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AGE GROUP DETERMINATION BY ANALYSIS OF THE CUTTLEBONE OF THE CUTTLEFISH *SEPIA OFFICINALIS* L. IN REPRODUCTION IN THE BAY OF BISCAY.

R. LE GOFF[1], E. GAUVRIT[2], G. PINCZON DU SEL[2] & J. DAGUZAN[2]
[1] *Communauté Urbaine de Brest, 3 rue Dupleix, 29200 Brest, France*
[2] *Station de Biologie Marine de Bailleron, Laboratoire de Zoologie et d'Ecophysiologie, 56860 Séné, France*
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ABSTRACT

Very few hard pieces of evidence can be used to determine the age of the cuttlefish *Sepia officinalis*. We have studied the cuttlebone of cuttlefish living in Southern Brittany over a complete life cycle (from 1988 to 1990) in order to separate clearly the two age groups existing in this area. Cuttlebone is formed with successive calcium carbonate lamellae formation during growth. The rhythm of lamellae deposition is not constant and appears to be closely linked with temperature. It reaches a maximum in summer (18.75 lamellae per month) and falls drastically in winter (1.6 lamellae per month). The lamellar thickness measured on the mid-sagittal surface of the hypostracum shows characteristic zones of narrow lamellae. The first series of narrow lamellae appears at the 9th lamella and is formed just after hatching. The second one, seen only in group II breeders, is located between the 45th and 60th lamellae. This takes place in May when this group begins its first spring migration to the coast. This second set of narrow lamellae is also visible on the siphuncular surface of the phragmocone by an inflexion point. A final series of narrow lamellae is formed during the season of reproduction and appears in both groups of breeders. During the breeding season, the separation of the two year-classes of breeders is also possible by counting the total number of lamellae formed. Group I has a minimum of 80 lamellae whereas group II has about 120.

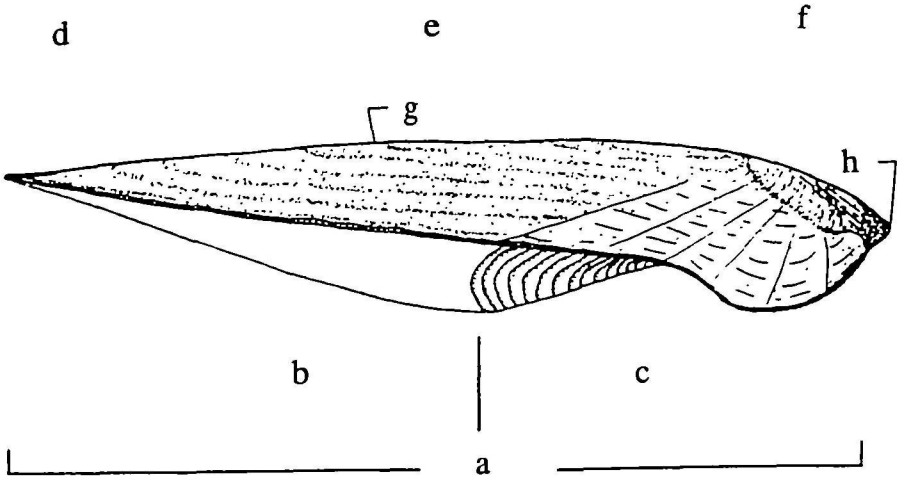
INTRODUCTION

Catches of *Sepia officinalis* (L.) throughout its geographical range show that two different life cycles exist. Whereas, in the English Channel, all the cuttlefish seem to breed around the age of 2 years (18–20 months) (Boucaud-Camou *et al.*, 1991); in southern waters, some are able to breed at around one year (i.e. 14–16 months; *Sepia officinalis hierredda* (Bakhayokho, 1983); *Sepia officinalis* (Le Goff & Daguzan, 1991)).

Individuals which breed when they belong to the second year class are called RGII ('reproducers of group II'), the others, which breed as soon as they belong to the first year class, are called RGI ('reproducers of group I').

