

Original article

Variability in age-at-recruitment and early growth in English Channel *Sepia officinalis* described with statolith analysis

Laurence Challier, Juliette Royer, Jean-Paul Robin *

Laboratoire de Biologie et Biotechnologies Marines, Institut de Biologie et Biochimie Appliquée, Université de Caen, esplanade de la Paix, 14032 Caen cedex, France

Received 26 October 2001; accepted 17 July 2002

Abstract

In the English Channel, the French cuttlefish landings of the smallest commercial category (body weight < 100 g) indicate that recruitment begins in autumn. Samples of recruits were collected in the Port-en-Bessin (Normandy) fish market once every fortnight from October to December 2000 to determine age-at-recruitment and juvenile growth variability. In addition, pre-recruit samples (mantle length: 53–90 mm) were collected in the Bay of Seine from August. Age was determined by statolith analysis: daily growth increments on the statolith lateral dome were observed under a light microscope. Increments were counted automatically, and manually when necessary, using the image analysis software TNPC. Readable statoliths were obtained in 236 cuttlefish, which revealed changes in age-at-recruitment during the study period. The cohort 2000 is recruited at an age ranging between 2 and 5.5 months (the bulk of recruits are 60–120 d old) and an increasing trend in average age is observed in autumn. The duration of the hatching period was derived from back-calculated hatching dates. A main hatching peak was observed between June and August. Juvenile growth variability was analysed at the individual level and between batches of hatchlings. Growth rates suggest that early hatched individuals grow faster than animals hatched later in the summer. © 2002 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Résumé

Variabilité de l'âge au recrutement et croissance des juvéniles de *Sepia officinalis* d'après l'analyse des statolithes. En Manche, les débarquements français de seiches, triés par catégories commerciales, indiquent à travers la catégorie des plus petits individus (poids < 100 g) que le recrutement a lieu en automne. L'échantillonnage des recrues a été réalisé à la criée de Port-en-Bessin tous les quinze jours d'octobre à décembre 2000 pour déterminer l'âge au recrutement et les variations de croissance des juvéniles. Des juvéniles au stade pré-recrue ont aussi été récoltés en baie de Seine d'août à octobre. L'âge des animaux a été déterminé à partir des statolithes. Les stries de croissance, journalières chez *Sepia officinalis*, sont observées au microscope optique sur le dôme latéral du statolithe. Leur nombre est ensuite évalué automatiquement et parfois manuellement par analyse d'images numérisées. Des statolithes lisibles ont été prélevés chez 236 seiches, ce qui a permis d'étudier la variabilité de l'âge au recrutement. L'âge au recrutement de la cohorte 2000 a été déterminé, et se situe entre 2 et 5 mois et demi (l'essentiel des recrues ayant de 60 à 120 jours). On observe aussi que l'âge moyen des recrues augmente au cours de l'automne. La période des éclosions est déduite par rétrocalcul. Le pic des éclosions est observé entre juin et août. La croissance des juvéniles a été étudiée en prenant en compte des différences individuelles et celles existant entre groupes d'animaux nés en même temps. Les taux de croissance indiquent une croissance plus rapide pour les animaux nés au début de la période d'éclosion que pour ceux nés à la fin de l'été. © 2002 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS. Tous droits réservés.

Keywords: Recruitment; Statolith; Age reading; Early growth; *Sepia officinalis*

1. Introduction

Recruitment, the renewal of harvestable stages in a population, is a key parameter of stock dynamics and

recruitment variability is often considered as the most difficult problem in stock assessment (Hilborn and Walters, 1992). In cephalopods, the short life span makes commercial catches more dependent on the number of recruits than in most teleost fish. Early life stages are likely to incur higher mortalities than adult stages (Caddy, 1996) and are generally described as extremely responsive to environmental

* Corresponding author.

E-mail address: robin@ibba.unicaen.fr (J.P. Robin).

variables (Boyle and Boletzky, 1996). In this context, changes in age-at-recruitment and juvenile growth are important parameters relative to recruitment strength.

Cuttlefish population structure may superficially look simple, with seldom more than two annual cohorts overlapping (Boletzky, 1983). However, *Sepia officinalis* spawning occurs over a long time period when compared to life span, and the recruitment of successive broods reveals subgroups (“micro-cohorts”) (Guerra and Castro, 1988) which represent a more complex underlying pattern.

The need to take into account such subgroups in growth studies was stressed by Caddy (1991). Cephalopod growth studies were first based on length–frequency data and modal progression analysis. *Sepia officinalis* samples from the Bay of Biscay and the English Channel were split by cohorts assuming that all individuals in the same length had the same age (Gi-Geon, 1982; Medhioub, 1986; Dunn, 1999). This method is also susceptible to sampling bias due to selection by fishing gear. Important advances in cephalopod growth analysis have been made in the last two decades with individual age determination through quantification of statolith increments. Daily periodicity of statolith increments has been demonstrated in a number of squid and sepioid species (Jackson, 1994) using chemical marking (in *Illex illecebrosus*, Hurley et al., 1985, in *Idiosepius pygmaeus*, Jackson, 1989, in *Loligo chinensis* and *Loliolus nocticula*, Jackson, 1990, in *Todarodes pacificatus*, Nakamura and Sakurai, 1991, in *Loligo vulgaris*, Lipinski et al., 1998) and in rearing experiments (in *Abralia trigonura*, Bigelow, 1992, in *Sepioteuthis lessoniana*, Jackson et al., 1993).

In *Sepia officinalis*, statolith increments are difficult to visualise and initially other hard structures such as the cuttlebone were examined (Le Goff et al., 1998). Recent rearing experiments have both verified the daily periodicity of statolith rings and invalidated the use of other hard structures (cuttlebone, eye lens, beaks) (Bettencourt and Guerra, 2001). These authors indicated, however, that in cuttlefish more than 240 d old, statolith rings were hardly visible, and thus that the technique is useful only for the study of juveniles.

