has been described in several·species: *Sthenoteuthis oualaniensis, Opisthoteuthis vossi, Loligo bleekeri* and *Idiosepius pygmaeus* (Harman et al. 1989, Villanueva 1992, Hun Baeg et al. 1993, Lewis and Choat 1993). At the same time, the fractioned final spawning·occurs when egg-laying takes placé in multiple, quite separate batches, during the spawning period. This period is usually relatively long, although in no case does it represent the greatest fraction of the animal's life ending in its subsequent death. Several species have been found which present this type of spawning, e.g. *Octopus chierchiae, Sepia officinalis* and *S. oualaniensis* (Rodaniche 1984, Boletzky 1987, 1988, Harman et al. 1989). The species presenting these two characteristics have been defined as "multiple spawners" (Harman et al. 1989, Mangold et al. 1993).

In the case of *Loligo vulgaris* and *Loligo forbesi*, reproduction has been studied by several authors, covering various aspects such as maturation, spawning period, fecundity, etc. (Mangold-Wirz 1963, Worms 1983, Baddyr 1988, Lum-Kong et al. 1992, Boyle and Ngoile 1993, Coelho et al. 1994, Guerra and Rocha 1994, Moreno et al. 1994, Pierce et al. 1994). But only recently has the possibility been pointed out that both species mature presenting partial ovulation and a fractioned final spawning, taking into consideration the presence of various sized oocytes in the gonad and the extended spawning period (Lum-Kong et al. 1992, Lum-Kong 1993, Guerra and Rocha 1994, Pierce et al. 1994).

Most works to date on cephalopod reproduction have concentrated solely on females (Mangold et al. 1993), although the role of males in this process should not be overlooked.

This study examines the reproductive pattern presented in both sexes of *Loligo vulgaris* and *Loligo forbesi* in the waters off NW Spain, completing various aspects of the reproductive biology of these species presented in Guerra and Rocha (1994), particularly regarding the duration of mating and types of ovulation and spawn.

MATERIALS AND METHODS

A total of 1,787 individuals of *Loligo vulgaris* were studied (862 males and 925 females), and 755 *Loligo forbesi* (399 males and 356 females), with a dorsal mantle length (ML) of between 98 and 475 mm ML in *L. vulgaris*, and 100 and 685 mm ML in *L. forbesi*. The individuals were obtained from monthly samples from commercial catches landed at ports and fishing sites in Galicia, between February, 1991 and February, 1993. Samples were frozen at -30°C, duly labeled and preserved for subsequent laboratory analysis. The individuals were defrosted at room temperature, their sex was determined and total weight (BW) and dorsal mantle length (ML) noted. Measurements were made in millimetres and grammes, with a margin of error of ±0.5 mm and ±0.01 g, respectively.

Maturity in males and females was estimated using a 5 stage maturity scale, as indicated by Boyle and Ngoile (1993). A gonadic-somatic index (GSI) was calculated by the expression:

$$GSI = \frac{GW}{BW} * 100 \tag{1}$$

where GW is the weight of the gonad (testis or ovary).

The reproductive system was dissected from the females, weighing the ovary (OW), the oviductal complex (oviducts, nidamentary glands and accessory reproductive glands: (OCW), the proximal oviduct (POW) and the nidamentary glands (NGW). In the males, the reproductive system was dissected, determining the weight of the testis (TW) and the weight of the spermatophoric complex (SCW).

Female fecundity was estimated for mature individuals (stages IV and V), of which subsamples of between 0.3 and 1.0 g of ovary and proximal oviduct from the end and medial zones were obtained. Each sample was weighed and preserved in Gilson solution, before which the oocytes from the sample were released from the surrounding tissue by brusque movements in water. The oocytes present in each ovary and oviduct subsample were counted, measuring the largest diametre using a binocular microscope with an ocular micrometre. Damaged or deformed oocytes were not measured. The size distribution of oocytes in the ovaries of both species was subjected to a size frequency distribution analysis using the method by Bhattacharya (1967) to check for the presence of different size groups.

The spermatophoric complex in mature males (stages IV and V) of different sizes was labeled and preserved in formaline (5%). A subsample of each complex was weighed, counting and measuring the spermatophore length (SL) which they contained. Deteriorated spermatophores were excluded from the measuring. In each case, the total number of spermatophores (SN) was estimated.