The aim of the paper is to describe a simple method allowing an approximate determination of the age of the cuttlefish and especially to separate clearly RGI from RGII by studying their cuttlebones.

The cuttlebone is composed of a dorsal shield and ventral phragmocone (Fig. 1). The shield comprises three sheets of very hard and thin calcium carbonate (Naef, 1923); the external 'periostracum', the intermediate 'ostracum' and the 'flat hypostracum'. The phragmocone is composed of successive calcareous lamellae deposited on each other during growth. The ventral phragmocome face shows a smooth area (the last septum of the dorsal part of the phragmocone) and a striated area, or siphuncular region, which is composed of a outer surface, of all the lamellae deposited before the last one. Two consecutive lamellae delimit a chamber. The last chamber formed, and the older ones located in posterior extremity of the animal, contain only liquid. In the middle part, chambers are full of gas, mainly nitrogen (Denton & Taylor, 1964). Between these gas chambers and the others, there are intermediate chambers, with both gas and liquid, in contact with the siphuncular epithelium. It is in this area that exchange of liquid, between the siphuncular epithelium and chambers, takes place, permitting the animal to control its buoyancy (Denton & Gilpin-Brown, 1961c). This study considers the number of lamellae visible on the siphuncular surface, as well as the lamellar thickness measured in the mid-sagittal surface of the hypostracum.



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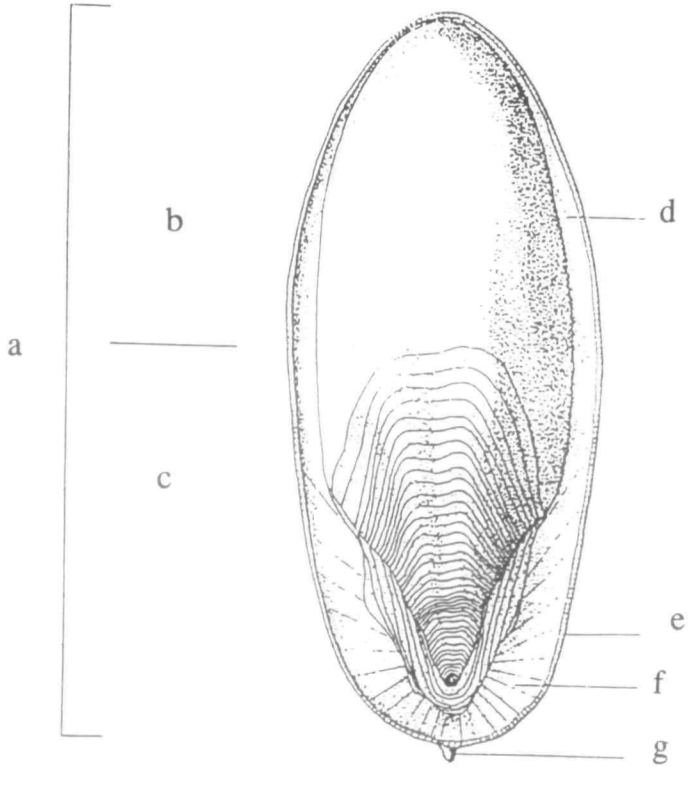

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Figure 1. Cuttlebone of *Sepia officinalis* L.
A. lateral view. Abbreviations: a, phragmocone; b, smooth part (last lamella deposited); c, striated part (siphuncular surface); d, anterior region; e, dorsal region; f, posterior region; g, shield; h: rostrum.
B: ventral view. Abbreviations: a, phragmocone; b, smooth part; c, striated part; d, lateral side; e, chitinous rim; f, calcareous part; g, rostrum.

MATERIAL AND METHODS

Cuttlebones were collected throughout the life cycle of the generation born in June, July, August and September 1988.

From 15 June to 1 November 1988, experimental trawls were made within Morbihan Bay and in the Morbraz coastal area using a trawl net with a small mesh and 133 juveniles were caught. From September 1988 to February 1990, 420 cuttlebones were collected from animals caught by fishermen in the Morbraz area and in deeper waters to the south-west of Belle Ile (wintering area). From mid-March to mid-June 1990, shells (191) were collected from animals caught during the breeding season with basket traps in Morbihan Bay and in the shallow waters of the Morbraz coastal area.

