AGE AND GROWTH OF THE SQUID LOLIGO VULGARIS OFF THE SOUTH COAST OF PORTUGAL, USING STATOLITH ANALYSIS

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ABSTRACT
Age composition and growth rates of the squid Loligo vulgaris (Lamark, 1797) were studied by examination of growth increments within statoliths of 419 specimens (mantle length, ML, ranging from 32 to 400 mm). The squid were obtained by monthly sampling from the catches of commercial trawls off southern Portugal between March and September, 1993. The total number of growth increments in the mounted and ground statoliths was counted using a semi-automatic image analysis system. ML was significantly correlated with both the statolith length, TSL, and the number of increments, NI. The female statolith was slightly larger than the male statolith for the same mantle size. Growth rates of individual showed high variability with an average estimated at 34.6 mm month⁻¹ for males and 33.5 mm month⁻¹ for females. Growth in length between 70 and 280 days was best described by a power function for both sexes. The growth index of the statolith (TSL/NI) decreased with individual growth. This result may be related with the onset of sexual maturation. L. vulgaris hatched throughout the year with two distinct peaks, in spring which is the main breeding period, and in autumn. The life cycle of the L. vulgaris population on the south Portuguese shelf was completed in one year.

INTRODUCTION
Interest in cephalopods has increased during the last three decades, not only because of increased exploitation (Caddy, 1995), but also since it has been recognised that they play an important role in the marine ecosystem (see Okutani, O'Dor & Kubodera, 1993).

To evaluate population biomass changes caused by natural or fishing mortality it is necessary to know the demographic structure, which implies a precise study of the age and growth of individuals. Furthermore, it is important to study the age and growth of cephalopods since knowledge of these parameters makes it possible to compare different maturation, feeding and ecological patterns of a species during its life cycle (González, Castro & Guerra, in press).

Traditional, indirect methods to determine age of cephalopods such as length frequency analysis cannot be used due to the rapid growth of individuals, their short life span, protracted spawning season, along with the migrations undertaken by many species (Caddy, 1991; Natsukan & Komine, 1992; Natsukan, Mukai, Nakahama & Kubodera, 1993; Guerra & Rocha, 1994). The search for a method to measure the age of cephalopods has included analysis of periodic depositions on different hard structures such as beaks, radulae, gladius, cuttlebones and statoliths (Rodhouse & Hatfield, 1990). The statolith has been proven to be the most promising for age and growth studies (Jereb, Ragonese & Boletzky, 1991).

Information from studies of statolith increments can contribute not only to establishing the age of the specimens, but also their life span, specific information on growth and maturation process, growth modelling, cohort analysis, hatching dates, migrations and ecological information on the life history of some species (see Jackson, 1994 for a review). Although in the last two decades several studies have been carried out, there is a need to continue work on more species from other geographical areas.

Studies on the biology of Loligo vulgaris (Natsukan & Komine, 1992; Coelho, Quintela, Bettencourt, Silva & Villa, 1994; Rocha, 1994) indicate that this species has a short life cycle, highly variable growth rates and an extended spawning season that contributes to a complex population structure.

Published information on the age and growth of L. vulgaris is restricted to Natsukan...
& Komine (1992) in the Mediterranean (South of France), Guerra & Rocha (1994) in Galician waters (Northwest of Spain) and Arkhipkin (1995) on the West Saharan shelf (Northwest of Africa). These studies show that the analysis of the increments of the statoliths can be used in ageing and estimations of growth rates of this species. Studies with other species of cephalopods showed that sexual maturation can influence the growth in weight and length of statoliths (Guerra & Sánchez, 1985).

The aim of this paper is to evaluate the age and growth of L. vulgaris based on statolith analysis in an area located among the other three previous studies. The growth of the statolith and the number of increments are related to the growth, sex and maturity stage of the individuals.

MATERIAL AND METHODS

A total of 419 European squid, Loligo vulgaris, was collected in monthly samples from commercial catches in Olhão port, South of Portugal (Figure 1), between March and September of 1993, but as there were no landings in April and June it was not possible to obtain specimens during these months. Squid were measured (ML—mantle length, the distance between the anterodorsal protuberance and the apex of the tail fin) to the nearest millimetre and sexed. Maturity stages were determined using the scale developed by Boyle & Ngoile (1993) for Loligo forbesi and adopted for L. vulgaris.

The statoliths were removed from all specimens, collected by dissection (Jereb et al., 1991) and total statolith length (TSL) was measured from dorsal dome to the tip of the rostrum (Clarke, 1978) with a stereological microscope equipped with a micrometer eye-piece. After cleaning, the statoliths were mounted in Pro-texx, ground and polished on both sides following the method developed by Arkhipkin (in Jereb et al., 1991) using a commercial waterproof sand paper 3M, coarse (30 μm) and fine grain (1 μm), respectively.