RESULTS

In male *Loligo vulgaris* at stage V of maturity, a large variability in the SCW was observed, expressed as a fraction of the total weight, relative to the ML (Fig.1a). Individuals with a GSI of over 1.5% presented sizes below 310 mm ML, whereas those with a GSI of below 1.0% showed a more empty spermatophoric complex and a greater size (Fig.1a).

There was also a large variation between the SL and the SCW in male *Loligo vulgaris* at stage V of maturity (Fig.1b). It was observed, however, that individuals with a GSI of over 1.5% had smaller spermatophores and larger spermatophoric complexes than those with a GSI of below 1.0% (Fig. 1b).

In male *Loligo forbesi* at stage V of maturity, a gradual decrease in the SCW was found, as a fraction of the total weight, relative to the ML (Fig. 2a). Stage V males with a GSI of over 1.5% presented sizes of below 300 mm ML, whereas those with a GSI of below 1.5% had sizes of over 300 mm ML and smaller spermatophoric complexes (Fig. 2a).

The SCW also decreased with the SL (Fig. 2b). It was noted that male *Loligo forbesi* with a GSI of over 1.5% presented a larger complex and a smaller sized spermatophore, than the males with a GSI of below 1.5% (Fig. 2b).
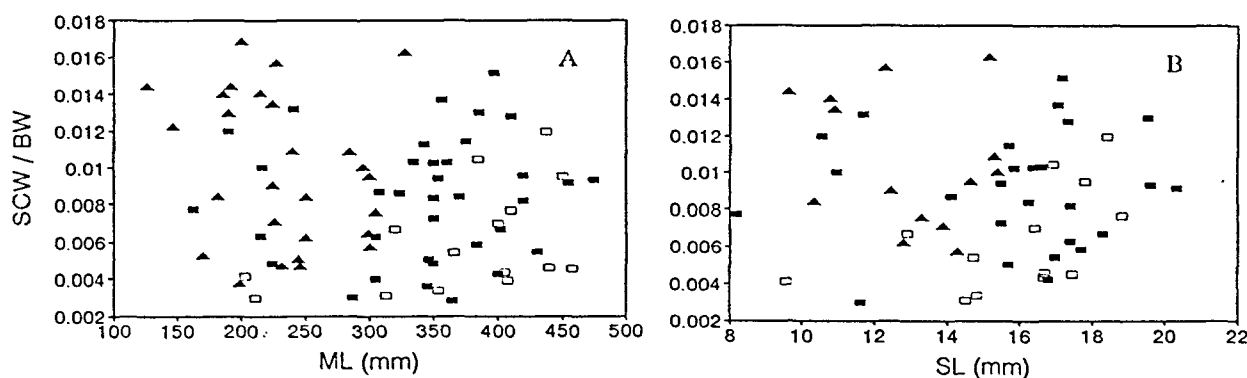

Figure 1. Relationship between the dorsal mantle length (ML) and the spermatophoric complex weight (SCW) (A) and between spermatophore length (SL) and the spermatophoric complex weight (B) in male *Loligo vulgaris* at stage V of maturity. BW: Body Weight. Triangle: GSI above 1.5%; Filled in rectangle: GSI between 1.5 and 1.0%; Empty rectangle: GSI of below 1.0%.
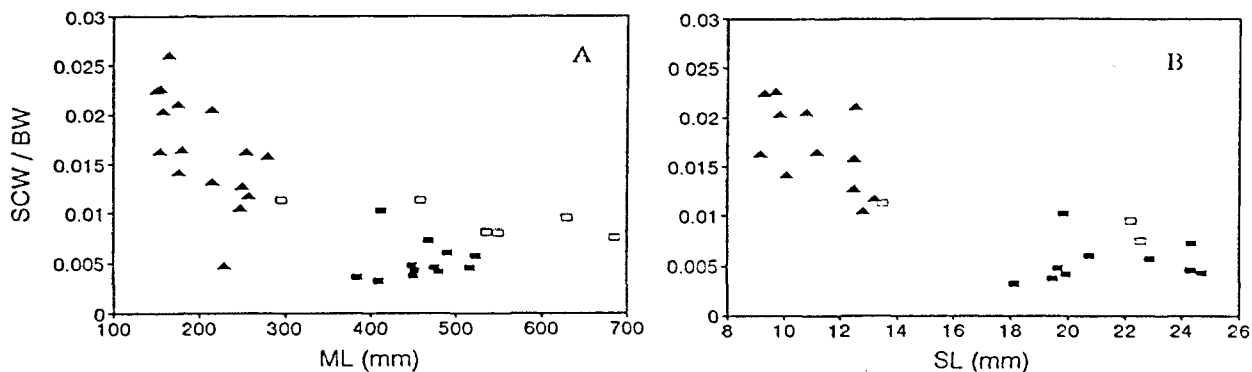
Figure 2. Relationship between the dorsal mantle length (ML) and the spermatophoric complex weight (SCW) (A) and between spermatophore length (SL) and the weight of the spermatophoric complex (B) in male *Loligo forbesi* at stage V of maturity. BW: Body Weight. Triangle: GSI above 1.5%; Filled in rectangle: GSI between 1.5 and 1.0%; Empty rectangle: GSI of below 1.0%.