The present study is the first attempt to apply statolith analysis to juvenile *Sepia officinalis* collected in the wild. Special attention was given to methodological aspects of statolith reading; in particular, the influence of the observer was tested. Juvenile cuttlefish of the cohort 2000 collected in the Bay of Seine (English Channel) were analysed to determine the hatching time and age-at-recruitment in the commercial fishery. The duration of the hatching period and the recruitment period are compared to test the hypothesis of constant age-at-recruitment (differences in the duration of each step should correspond to variations in growth rate).

Age and length data were also integrated in a model of juvenile growth. The model includes a parameter related to hatching time and differences in growth rates between batches of animals hatched at the same time were tested.

2. Materials and methods

2.1. Sampling

Collected samples have two different origins. Firstly, animals entering the exploited stage (recruits) were obtained from commercial landings. Secondly, research trawl fishing was carried out to catch pre-recruits.

Commercial landings were sampled in the Port-en-Bessin fish market (Calvados, Basse-Normandie) where cuttlefish are sorted into four commercial categories based on animal weight (Table 1). Recruits (as defined above) are observed in autumn (Dunn, 1999) and fishery statistics per commercial category confirm that recruitment occurs during the last quarter of the year (Fig. 1). Recruits are cuttlefish of the smallest category landed and thus in the period October–December 2000, one sample of about 30 specimens of the T4 category was collected every fortnight. The first October sample belongs to the T3 category since no category 4 animals were landed on the day of sampling.

Pre-recruit samples were also caught fortnightly in the Bay of Seine between August and October using a small beam trawl (2 m width and 20 mm mesh size). The trawl was towed by a small boat of the University Marine Station in Luc-sur-Mer. All samples were kept frozen until processing in the laboratory.

2.2. Basic biological data

All specimens were thawed and measured (dorsal mantle length, ML, to the nearest millimetre). Body weight (in

Table 1
Boundaries of commercial categories applied in the French cuttlefish fishery

| Commercial categories | Body weight (kg) |
|-----------------------|------------------|
| T1 | ≥ 0.5 |
| T2 | [0.3–0.5] |
| T3 | [0.1–0.3] |
| T4 | < 0.1 |

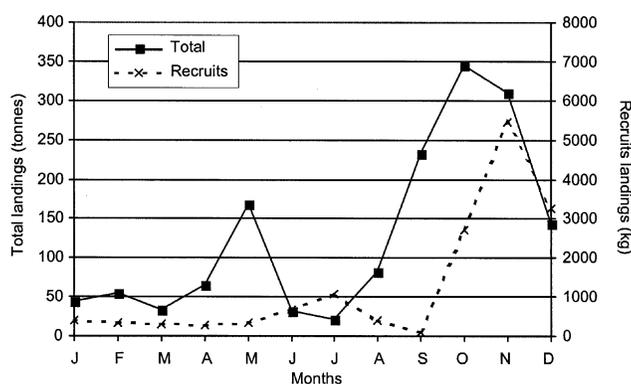


Fig. 1. Monthly variations in Port-en-Bessin cuttlefish landings averaged for the period (1999–2000). Solid line shows total landings of all commercial categories. Dotted line indicates the cuttlefish landings at recruitment (individual body weight < 100 g).

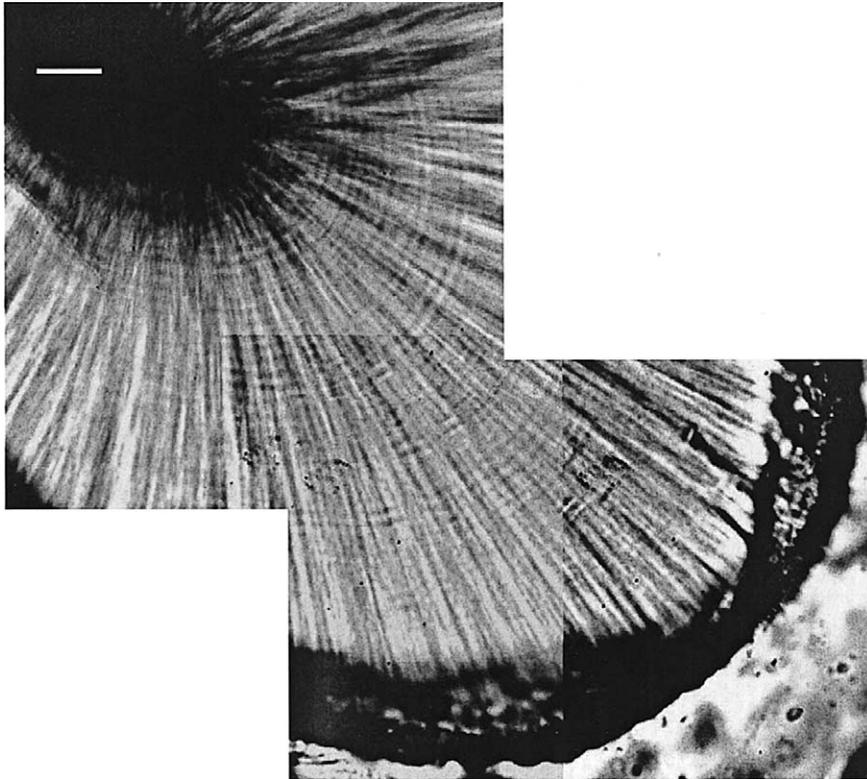


Fig. 2. Mosaic-picture of the lateral dome of a statolith in *Sepia officinalis*. Bar: 20 μm .

grams) was also recorded. Sex was not considered since the study is focused on immature stages (juveniles). In the analysis of length data, basic assumptions were tested using the Shapiro–Wilks test for normality and Bartlett’s test for homoscedasticity. Either analysis of variance or the non-parametric Kruskal–Wallis test was subsequently applied depending on the nature of the data.

