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Growth and cuttlebone microstructure of juvenile cuttlefish, *Sepia officinalis* L., under controlled conditions

Pedro Ré*, Luis Narciso

Laboratório Marítimo da Guia, Departamento de Zoologia e Antropologia, Faculdade de Ciências da Universidade de Lisboa, Estrada do Guincho, 2750 Cascais, Portugal

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Abstract

The periodicity of deposition of growth increments in the cuttlebone of juvenile *Sepia officinalis* was investigated under controlled conditions for a short period after hatching (19 days). The slope of time elapsed and increment counts was significantly different from 1 indicating that lamellae are not deposited on a daily basis. The relationship between increment counts and juvenile length was, however, highly significant. Also significant were the relationships between the number of growth increments and shell length and increment counts and cuttlebone area. These data show that the number of growth increments is related primarily to the growth rate of the juvenile rather than to its chronological age. Taking into account these data, the cuttlebone lamellae cannot be used for age determination in the juvenile cuttlefish just after hatching.

Key words: Cuttlebone microstructure; Growth; Growth increment; Juvenile; *Sepia officinalis*

1. Introduction

The cuttlefish, *Sepia officinalis* L., is a common demersal neritic species occurring predominantly near sandy and muddy bottoms up to a depth of 200 m. It is widely distributed in the Eastern Atlantic and ranges from the Baltic and North Sea to South Africa including the Mediterranean. Larger individuals exhibit offshore-inshore seasonal migration patterns mainly related to spawning (Roper et al., 1984), which occurs in shallow waters and peaks in spring and summer. This species represents an important commercial resource throughout its range and world cuttlefish catches varied from 8500 to 14000 metric tons in the 1980s (Roper et al., 1984). During a recent interna-

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* Corresponding author.

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tional symposium (Boucaud-Camou, 1991) the importance of the rearing prospects for cuttlefish was widely recognized.

Cuttlebone growth patterns are relatively well documented and suggest that there is a close relationship between shell growth and the periodicity of physiological processes (Choe, 1963). Choe (1963) also mentions that three species of cuttlefish (*Sepia esculenta*, *Sepia subaculeata* and *Sepiella maindroni*) deposit daily growth increments (lamellae or striations) in the cuttlebone. The objective of the present paper was to investigate the periodicity of deposition of lamellae in the cuttlebone of juvenile cuttlefish (*Sepia officinalis*) under controlled conditions for a short period after hatching.

2. Materials and methods

Juvenile cuttlefish were hatched from wild eggs collected in Sado estuary (central Portugal) at low tide in July 1992. Clusters of eggs were randomly collected from a small area of the estuary and most probably did not belong to the same brood stock. The clusters of eggs were incubated in the laboratory, in seawater at a temperature of 21 ± 1 °C and a salinity of 33 ± 1‰ in a small tank (80-l capacity) with bottom aeration and 16L:8D photoperiod, until hatching. In order to simulate natural conditions clusters of eggs were suspended on ropes.

Newly hatched juveniles were reared in 5-l tanks, under the same conditions, in filtered ultra violet sterilized seawater. Water was changed daily. Adult brine shrimp *Artemia franciscana* (Utah strain) fed on *Chlorella* sp. was supplied ad libitum twice a day (1000 and 1900). The lighting was provided by fluorescent daylight strip lamps (18 W) suspended over the tanks. Rearing tanks were provided with bottom sand filters. Sand was used to enable juveniles to burrow, and thereby reduce stress.

Several juveniles (three to eight) were sacrificed daily for a period of 19 days after hatching. Fixation and preservation was performed using borate-buffered formalin (pH 8.5–9). All measurements and weighings were made on preserved material. No shrinkage correction was applied to the lengths of the juveniles. Cuttlebone dissection was performed using a stereoscopic microscope and fine forceps under a magnification of 6 to 12 ×. Several measurements (juvenile length, juvenile width, cuttlebone length and cuttlebone maximum width) were made on each individual with the aid of a stereoscopic microscope and a calibrated micrometer eyepiece. Juvenile length corresponded to total length measured from the posterior portion of the mantle to the end of extended tentacles. Juvenile width correspond to maximum width usually measured as the distance between the eyes. Cuttlebone measurements corresponded only to the inner cone (terminology of Sweeney et al., 1992). Other cuttlebone measurements (perimeter, area and growth increment widths) were performed using a Video-Microscope-IBM/PC image analysis system (IAS). The IAS used consisted of an IBM compatible microcomputer, a Sony video camera, a video monitor, image analysis software (Image-Pro Plus) and hardware (digitizer board PCVISION plus) interfaced with a stereoscopic microscope. This IAS was used mainly to measure the widths of growth increments (lamellae) and to study cuttlebone microstructure using its image processing capabilities.