The diametre of oocytes in the *L. vulgaris* ovary varied between 0.1 and 4.1 mm., whereas in the ovary of the female *Loligo forbesi*, this varied between 0.1 and 4.3 mm. In both species, the mature female ovaries of all the sizes studied showed slightly different polymodal distributions of oocytes, according to the individual examined (Fig. 3). Although the distribution of diametres in oocytes in each ovary vary from individual to individual within the same species, these may be represented by an average distribution and the standard deviation for each species (Fig. 4).

Analysis of size distribution of oocytes in the ovaries of 9 female *Loligo vulgaris* showed the presence of 3 to 5 modal groups of oocytes, according to the individual studied. A minimum of 3 and a maximum of 7 modal size groups of oocytes were found in the ovaries of 14 mature female *Loligo forbesi* (Table 1).

The number of oocytes in the proximal oviduct of mature females in both species varied according to the ML (Fig. 5), increasing the maximum amount they may contain with an increase in the ML. In *Loligo vulgaris*, the maximum amount of oocytes present was 10,000, whereas in the case of *Loligo forbesi*, the amount rose to 4,700. The presence of proximal oviducts with a low number of oocytes in large size mature females was noted.
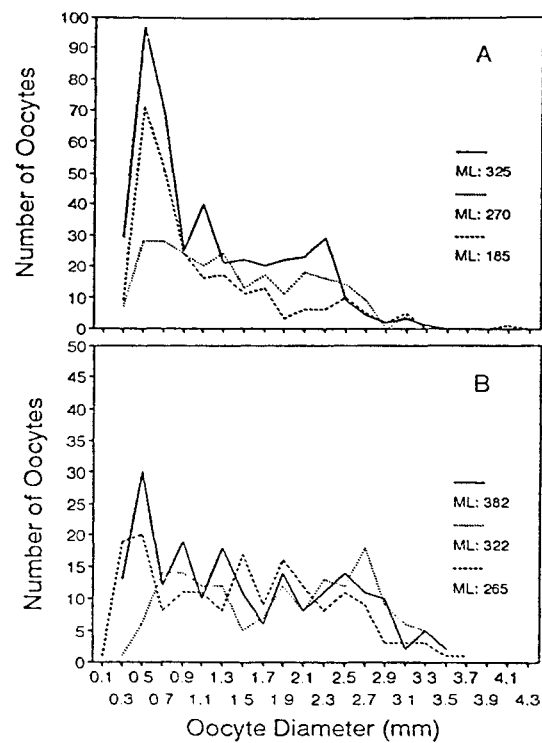
Figure 3. Frequency distribution of oocytes in the ovary of three individuals of *Loligo vulgaris* (A) and *Loligo forbesi* (B), according to size intervals of 0.2 mm (in the figure, the upper limit of the interval is shown). ML: Dorsal Mantle Length.
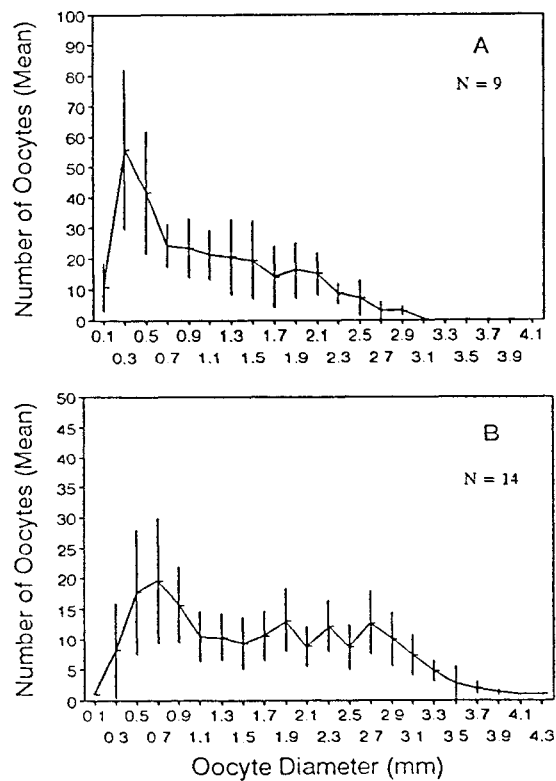