2.3. Statolith processing

2.3.1. Extraction

Statoliths were extracted as described by Lipinski (1986) and preserved in 95% ethanol. The mounting and grinding procedure is similar to the “method of Arkhipkin and Villanueva” (Dawe and Natsukari, 1991) in which both sides of the statolith are ground, starting with the concave side. The main difference with Arkhipkin’s protocol is that statoliths were mounted with a heat-melting resin (Crystal Bond™). A specific grinding plane was sought to reveal rings as clearly as possible, the best position was obtained by cutting the rostrum after fixing the lateral dome to the slide with the wing upward. Grinding papers had grain sizes ranging from 1 to 0.3 μm .

2.3.2. Image analysis and statolith reading

Statoliths were observed by light microscopy (magnification at 400 \times). Series of up to four images were necessary to cover the lateral dome of each statolith. Corrections in contrast and luminosity were applied to improve ring

visibility and each series was merged automatically into one mosaic using image analysis procedures (T.N.P.C.© software) (Fig. 2).

Growth rings were counted along an observer-defined radius. Grey level peaks extracted by T.N.P.C. (Calcified Structure Digital Processing) along the radius give the number of rings. In the second step, extrapolating increments in dark “unreadable” zones completed counts. Increments were also extrapolated on the dome edge. Extrapolation was necessary in 143 out of 236 statoliths and the average distance extrapolated represented 18% of transect length (between 4% and 25%).

The consequences of observer’s choices in radius position and dark zone processing were tested on a subsample of 60 statoliths read by four different observers.

2.4. Age and length-at-age data analysis

2.4.1. Validation of counts of rings

The analysis of discrepancies between observers reflects a cautious attitude in the transfer of statolith techniques to a new species whose statoliths are often considered as poorly marked.

Validation procedures usually rely on Friedman’s non-parametric “test of k related samples” (as stated in the European Fish Ageing Network recommendations which are available on the EFAN website). In addition, two more powerful parametric methods were applied to identify

differences between observers and also to quantify observer-related reading errors.

An analysis of variance was carried out with two factors: the observer and the statolith identification code. Normality and homoscedasticity were checked before applying parametric statistics (see above mentioned tests). Following the ANOVA, homogeneous groups of observers were sought with Newman–Keul’s test.

The quantification of observer-related error was based on a linear model with mixed effects fitted with the “lme” function implemented in S-Plus© (Laird and Ware, 1982) (we used the freeware equivalent of S-plus© named “R”). Two different effects are considered to have an effect on the age of the specimen: the statolith’s number and the observer. Statistical errors related to each affect add up in this model:

$$Y_{i,j} = \mu + \sigma_i + \sigma_j + \sigma_{i,j}$$

where $Y_{i,j}$ is the real age (in days) of statolith j read by the observer i , μ is the average across all ages, σ_i is the standard error of “observer i ” effect, σ_j is the standard error of “statolith number j ” effect and $\sigma_{i,j}$ is the residual standard error (i.e. the usual error on counts whatever the observer).

Such mixed-effect models allow the computation of confidence limits, which correspond to the interval between two counts that can be considered as statistically different; 95% confidence limits indicate that statoliths j and k read by the same observer i have a significantly different age if the observed difference falls outside the interval:

$$\Pr\left(\mu_j - \mu_k \notin \left(|Y_{i,j} - Y_{i,k}| \pm 1.96\sqrt{2\sigma_{i,j}^2}\right)\right) = 0.95$$

The estimation of statistical errors in age reading was then used to define time periods for animals “born at the same time” within the hatching period.

2.4.2. Age-at-recruitment and hatching period

The influence of the sampling date on the average age-at-recruitment was tested with an analysis of variance, or better with Kruskal–Wallis’s non-parametric equivalent of the ANOVA.

Individual age of studied specimens allowed back-calculation of hatching dates.

2.4.3. Growth

A growth model was fitted to length-at-age data collected for juveniles of the cohort 2000. In order to test the influence of the hatching period, all aged specimen were grouped in clusters of animals born at the same time. Linear relationships between mantle length and age are thus derived from our model:

$$L_{i,m} = (\beta_a + \delta_{a,m}) A_{i,m} + (\beta_0 + \gamma_m)$$

where $L_{i,m}$ is the dorsal mantle length (DML) (in mm) of a cuttlefish in age i among those which hatched in month m and $A_{i,m}$ is the cuttlefish age (in days).

Estimated parameters are:

- the growth rate ($\beta_a + \delta_{a,m}$) which is the combination of β_a , average growth rate and $\delta_{a,m}$: the influence of the hatching month on growth rate,
- the theoretical hatchling length ($\beta_0 + \gamma_m$) (or the origin ordinate) which combines β_0 , average hatching length and γ_m : influence of the hatching month on hatching length.

Model estimates and analysis of variance were computed with the “lm” procedure implemented in S-plus.

3. Results

3.1. Observer-related counting errors

The Friedman non-parametric test did not reveal significant differences between observers ($W = -4.8$, probability > 0.05). On the studied subsample, preliminary tests of normality and homoscedasticity allowed the use of parametric methods (Shapiro–Wilks statistic = 0.98 with $P > 0.05$ and Bartlett’s statistics = 1.013 with $P > 0.05$). The two-way analysis of variance indicated on the contrary that the observer had a significant effect on age reading (Table 2). The Newman–Keul’s procedure suggested, however, that this might be related to observer number 2 whose results are significantly different from the homogeneous group of the three other observers.

Quantification of errors was derived from the mixed-effect model (Table 3). The observer component of statistical error σ_i indicated that whoever the observer, readings were biased by 3.3 d on average. However, this was much less than the residual error in readings, which was 9.5 d. Confidence intervals computed with such standard errors indicate that two statoliths read by the same observer could be considered having a different age when their number of growth rings differed by more than 13 rings. This estimation allowed to define monthly periods for animals born within the hatching period. The rest of the age estimates in this study was based on readings made by the same observer.