Growth increments in the cuttlebone were counted directly through a stereomicro-

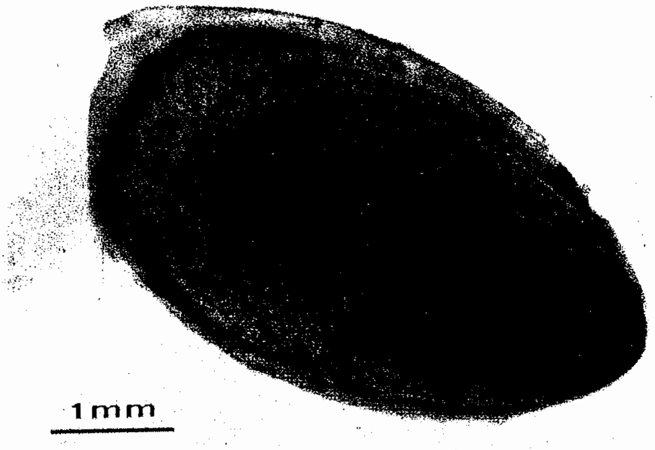

Fig. 1. Microstructure of the cuttlebone of juvenile *Sepia officinalis* (processed image): cuttlebone with eight growth increments; juvenile length 9.4 mm; age 11 days.

scope using magnifications that ranged from 12 to 50 × . Lamellae consist of two zones with different development. When viewed through the stereomicroscope with transmitted light each lamellae consists of a wide translucent zone and a narrow opaque zone (Fig. 1). Total counts of lamellae were made only in the inner cone of the cuttlebone. Juvenile and cuttlebone dry weights were determined after freeze-drying using a Cahn 21 automatic electrobalance with a precision of 0.1 μg.

3. Results

Juvenile weight varied markedly from day to day whereas total length, cuttlebone length and cuttlebone weight remained almost constant during the experiment with a

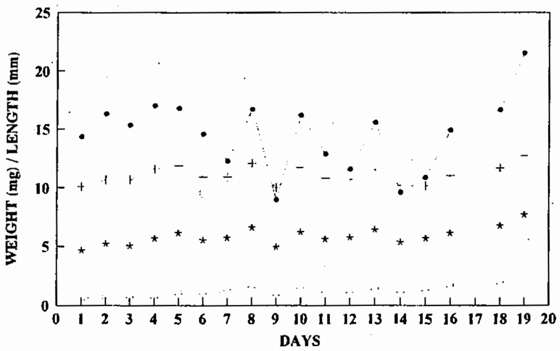

Fig. 2. Variation in juvenile weight (●), juvenile length (+), cuttlebone length (∗) and cuttlebone weight (□) throughout the experimental period. Each data point represents the mean of three to eight individuals.

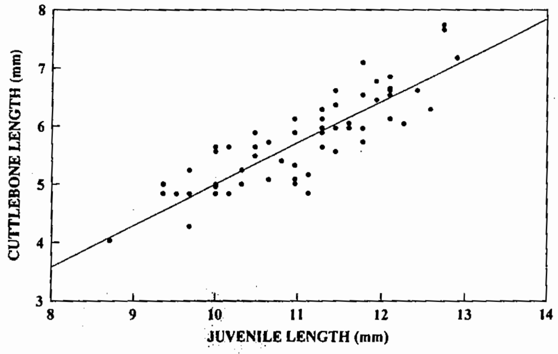

Fig. 3. Relationship between juvenile length and cuttlebone length, together with linear-regression analysis and fitted line.

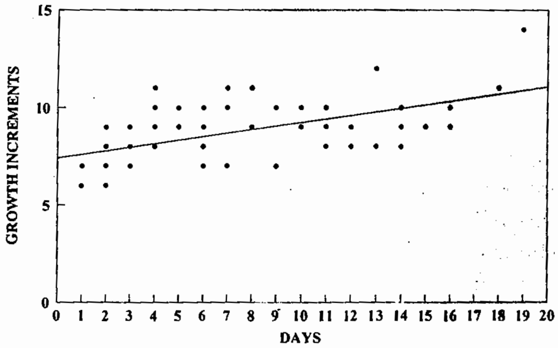

Fig. 4. Relationship between the number of days after hatching and the number of growth increments deposited in the cuttlebone, together with linear-regression analysis and fitted line.