Figure 4. Average distribution of diametres of oocytes and standard deviation in the ovary of mature *Loligo vulgaris* (A) and *Loligo forbesi* (B) females, according to size intervals of 0.2 mm (in the figure, the upper limit of the interval is shown). N: Number of Ovaries.

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Table 1. Number of oocyte modal size groups found in ovaries of *Loligo vulgaris* and *Loligo forbesi* by polymodal analysis.

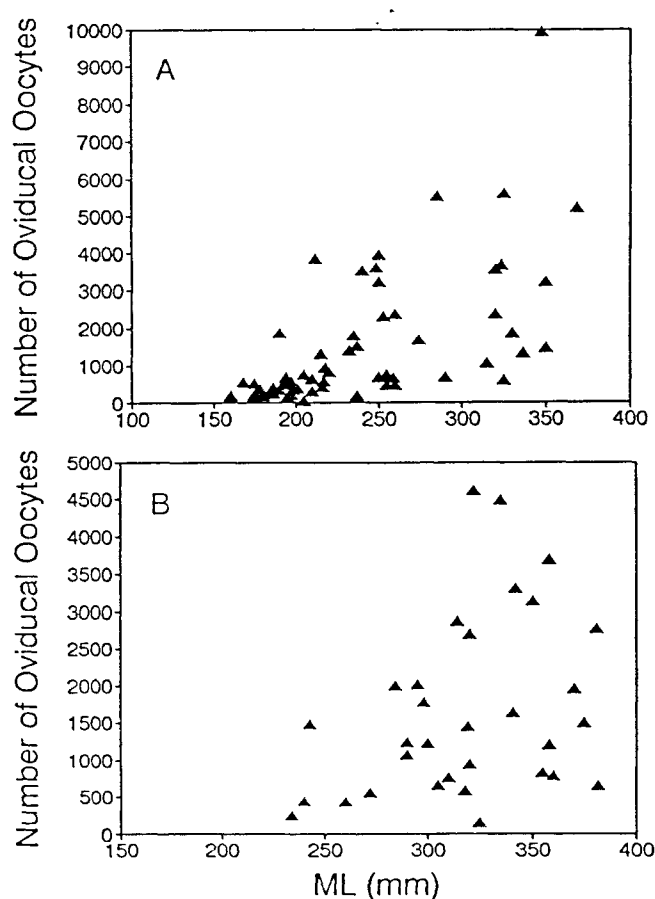| Modal Sizes	*Loligo vulgaris* Percentage	*Loligo forbesi* Percentage
3	44.5	7.1
4	33.3	42.9
5	22.2	35.8
6	-	7.1
7	-	7.1
Number	9	14

Figure 5. Relationship between the dorsal mantle length (ML) and the number of oocytes present in the proximal oviduct of *Loligo vulgaris* (A) and *Loligo forbesi* (B).

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Nidamentary gland weight increased with the size of mature females in both species (Fig. 6). In the case of females with a GSI of below 4.0, however, this weight was less than that for females of the same size, but with an average GSI of between 4.0 and 7.0, or of over 7.0. This ratio was exponential, in each case (Table 2), and, although both in *Loligo vulgaris* and in *Loligo forbesi* there were values not far removed from the function calculated, these do not alter the general trend observed in both species.