3.2. Length structures

Length–frequency distributions of all studied samples are plotted in Fig. 3. Modal progression observed in pre-recruitment scientific samples (10–58 mm) collected in August–September and in recruited commercial samples of category 4 animals (53–90 mm) suggest that these two samples belonged to the same cohort. On the other hand, the October commercial sample of category 3 animals (103–128 mm) was clearly from another group of larger animals. Changes in average length were tested in pre-recruitment samples and in the category 4 samples.

Table 2
Two-way analysis of variance and Newman–Keuls’s test for the validation of age reading on *Sepia officinalis* statolith

| Factors | df | SEM | Mean square | F-ratio | P-value |
|----------------------|-------|----------------------|-------------|--------------------|---------|
| Analysis of variance | | | | | |
| Statolith number | 68 | 60.552 | 890.5 | 8.57 | 0 |
| Observer | 3 | 1.272 | 424.2 | 4.08 | 0.008 |
| Residuals | 136 | 15.449 | 113.6 | | |
| Total | 206 | 63.943 | | | |
| Newman–Keuls’s test | | | | | |
| Observer | Count | Mean number of rings | | Homogeneous groups | |
| 2 | 69 | 108.9 | | X | |
| 4 | 69 | 113.7 | | X | |
| 3 | 69 | 113.8 | | X | |
| 1 | 69 | 114.3 | | X | |

Table 3
Coefficients (in increment number) of the linear mixed-effect model relating error estimations of age reading by observers on *Sepia officinalis* statolith

| Parameters | Number of rings |
|-------------------------|-----------------|
| μ | 107 |
| σ_i | 3.3 |
| σ_j | 15.3 |
| $\sigma_{i,j}$ | 9.5 |
| 95% Confidence interval | 13.4 |

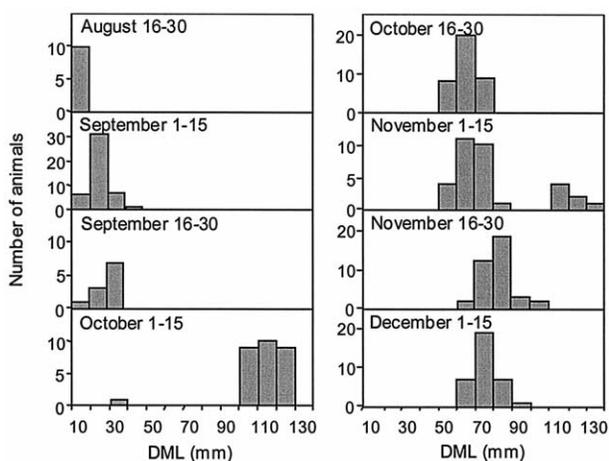


Fig. 3. Length–frequency distribution of juvenile cuttlefish sampled from August to December 2000 in the English Channel. DML: dorsal mantle length.

Pre-recruitment mean size increased from 13 to 30 mm (ML) between August and September. In this series, the Kruskal–Wallis test used to compare samples showed highly significant differences in average length (probability $< 3 \times 10^{-7}$).

Average length increased from 65 to 81 mm between October and December in the category 4 animals. Hypotheses of normality and homoscedasticity could not be rejected (Shapiro–Wilks statistic = 0.97 with $P > 0.05$ and Bartlett’s statistics = 1.063 with $P > 0.05$ in the data set). The analysis of variance showed that sampling time had a significant effect on the average length of recruits (Table 4). Besides, Newman–Keul’s test suggested that each sample was significantly different from all others.

3.3. Age-at-recruitment

Age distribution of recruits sampled in autumn 2000 derived from statolith information is plotted in Fig. 4. The age of “category 4” samples ranged between 2 and 5.5 months (84% of recruits were between 60 and 120 d old). The age distribution pattern appears unimodal and modal progression was confirmed by a Kruskal–Wallis test showing that average age was significantly different in the four samples. However, differences in average age in successive samples are not correlated with time intervals between samples. By the end of the study period, no animal younger than 70 d old was caught, which suggests that

Table 4
One-way analysis of variance and Newman–Keuls’s test for mantle length variations at recruitment for *Sepia officinalis*

| Factors | df | SEM | Mean square | F-ratio | P-value |
|----------------------|-------|-----------|-------------|--------------------|---------|
| Analysis of variance | | | | | |
| Collecting dates | 3 | 5.256 | 1.752 | 36.9 | 0 |
| Mantle length | 128 | 6.061 | | | |
| Total | 131 | 11.316 | | | |
| Collecting dates | | | | | |
| Newman–Keuls’s test | Count | Mean (mm) | | Homogeneous groups | |
| 22 October | 35 | 64.6 | | X | |
| 12 November | 26 | 68.7 | | X | |
| 26 November | 34 | 74.0 | | X | |
| 10 December | 37 | 80.9 | | X | |

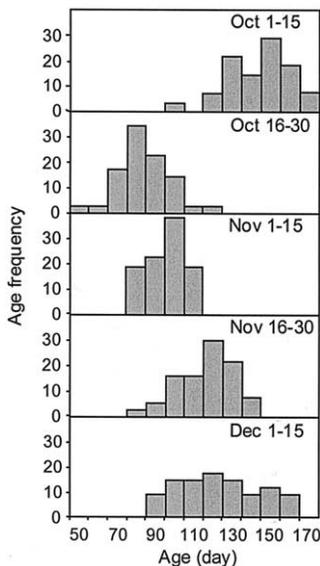


Fig. 4. Age–frequency histograms of juveniles sampled from commercial categories 3 and 4 in the period from October to December 2000 in the English Channel.

hatching of this micro-cohort was finished at the beginning of autumn.

In October 2000, the smallest cuttlefish landed in Port-en-Bessin was a category 3 specimen. These animals were between 4 and 7 months old, which suggest that they belonged to a different micro-cohort of the 2000-year class. Additional samples of this micro-cohort would have been necessary to describe this previous pulse of recruitment. Nevertheless, it seems logical to consider that these animals reached the size at recruitment in category 4 during the summer.