Finally, to complete the data collection, 12 Mediterranean cuttlebones and 27 from breeders caught in Morbihan Bay in 1986 and 36 very large shells of breeders were examined.

The dorsal mantle length (DML) of each animal, the lamellae number (the term streak is employed to determine the surface outcrop of lamellae) and the lamellae thickness were measured in the mid-sagittal surface of the hypostracum. The phragmocome was removed initially with a spoon and then with a scalpel, once the hypostracum was reached (Fig. 2). Lamellae thickness is measured to the nearest 0.01 mm with a microscope equipped with a ocular micrometer.

The beginning of the sexual maturation is determined by the differentiation of an hectocotylus arm for the males and the colour of the accessory nidamental gland (beige to yellowish) for the females (Le Goff, 1991) and allowed us to identify the future group I breeders (RGI) and group II breeders (RGII) in the annual cohort (Le Goff & Daguzan, 1991).

RESULTS

Streak formation

The streaks appearing on the 675 shells collected in this study were counted. Means, minimum and maximum streaks numbers are summarized in Fig. 3.

The first hatching occurs in early June, the last in late August or early September (Le Goff

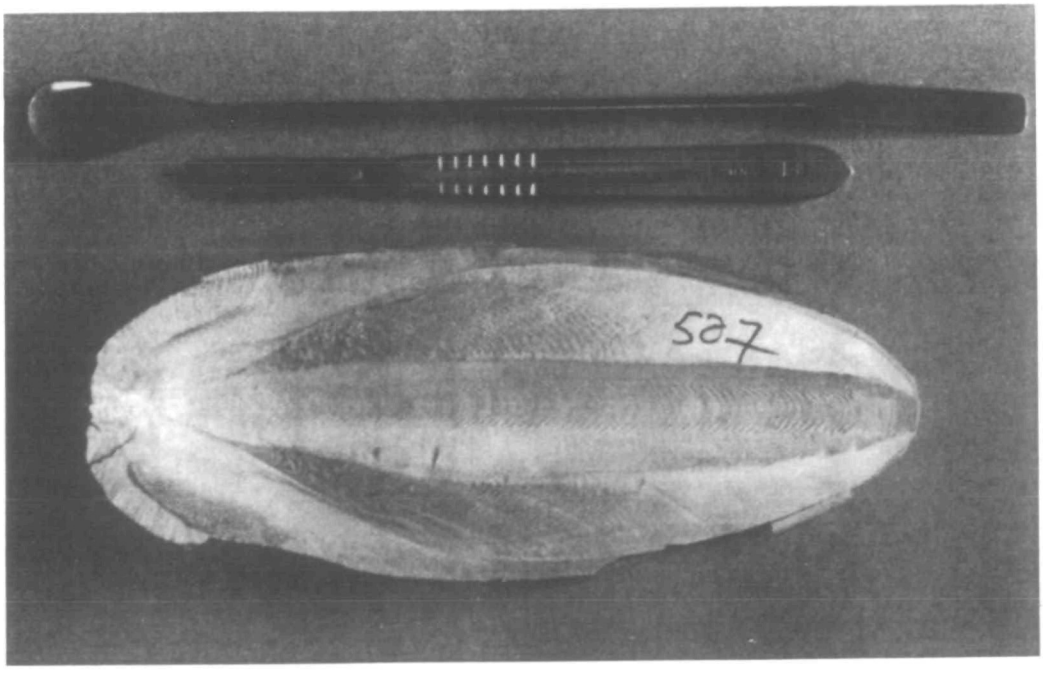

Figure 2. Preparation of the cuttlebone to the interstreak length measures.