Additional information was available on 5 egg masses of *Loligo vulgaris* found and recovered from the inner part of the Galician rias. Four of these were kept in laboratory and produced 1,240, 3,000, 6,000 and 8,400 newhatchs, respectively. The fifth contained some 2,600 eggs which hatched when caught.
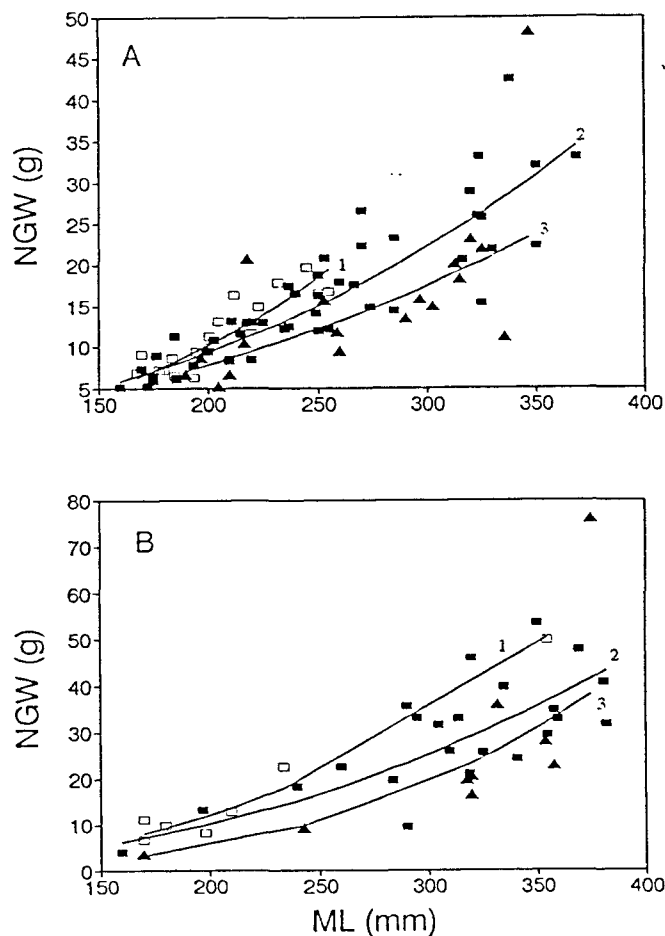

Figure 6. Relationship between the dorsal mantle length (ML) and nidamentary gland weight (NGW) in mature female *Loligo vulgaris* (A) and *Loligo forbesi* (B), according to their GSI. Empty rectangle: GSI $\geq$ 7.0 (1); Filled in rectangle: 4.0 $\leq$ GSI < 7.0 (2); Triangle: GSI < 4.0 (3).

Table 2. The relationship between the nidamentary gland weight (NGW) and dorsal mantle length (ML) in mature female *Loligo vulgaris* and *Loligo forbesi*, according to the Gonado-somatic index (GSI). Equation: $NGW = nML^m$; r^2: determination coefficient; N: number of individuals.

	n	m	r^2	N
Loligo vulgaris				
GSI $\geq$ 7.0	0.0000062	2.699	0.77	22
4.0 $\leq$ GSI < 7.0	0.0001151	2.134	0.83	49
GSI < 4.0	0.0002112	1.985	0.56	18
Loligo forbesi				
GSI $\geq$ 7.0	0.0000228	2.488	0.88	7
4.0 $\leq$ GSI < 7.0	0.0000719	2.238	0.67	23
GSI < 4.0	0.0000004	3.117	0.86	9

DISCUSSION

The ML size variation in the spermatophoric complex in *Loligo vulgaris* males at stage V of maturity is the main sign of the presence of a lengthy mating period in males. In these individuals, the spermatophores spent in mating are replaced in the spermatophoric complex, the testis being spent and the GSI decreasing. If mating is prolonged in time and somatic growth occurs, it is to be expected that the larger sized individuals will have a smaller GSI than the small ones, as actually occurs.

A similar deduction arises when comparing the spermatophore length with the size of the spermatophoric complex in mature *Loligo vulgaris* males at stage V. The fact that the individuals with larger sized spermatophores have a low GSI is a sign that these animals have mated previously. Since the SL/ML ratio is linear (Guerra and Rocha 1994), it is possible that when spermatophores are renewed, that their length increases in the same given mature animal while growing somatically.