3.4. Hatching period

All sampled cuttlefish belonged to the year 2000 cohort and by back-calculation, the hatching dates of these 236 animals were estimated. Hatching was spread from April 24th to September 11th (Fig. 5). However, most of the animals were hatched within 4 months (May–August) and 75% between July and the middle of August. Age-at-recruitment of category 4 individuals increased with time (Fig. 6). More August born animals were recruited later in the season. The sample of the category 3 individuals showed a different pattern with almost all specimens hatched before mid-June.

3.5. Growth model

The linear growth model fitted to length-at-age data indicated that hatching month had a highly significant effect on growth rate (Table 5). As a consequence, three curves describing the length/age relationship of cuttlefish hatched in June, July and August were plotted (Fig. 7) which corresponded to three sets of parameters (Table 6). Growth

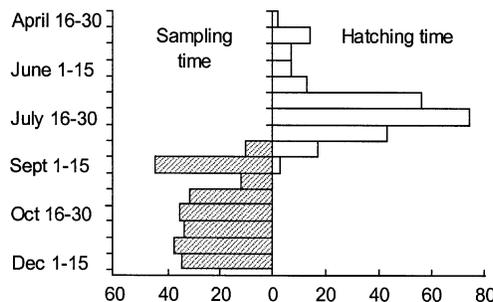


Fig. 5. Comparison between temporal distribution of samples and hatching of juvenile cuttlefish from English Channel ($n = 236$).

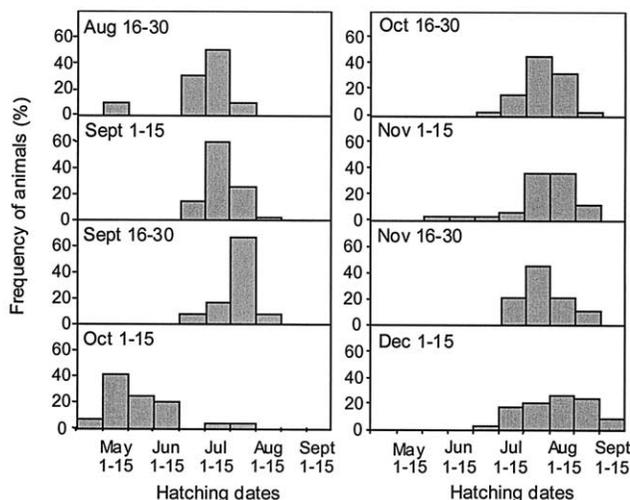


Fig. 6. Hatching date–frequency histograms of juvenile cuttlefish sampled from August to December 2000 in the English Channel.

Table 5
Analysis of variance. Regression coefficients of the growth linear model of juveniles hatched in three different months for *Sepia officinalis* in 2000

| Factors | df | SEM | F-ratio | P-value |
|---|----|---------|---------|---------|
| Analysis of variance | | | | |
| Average growth rate | 1 | 114 389 | 717.9 | *** |
| Influence of the hatching month on: hatching length | 2 | 2 525 | 15.8 | *** |
| Growth rate | 2 | 3 513 | 22 | *** |

*** $P < 0.001$.

rate decreased from early hatched (June 1.2 mm d^{-1}) to late hatched animals (July 0.7 mm d^{-1} and August 0.5 mm d^{-1}).

The ordinate origin coefficients were also significantly different according to the hatchling month (Table 5). However, this parameter should be considered here as a theoretical “length at hatchling” since it was estimated with cuttlefish ranging from 30 to 160 d old and not with hatchlings.

4. Discussion

The analysis of statolith increments to derive age estimates is now routinely applied in studies on squid (Jackson,

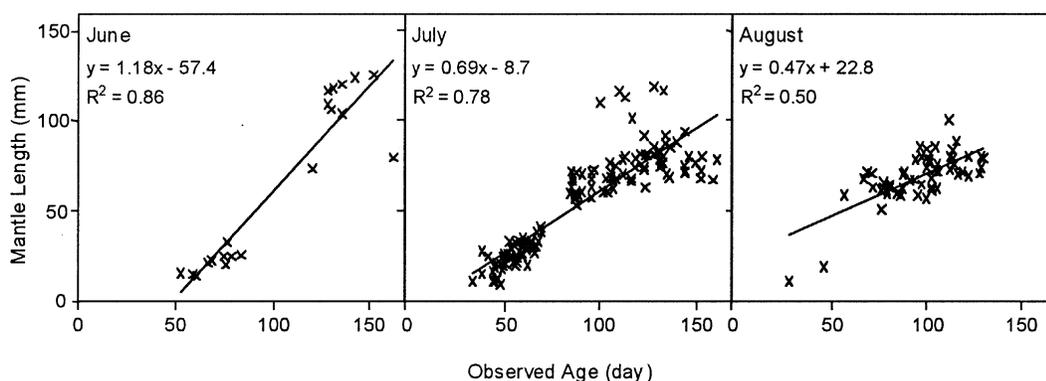


Fig. 7. Relationships between the number of growth increments (age) and mantle length (ML) in *Sepia officinalis* supposed to hatch in three different months (June–August).

Table 6
Two-way analysis of variance for growth variations of juveniles for *Sepia officinalis* in 2000

| Explanatory variable | Regression coefficient | Standard error | <i>t</i> | <i>P</i> -value |
|---------------------------------|------------------------|----------------|----------|-----------------|
| Origin ordinate | -57.4 | 9 | -6.6 | *** |
| Average growth rate | 1.2 | 0.1 | 14.7 | *** |
| Influence of the hatching month | | | | |
| July on hatching length | 48.5 | 9 | 5.2 | *** |
| August on hatching length | 81.4 | 12 | 5.2 | *** |
| July on growth rate | -0.5 | 0.1 | -5.7 | *** |
| August on growth rate | -0.7 | 0.1 | -6.3 | *** |

*** $P < 0.001$.

1994). Evidence for daily statolith ring periodicity has been obtained for a number of cephalopod species, including recently for *Sepia officinalis* by rearing experiments (Bettencourt and Guerra, 2001). Although cuttlefish are easier to rear than squid, the validation of daily ring deposition was obtained later in *Sepia* due to the fact that cuttlefish statolith increments are less visible and hence more difficult to read than squid statoliths. The poor definition of growth rings in *Sepia officinalis* seems to be related to the low percentage of organic matter in the statoliths (Bettencourt and Guerra, 2000). In this context, reading errors are likely to be higher (Lipinski et al., 1998).