The reading of the number of increments (NI) in the statoliths was taken using a JAVA image analyser system and a transmitting light microscope with a 400x magnification. Each increment was considered the paired 'dark ring' and the adjacent 'light ring' as proposed by Lipinski, Dawe & Natsukari (1991). Increments were counted from the nuclear zone (distance between the focus and the natal ring) to the top of the rostrum. The natal ring was identified as being darker and heavier and corresponds to individual hatching. Since some statoliths showed zones that were impossible to count (white zones), the total number of increments was calculated by adding the number of increments read to those estimated by backcalculation in the white zone (Natsukari, Nakanose & Oda., 1986; Villanueva, 1992; Gonzalez et al., in press). All the statoliths that showed 20% or more of white zone were rejected.

Data were analysed separately by sex. ML/TSL and ML/NI relationships were determined and the best fits, using the least squares method (Sokal & Rohlf, 1981), were obtained. Data on statoliths of indeterminate sex (21 specimens) were added to data on both male and female growth curves. To test differences between males and females, for all fits, covariance analyses and the F test (Sokal & Rohlf, 1981) were applied. Assuming the daily periodicity of increment depositions, the absolute growth rates AGR (mm month⁻¹) were calculated with the formula AGR = (ML - MLᵢ / NIᵢ - NIᵢ) * 100. The AGR was determined for each 30 days period and an average was taken.

In order to analyse the effect of sexual maturation on growth, the growth index (GI) of each statolith, GIᵢ = (TSL / NIᵢ) * 100, was related to the ML in immature and mature individuals separately.

The hatching month was determined by backcalculation of statolith increments, starting on the sampling month. The Bhattacharya method (Bhattacharya, 1967) for identifying modes in frequency distribution, implemented in the Compleat ELEFAN package (Gayanilo, Soriano, & Pauly, 1988) was used in this analysis.

RESULTS

All individuals with ML from 32 mm to 400 mm showed the same statolith morphology, consisting of a very well-developed lateral and a dorsal dome and an extended rostrum with a finger-like shape. The wing, or attachment area was white and opaque and easily removed from the remaining statolith.

The smallest statolith, TSL = 0.93 mm, was collected from an individual of indeterminate sex, ML = 32 mm. The largest statolith, TSL = 2.24 mm, was collected from a female, ML = 345 mm, while the largest male statolith, TSL = 2.16 mm, belonged to an individual with a ML = 386 mm. The mean length calculated for the nuclear zone was 55.55 μm with a standard deviation of 4.50 μm.

The ML/TSL relationship is shown in Figure 2. A single power function was the best fit to the present data for both male and female, expressed as follows:

Males: ML = 39.39 TSL⁻⁰.⁷⁷ p<0.001 n = 129
Females: ML = 41.98 TSL⁻⁰.⁸⁰ p<0.001 n = 142

There is a significant correlation between these two variables, with higher values of r² for females. There is a significant difference (F =
Figure 1. Map of East Atlantic ocean and Mediterranean sea showing the sampling area (A), and the area studied by Natsukari and Komine (1992) (B), Guerra and Rocha (1994) (C), and Arkhipkin (1995) (D).

Figure 2. Relationship between total statolith length (TSL) and mantle length (ML) of males, females and indeterminate sex of L. vulgaris.
Figure 3. Relationship between number of increments (NI) and mantle length (ML) of males, females and indeterminate sex of *L. vulgaris*.

14.87, *p*<0.0001) between the two fitted curves.

Figure 3 shows the relationship between ML and NI. The best fit was a power function expressed separately for males and females as follows:

Males: \( ML = 0.197 \text{NI}^{1.0} \) \( r^2 = 0.68 \), *p*<0.001, *n* = 71

Females: \( ML = 0.143 \text{NI}^{1.1} \) \( r^2 = 0.74 \), *p*<0.001, *n* = 69

Although the correlation coefficient is better for females than for males, both are significant. No significant differences between both curves were found (\( F = 0.78; p>0.38 \)). It was estimated that the oldest male and female found were 9 and 8 months old, respectively, and the average growth rates calculated for males were 34.6 mm month\(^{-1}\) (± 5.9) and 33.5 mm month\(^{-1}\) (± 6.6) for females.

Figures 4a and 4b show the relationship between GI and ML for immature and mature individuals, respectively. A linear relationship was found for both groups.

Immature juv.:
GI = 1.44 - 0.0032 ML \( r^2 = 0.27 \), *p*<0.001, *n* = 119

Mature adults:
GI = 1.15 - 0.0009 ML \( r^2 = 0.25 \), *p*<0.001, *n* = 33

In both groups, there is a small but significant correlation. The regression coefficient indicates that the statolith growth index is higher in immature animals, decreasing in mature ones.

The relative frequencies of the hatchlings in each month are shown in Figure 5. Although hatching occurs all year round, two peak seasons were obtained (\( \chi^2 = 4.261 \) with *p* = 95%): one very well defined between January and April with a maximum in March and the other more extended between July and November.