In male *Loligo forbesi* at stage V of maturity, the spermatophoric complex weight varied with the same ML, experiencing a gradual decrease in size as the ML increased in individuals. This may indicate that there is a certain replacing of spermatophores spent during mating, with a subsequent decrease of the GSI due to testis deterioration. If this were prolonged in time and somatic growth occurred, it is to be expected that the larger sized individuals had a lesser GSI than the small ones, as actually occurs.

Likewise, following the same line of reasoning as for *Loligo vulgaris*, in the case of large sized *Loligo forbesi*, the presence of large spermatophores in mature males at stage V with a low GSI, may imply that the length of the spermatophore produced by the same given animal increases when experiencing somatic growth. It must be considered that a positive, linear relationship was also found here between the SL and the ML (Guerra and Rocha 1994).

The presence of an asynchronic development in loliginid oocytes is histologically evidenced in *Loligo vulgaris reynaudii* (Sauer and Lipinski 1990), in *Loligo forbesi* (Lum-Kong 1993) and in *Loligo bleekeri* (Hun Baeg et al. 1993). This confirmed the certainty of multiple spawning in all these species, as proposed for other cephalopod species (Boletzky 1987, 1988, Harman et al. 1989). Nevertheless, as indicated by Mangold et al. (1993), the mere presence of oocytes at different stages of maturity during the spawning process is insufficient to identify a multiple spawning. A single spawner such as *Loligo opalescens* also shows all stages of oocytes present in the ovary of both spawning and spent females (Knipe and Beeman 1978). In this case, the difference lies in the fact that a single spawner presents most of its mature oocytes and only a low number of oocytes in an early maturation phase in the spawning or spent females. Conversely, a multiple spawner presents a large amount of immature or maturing oocytes, which is reflected in the size distribution of the oocytes in the ovary (Mangold et al. 1993). In the case of *Loligo vulgaris* and *L. forbesi*, the size distributions of the oocytes in mature female ovaries indicates the presence of an important fraction of hardly developed oocytes. This was corroborated histologically by Lum-Kong (1993) regarding *L. forbesi*. It may be deduced from this that both species are multiple spawners.

Furthermore, there are other signs which support the existence of multiple spawning in *Loligo vulgaris* and *Loligo forbesi*: (a) the wide variation in the total number of oocytes in the oviduct and ovary in females at the same stage of maturity and of the same size; (b) the wide ML range covered by stages IV and V of maturity in *L. vulgaris* and *L. forbesi* (Guerra and Rocha 1994). Both of these signs indicate a continual production and egg-laying during the spawning period, as noted by Harman et al. (1989) regarding *Sthenoteuthis oualaniensis*.

In the case of a single spawner species, the entire oocyte production must be available for the egg-laying within a very brief period of time; this occurs in *Loligo opalescens* (McGowan 1954). In *Loligo vulgaris* and *Loligo forbesi*, this is improbable due to the fact that all the oocytes do not mature at the same time (partial ovulation), and that the oviduct would be unable to manage the entire production all at once.

During egg-laying, females of both species secrete substances from the nidamentary gland to cover and protect the eggs (Lum-Kong 1992). It is logical, therefore, to suppose that nidamental gland weight would decrease in females which have spawned on one or more than one occasion prior to being caught.

Finally, the individual egg masses of *Loligo vulgaris* found in the course of this study and estimates by Mangold-Wirz (1963) indicate that females lay between 3,000 and 6,000 eggs at a time. Conversely, *Loligo forbesi* lays an average of 1,853 eggs per egg mass (Lum-Kong 1993). In both species, the number of oocytes found in the

ovaries (Guerra and Rocha 1994) is higher than the number of eggs per egg mass. Assuming that the majority of mature oocytes are fertilized and laid, the only way in which *L. vulgaris* and *L. forbesi* may spawn is for the females to produce several series of eggs over prolonged period in time.

In conclusion, there are signs in *Loligo vulgaris* and *Loligo forbesi* to indicate an extended spawning period for both sexes. Furthermore, the females of both species would present an asynchronous development of oocytes with a continual ovulation when spawning. Thus, both species should be considered as multiple spawners, a model previously suggested by Lum-Kong et al. (1992) for *L. forbesi*, although this is indicated for the first time for *L. vulgaris*.

ACKNOWLEDGEMENTS

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