The present study is the first attempt to apply statolith-ageing techniques to *Sepia officinalis* collected in the wild. Two complementary approaches were employed to address the problem of errors in statolith readings due to the difficulty in observing increments. Firstly, a grinding plane was defined so as to reveal increments as clearly as possible. Fig. 2 is an example of the quality of images that were digitised. Secondly, reading errors between observers were statistically evaluated. The recommendations of the European Fish Ageing Network were a useful starting point for a study of the validation procedures of age reading. However, the statistical methods employed in this programme were not powerful enough to analyse problems associated with cuttlefish statoliths. For most cephalopods, growth increments are daily and are condensed into a smaller structure than the annual rings of fish otoliths, resulting in higher observer-related errors. By comparing two methods of age reading (manual and semi-automatic) with the known

age of *Illex coindetii* and *Sepioteuthis lessoniana*, Gonzalez et al. (1998) showed that there was no statistical difference between the semi-automatic method and the true age of animals, and that reading errors were reduced with the semi-automatic compared to the manual method. The semi-automatic age reading method used in the present study can thus be considered to have provided better results than the manual method, despite differences between observers.

Cuttlefish population studies in the English Channel have deduced from spawning and recruitment that hatching occurred in August (Boucaud-Camou and Boismery, 1991) or in July and August (Medhioub, 1986). Age determination of individuals showed that in 2000 hatching was spread over more than 4 months, a period similar to that in Bay of Biscay populations where hatching commenced 1.5 months earlier (Bouchaud and Daguzan, 1989).

The general pattern of juvenile growth described with statolith analysis is consistent with previous studies based on length–frequencies in the English Channel (Medhioub, 1986). The growth curve was linear during the study period (from 40 to 170 d). Since growth was not measured before or after this period, the overall nature of the growth curve (i.e. exponential, linear, Gompertz, etc.) is unknown. Several types of growth curve function have been applied in cephalopod growth studies, perhaps reflecting the fact that these organisms show large inter-individual growth variations: the growth curve was exponential in *Beryteuthis magister* (Arkhipkin et al., 1998), logistic in *Onychoteuthis banksi* (Arkhipkin and Nigmatullin, 1997), a power growth function was fitted in *Loligo vulgaris* (Arkhipkin, 1995),

and a Gompertz function was applied in *Martialia hyadesi* (Arkhipkin and Silvanovich, 1997). The growth of *S. officinalis*, until present has only been recorded in studies on reared animals, is apparently different from that of squid. Richard (1971) and Boletzky (1979) showed that reared *S. officinalis* growth was sigmoid. Forsythe and Van Heukelem (1987) described a two-phase curve with an exponential phase followed by a logarithmic phase.

The large inter-individual growth variation in cephalopods is probably related to environmental conditions (Brodziak and Macy, 1996). Our results provide the first evidence for a reduced growth rate in juveniles born in late summer compared with those born early in the hatching period (1.2 mm d⁻¹ in June, 0.7 mm d⁻¹ in July and 0.5 mm d⁻¹ in August). Certain authors have also reported a different growth rate by hatching month in *Sepia officinalis* (Bouchaud and Daguzan, 1989; Le Goff and Daguzan, 1991) and in *Loligo gahi* (Hatfield, 2000). In each of these studies, temperature fluctuations seemed to be the most important environmental factor, which caused seasonal growth variations (Hatfield, 2000; Forsythe et al., 2001; Hatfield et al., 2001). The effect of temperature on hatching and growth of various cephalopod species has been investigated in several laboratory studies. Forsythe et al. (2001) demonstrated a relationship between growth rate and temperature in juveniles of *Sepioteuthis lessoniana*; growth rate at 27 °C was nearly two times that at 20 °C. A similar relationship was obtained in juveniles of *Sepia elliptica* (Martinez et al., 2000). In our study, juvenile cuttlefish hatched within the same spring–summer period showed in autumn a size range with two modes (53–90 and 103–128 mm). Animals born early in the hatching period were larger than animals of equivalent age born later, a phenomenon apparently related to the length of time spent at higher temperatures. However, reared *S. officinalis* also showed strong growth variations (Richard, 1969; Domingues et al., 2001). At constant temperature, a range of growth stages was observed, an observation, which may limit interpretations of the influence of environmental parameters on cuttlefish growth variation.

Recruitment variability is a complex phenomenon. It can either be related to abiotic factors affecting juvenile growth (and thus age-at-recruitment) or to density-dependent factors. Temperature used as a proxy of environmental conditions showed significant effect on recruitment success in *Illex illecebrosus* (Dawe and Warren, 1993; Dawe et al., 2000) and in Loliginid squid (Robin and Denis, 1999). On the other hand, density-dependent survival seemed to play a more significant role in recruitment in *Loligo gahi* (Agnew et al., 2000). In this stock and at high biomass levels, a negative trend is observed between spawning biomass and subsequent recruitment. All of the factors must be taken into consideration in order to understand recruitment variations. Stock assessment based on recruitment predictions will only use a subset of predictable variables.

Variations in size showed that juveniles of *S. officinalis* born in the same hatching period belonged to two commercial categories (3 and 4). Animals, which experienced higher temperatures over longer periods, were recruited earlier in the stock as observed by Agnew et al. (2000). Consequently, we may conclude that juveniles of *S. officinalis* from the same hatching period were not recruited into the fishery at the same time. The hatching period was shorter than the recruitment period and growth variations seemed to be the cause of this difference. Cephalopod stock assessment may be biased when analysis of length–frequency is employed rather than a growth curve derived from statolith readings (Jackson et al., 1997). In *Sepia officinalis*, the comparison between data of length-at-age and length–frequency analysis realised by Medhioub (1986) showed that growth was different for juvenile. Our study has used statoliths to estimate age and growth in wild individuals. Age reading is difficult in this species but the results were accurate enough to reveal variations in growth rates and age-at-recruitment. Despite its importance, age is often a missed data for a reliable stock assessment. In this study, only one cohort has been considered. This first analysis suggests that other cohorts should be analysed and compared to show inter-annual growth variations in *S. officinalis*. Inter-annual growth variations in juveniles could cause stock fluctuations, and hence be an important factor in development of predictive models for fishery management advice.