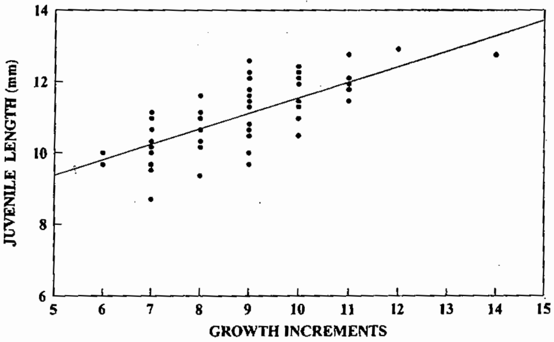

Fig. 5. Relationship between the number of growth increments deposited in the cuttlebone and juvenile length, together with linear-regression analysis and fitted line.

Table 1
Regression parameters ($y = a + bx$)

y	x	a	b	r^2	P	n
Cuttlebone length	Juvenile length	-2.098	0.710	0.742	0.001	64
Growth increments	Days	7.412	0.181	0.360	0.001	69
Juvenile length	Growth increments	7.203	0.434	0.539	0.001	69
Cuttlebone length	Growth increments	1.852	0.437	0.915	0.001	64
Juvenile weight	Growth increments	3.423	1.285	0.304	0.001	69
Cuttlebone area	Growth increments	4.189	2.146	0.814	0.001	55
Cuttlebone weight	Growth increments	1.223	0.259	0.658	0.001	69

slight increase from day 15 onwards (Fig. 2). The relationship between cuttlebone length and juvenile length was highly significant ($p < 0.001$) (Fig. 3).

On Day 1 juveniles did not feed on *Artemia* that was supplied ad libitum and did not attempt to actively catch these prey. On Day 2 the first attempts to catch prey items were observed but were rarely successful. From Day 3 onwards juveniles began to exhibit a normal feeding behaviour and actively captured *Artemia*.

Growth increments were enumerated in 69 cuttlebones. The relationship between the number of days after hatching and the number of growth increments deposited in the cuttlebone is shown in Fig. 4. The slope of the regression of increment counts on time was significantly different from 1 (t-test) indicating that lamellae were not deposited on a daily basis. However the relationship between increment counts and juvenile length was highly significant ($p < 0.001$) (Fig. 5). Also significant ($p < 0.001$) were the relationships between the number of growth increments *versus* cuttlebone length and increment counts *versus* cuttlebone area. The relationships between growth increments and weight (juvenile weight and cuttlebone weight) are also significant (Table 1).

The mean widths of growth increments are represented in Fig. 6. In general the widths obtained for each growth increment are similar (with the exception of the first striation) and show a low degree of dispersion.

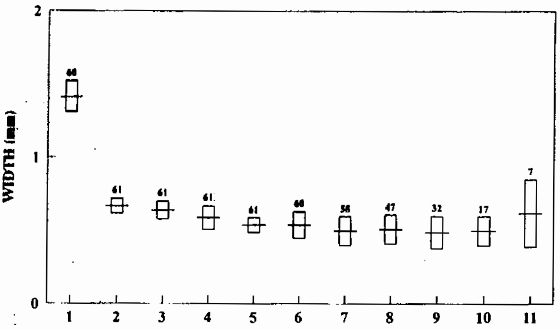

Fig. 6. Width of successive growth increments deposited on the cuttlebone (mean values ± 1 SD). Numbers indicate sample size.

4. Discussion

According to Choe (1963) the growth increments in the cuttlebone of several species of Sepiidae are produced daily but the periodicity of the formation of lamellae can be disrupted when feeding and/or environmental conditions are inadequate. The average number of lamellae at hatching varies among species: *Sepia esculenta* has 7, *Sepia subaculeata* 7.8 and *Sepiella maindroni* 8. In *Sepia officinalis* the number of lamellae immediately after hatching ranges from 7 to 8 (Choe, 1963). The data presented here show that growth increments are not deposited on a daily basis in the cuttlebone of *Sepia officinalis* because no significant relationship was found between days after hatching and increment counts. However, the relationships between the number of growth increments and several meristic characters (e.g. juvenile length, shell length and shell area) are highly significant. These data show that the number of growth increments is primarily related to the growth rate of the juvenile rather than to its chronological age. Larger juveniles exhibit the highest number of lamellae independently of their age.

Taking into account these data, the cuttlebone lamellae cannot be used for age determination in juvenile cuttlefish, at least during the first few days after hatching and under the experimental conditions used.

5. References

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