**DISCUSSION**

In this work, it was assumed that the growth increments in *L. vulgaris* are laid down daily. Natsukari & Komine (1992), Guerra & Rocha (1994) and Arkhipkin (1995) conducted their studies on statolith analysis of *L. vulgaris* under the same assumption. In addition, the microstructural appearance of the statolith of the studied species is very similar to that of other loliginids, such as *Alloteuthis subulata, Loligo opalescens* and *Photololigo edulis*. The daily periodicity of the increments has been validated directly or indirectly for these loliginids (Lipinski, 1986; Yang, Hixon, Turk, Krejci, Hulet & Hanlon, 1986; Natsukari, Nakanose & Oda, 1988) and other long-finned squids, *L. chinensis, Lolilolus noctiluca, Sepioteuthis lessoniana* (Jackson, 1990a, 1990b and Jackson, Arkhipkin, Bizikov & Hanlon, 1993).

Previous studies using modal progression analysis (Tinbergen & Verwey, 1945; Man-
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Gold-Wirz, 1963 and Worms, 1979) suggested a life span for *L. vulgaris* ranging from 2 to 4 years. Currently, authors point to life cycles of about 1 year, either by ring counts in statoliths (Natsukari & Komine, 1992 and Arkhipkin, 1995), or using both ring counts and modal progression analysis (Guerra & Rocha, 1994 and Rocha, 1994). The same seems to occur in laboratory reared individuals (Turk, Hanlon, Bradford & Yang, 1986). Statolith growth ring analysis in this study confirms that *L. vulgaris* has an approximate one year life cycle and hatches all year round, with a main period in spring and a further less well defined period in autumn. The presence of these two maxima of reproduction in such environments that allow
reproduction all year round, supports the finding of mature individuals with different lengths, showing growth variability within the populations. This could be a good indicator of the multiple microcohorts found by Rocha (1994) in the same population. Coelho et al., (1994) also refer to a less active period of reproduction in the summer and higher activity during the autumn, winter and spring for L. vulgaris off southern Portugal.

Average growth rates, both for males (34.6 mm.month\(^{-1}\)) and females (33.5 mm.month\(^{-1}\)) were, in general, similar to those estimated by other studies. Turk et al., (1986) found captive individual growth rates of 20 to 25 mm.month\(^{-1}\) but according to Natsukari & Komine (1992) slower growth is probably due to the suboptimal rearing conditions. Their estimates show one minimal growth rate of 23.7 mm.month\(^{-1}\) and a maximal of 43.8 mm.month\(^{-1}\). Coelho et al. (1994) found a very low growth rate of 20 mm.month\(^{-1}\) for mature individuals with a large mantle length. Rocha (1994) also estimated separate growth rates for females (30.6 mm.month\(^{-1}\)) and for males, 26.0-38.5 mm.month\(^{-1}\). Arkhipkin (1995) found higher growth rates in males than in females, increasing from 28.8 mm.month\(^{-1}\) at age 5 months to 58.2 mm.month\(^{-1}\) at age 12 months. The maximum growth rate in females occurred at the age of 12 months (38.1 mm.month\(^{-1}\)). This high variability in individual growth rates could be associated with temperature and the individual size and maturation. In other squid species, it has been shown that growth rates are influenced by water temperature and food availability (Forsythe & Van Heukelem 1987; Rodhouse & Hatfield, 1990). Additionally, Jackson & Choat (1992) suggested that tropical squid species have a higher growth rate and shorter life spans when compared with temperate species, perhaps as a function of temperature.

Natsukari et al., (1993) in a study of age and growth in Berryeuthis magister detected the existence of a considerable variation in individual growth rate. This result together with the growth rate variations reported for other loliginids; Allotheuthis subula, Photololigo edulis, Heterololigo bleeckert, L. forbesi and L. vulgaris (Lipinski, 1986; Natsukari et al., 1988; Kinoshita, 1989; Guerra & Rocha, 1994, Natsukari & Komine, 1992 and data from this work); suggested that this variation is a common characteristic not only of loliginids but also of gonatids.

Despite the high variability in growth of L. vulgaris, the growth curves found in this study are described by a power function. Arkhipkin (1995) also found a power function for both sexes. However, there are no significant differences in ring deposition between sexes, which agrees with Rocha (1994), who found a linear growth function. Natsukari & Komine (1992) empirically fitted a double exponential curve to their data. This seems to confirm a non-

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**Figure 5.** Frequency of back-calculated hatching date for L. vulgaris.
asymptotic growth for *L. vulgaris*, as proposed by Alford & Jackson (1993) for cephalopods in general. Differences between statoliths of male and female squid, though small, were significant, female statoliths being slightly larger. Similar results are reported by Lipinski & Durholtz (1994) for *L. vulgaris reynaudii*.

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