Acknowledgements

This work owes a lot to Hervé Troadec (LASAA, France) who unfortunately has left us too early. We wish to thank him for helping us to use his image analysis software T.N.P.C.© and for his advice in age reading. We would also like to thank Jean Lejeune (Department of Mathematics of the University of Caen) for his help in the mathematical aspect of the study.

References

- Agnew, D.J., Hill, S., Beddington, J.R., 2000. Predicting the recruitment strength of an annual squid stock: *Loligo gahi* around the Falkland Islands. *Can. J. Fish. Aquat. Sci.* 57, 2479–2487.
- Arkhipkin, A., 1995. Age, growth and maturation of the European squid *Loligo vulgaris* (Myopsida, Loliginidae) on the West Saharian shelf. *J. Mar. Biol. Assoc. UK* 75, 593–604.
- Arkhipkin, A., Bizikov, V., Verkhunov, A., 1998. Distribution and growth in juveniles of the squid *Beryteuthis magister* (Cephalopoda, Gonatidae) in the western Bering Sea. *Sarsia* 83, 45–54.
- Arkhipkin, A., Nigmatullin, C., 1997. Ecology of the oceanic squid *Onychoteuthis banksi* and the relationship between the genera *Onychoteuthis* and *Chaunoteuthis* (Cephalopoda, Onychoteuthidae). *J. Mar. Biol. Assoc. UK* 77, 839–869.

- Arkhipkin, A., Silvanovich, N., 1997. Age, growth and maturation of the squid *Martialia hyadesi* (Cephalopoda, Ommastrephidae) in the south-west Atlantic. *Antarctic Sci.* 9, 373–380.
- Bettencourt, V., Guerra, A., 2000. Growth increments and biomineralization process in cephalopod statoliths. *J. Exp. Mar. Biol. Ecol.* 248, 191–205.
- Bettencourt, V., Guerra, A., 2001. Age studies based on daily growth increments in statoliths and growth lamellae in cuttlebone of cultured *Sepia officinalis*. *Mar. Biol.* 139, 327–334.
- Bigelow, K.A., 1992. Age and growth in paralarvae of the mesopelagic squid *Abralia trigonura* based on daily growth increments in statoliths. *Mar. Ecol. Prog. Ser.* 82, 31–40.
- Boletzky, S.V., 1979. Growth and life-span of *Sepia officinalis* under artificial conditions (Mollusca, Cephalopoda). *Rapp. Comm. Int. Mer. Medit.* 24/25, 159–168.
- Boletzky, S.V., 1983. *Sepia officinalis*. In: Boyle, P.R. (Ed.), *Cephalopod Life Cycles*, vol. 1. Academic Press, London, pp. 31–52.
- Boucaud-Camou, E., Boismery, J., 1991. The migrations of the cuttlefish (*Sepia officinalis* L.) in the English Channel. In: Boucaud-Camou, E. (Ed.), *The Cuttlefish*. Université de Caen, pp. 179–189.
- Bouchaud, O., Daguzan, J., 1989. Etude du développement de l'œuf de *Sepia officinalis* (Mollusque, Sepiidae) en conditions expérimentales. *Haliosis* 19, 189–200.
- Boyle, P.R., Boletzky, S.V., 1996. Cephalopod populations: definition and dynamics. *Phil. Trans. R. Soc. Lond.* 351, 985–1002.
- Brodziac, J.K.T., Macy, W.K., 1996. Growth of long-finned squid, *Loligo pealei*, in the Northwest Atlantic. *Fish. Bull.* 94, 212–236.
- Caddy, J.F., 1991. Daily rings on squid statoliths: an opportunity to test standard population models? In: Jereb, P., Ragonese, S., von Boletzky, S. (Eds.), *Squid Age Determination Using Statoliths*, Proceedings of the International Workshop held in the Instituto di Tecnologia Della Pesca e del Pescato. NTR-ITPP, Spec. Publ. pp. 53–66.
- Caddy, J.F., 1996. Modelling natural mortality with age in short-lived invertebrate populations: definition of a strategy of gnomonic time division. *Aquat. Living Resour.* 9, 197–207.
- Dawe, E.G., Colbourne, E.B., Drinkwater, K.F., 2000. Environmental effects on recruitment of short-finned squid *Illex illecebrosus*. *ICES J. Mar. Sci.* 57, 1002–1013.
- Dawe, E.G., Natsukari, Y., 1991. Light microscopy. In: Jereb, P., Ragonese, S., von Boletzky, S. (Eds.), *Squid Age Determination Using Statoliths*, Proceedings of the International Workshop held in the Instituto di Tecnologia Della Pesca e del Pescato. NTR-ITPP, Spec. Publ. pp. 83–95.
- Dawe, E.G., Warren, W.G., 1993. Recruitment of short-finned squid in the northwest Atlantic Ocean and some environmental relationships. *J. Cephalopod Biol.* 2, 1–21.
- Dommingues, P.M., Kingston, T., Sykes, A., Andrade, J.P., 2001. Growth of young cuttlefish, *Sepia officinalis* (Linnaeus, 1758) at the upper end of the biological distribution temperature range. *Aquat. Res.* 32, 923–930.
- Dunn, M., 1999. Aspects of the stock dynamics and exploitation of cuttlefish, *Sepia officinalis* (Linnaeus, 1758) in the English Channel. *Fish. Res.* 40, 277–293.
- Forsythe, J.W., Van Heukelem, W.F., 1987. Growth. In: Boyle, P.R. (Ed.), *Cephalopod Life Cycles*, vol. 2. Academic Press, London, pp. 135–156.
- Forsythe, J.W., Walsh, L.S., Turk, P.E., Lee, P.G., 2001. Impact of temperature on juvenile growth and age at first egg-laying of the Pacific reef squid *Sepioteuthis lessoniana* reared in captivity. *Mar. Biol.* 138, 103–112.
- Gi-Geon, I., 1982. Etude des populations de seiche (*Sepia officinalis* L.) du golfe de Gascogne. Thèse de 3^e cycle, Université de Nantes.
- Gonzalez, A.F., Macy III, W.K., Guerra, A., 1998. Validation of a semi-automatic image analysis system to age squids and its application to age *Illex coindetii* statoliths. *ICES J. Mar. Sci.* 55, 535–544.
- Guerra, A., Castro, B.G., 1988. On the life cycle of *Sepia officinalis* in the Ria de Vigo. *Cah. Biol. Mar.* 29, 395–405.
- Hatfield, E.M.C., 2000. Do some like it hot? Temperature as a possible determinant of variability in the growth of the Patagonian squid, *Loligo gahi* (Cephalopoda: Loliginidae). *Fish. Res.* 47, 27–40.
- Hatfield, E.M.C., Hanlon, R.T., Forsythe, J.W., Grist, E.P.M., 2001. Laboratory testing of a growth hypothesis for juvenile squid *Loligo pealei* (Cephalopoda: Loliginidae). *Can. J. Fish. Aquat. Sci.* 58, 845–857.
- Hilborn, R., Walters, R.F., 1992. *Quantitative Fish Stock Assessment: Choice, Dynamics and Uncertainty*. Chapman & Hall, New York.
- Hurley, G.V., Odense, P.H., O'Dor, R.K., Dawe, E.G., 1985. Strontium labelling for verifying daily growth increments in the statolith of the short-finned squid (*Illex illecebrosus*). *Can. J. Fish. Aquat. Sci.* 42, 380–383.
- Jackson, G.D., 1989. The use of statolith microstructures to analyse life-history events in the small tropical cephalopod *Idiosepius pygmaeus*. *Fish. Bull. US* 87, 265–272.
- Jackson, G.D., 1990. The use of tetracycline staining techniques to determine statolith growth ring periodicity in the tropical Loliginid squids *Loliolus nocticula* and *Loligo chinensis*. *Veliger* 33, 389–393.
- Jackson, G.D., 1994. Application and future potential of statolith increment analysis in squids and sepoids. *Can. J. Fish. Aquat. Sci.* 51, 2612–2625.
- Jackson, G.D., Arkhipkin, A.I., Bizikov, V.A., Hanlon, R.T., 1993. Laboratory and field corroboration of age and growth from statoliths and gladii of the loliginid squid *Sepioteuthis lessoniana*. In: Okutani, T., O'Dor, R.K., Kubodera, T. (Eds.), *Recent Advances in Cephalopod Fisheries Biology*. Tokai University Press, Tokyo, pp. 189–199.
- Jackson, G.D., Forsythe, J.W., Hixon, R.F., Hanlon, R.T., 1997. Age, growth and maturation of *Lolliguncula brevis* (Cephalopoda: Loliginidae) in the northwestern Gulf of Mexico with a comparison of length–frequency versus statolith age analysis. *Can. J. Fish. Aquat. Sci.* 54, 2907–2919.
- Laird, N.M., Ware, J.H., 1982. Random-effects models for longitudinal data. *Biometrics*. 38, 963–974.
- Le Goff, R., Daguzan, J., 1991. Growth and life cycles of the cuttlefish *Sepia officinalis* L. (Mollusca, Cephalopoda) in south Brittany (France). *Bull. Mar. Sci.* 49, 341–348.
- Le Goff, R., Gauvrit, E., Pinczon Du Sel, G., Daguzan, J., 1998. Age group determination by analysis of the cuttlebone of the cuttlefish *Sepia officinalis* L. in reproduction in the Bay of Biscay. *J. Moll. Stud.* 64, 183–193.
- Lipinski, M., 1986. Methods for the validation of squid age from statoliths. *J. Mar. Biol. Assoc. UK* 66, 505–526.
- Lipinski, M.R., Durholtz, M.D., Underhill, L.G., 1998. Field validation of age readings from the statoliths of chokka squid (*Loligo vulgaris reynaudii* d'Orbigny, 1845) and an assessment of associated errors. *ICES J. Mar. Sci.* 55, 240–257.
- Martinez, P., Bettencourt, V., Guerra, A., Moltschanivskij, N.A., 2000. How temperature influences muscle and cuttlebone growth in juvenile cuttlefish (*Sepia elliptica*) (Mollusca: Cephalopoda) under conditions of food stress. *Can. J. Zool.* 78, 1855–1861.
- Medhioub, A., 1986. Etude de la croissance et du cycle sexuel de la seiche (*Sepia officinalis* L.) des côtes normandes. Thèse de 3^e cycle, Université de Caen.
- Nakamura, Y., Sakurai, Y., 1991. Validation of daily growth increments in statoliths of Japanese common squid *Todarodes pacificus*. *Nippon Suisan Gakkaishi* 57, 2007–2011.
- Richard, A., 1969. The part played by temperature in the rhythm of marking on the shell of cuttlefish (*Sepia officinalis* L.) (Cephalopoda, Mollusca). *Experimentation Basel* 25, 1051–1052.
- Richard, A., 1971. Contribution à l'étude expérimentale de la croissance et de la maturation sexuelle de *Sepia officinalis* L. (Mollusque : Cephalopode). [Thèse]. Doct. et Scien. Nat. Université de Lille.
- Robin, J.P., Denis, V., 1999. Squid stock fluctuations and water temperature: temporal analysis of English Channel Loliginidae. *J. Appl. Ecol.* 36, 101–110.