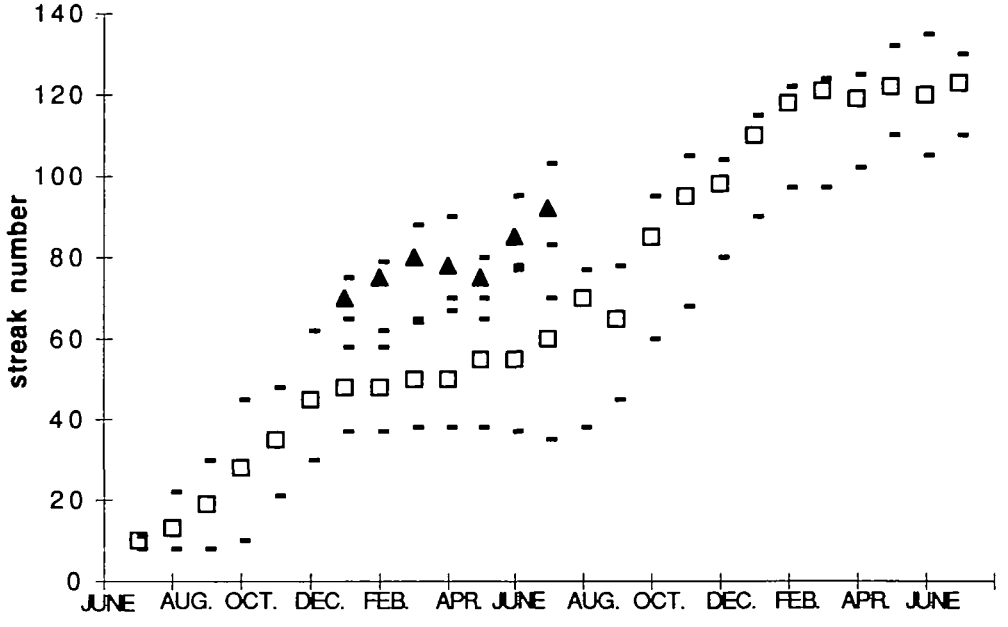

Figure 3. Variation in the number of streaks appearing in cuttlebones of group I (RGI) and II (RGII) breeders during the growth of the cuttlefish hatched from June to September 1988 in the northern part of the Bay of Biscay.
▲ mean RGI
□ mean RGII
– minimal and maximal values

& Daguzan, 1991). Newly hatched shells show 8 streaks (or 9) in South Brittany as well as in the English Channel (Richard, 1972) and Mediterranean Sea (Boletzky, 1974a). The sexual maturation of RGII becomes visible as early as August (second year of life) for all the males and in September for all the females. In RGI, sexual maturation is conspicuous later, in mid-September (first year of life) for the most precocious males and in February for the least precocious females. Consequently, before December, it is impossible to separate clearly the future RGI from the other individuals belonging to the same cohort (year class 0). On the other hand, during the breeding period (from March to June), the differences between RGI and RGII are clear. The cuttlebones of RGI have a minimum of 80 streaks, whereas the RGII cuttlebones have about 120. The extreme cases (larger RGI, smaller RGII), recognized by the streak counts were studied by cross-checking with the inter-streak distance analysis.

The number of streaks deposited per month on cuttlebones from hatching until death of the animal is described by Fig. 4. Streak deposition is maximal from June to August and decreases from February to April. The decrease continues until June for the RGI which breeds and dies soon after. For the RGII, a further increase in the rhythm takes place in May, when this group begins its first spring migration to the coast. The rhythm continues to increase up to a maximum (11 streaks per month) in August. Then, as at the beginning of the curve, the rhythm decreases regularly until the death of the animal, in the following spring or early summer.

Inter-streak distances

52 cuttlebones from RGII and 139 from RGI during the breeding period were used for this study. Each interstreak distance was measured from the last formed streak (Table 1).

For the RGII, the first interstreak maximum corresponds to the first streak whose distance is nearly 1 mm on the hypostracum. The second maximum is reached between the 25th and the 40th streak. The mean inter-streak distance is about 1.4–1.5 mm, the last maximum appears between the 75th and the 115th streak for a

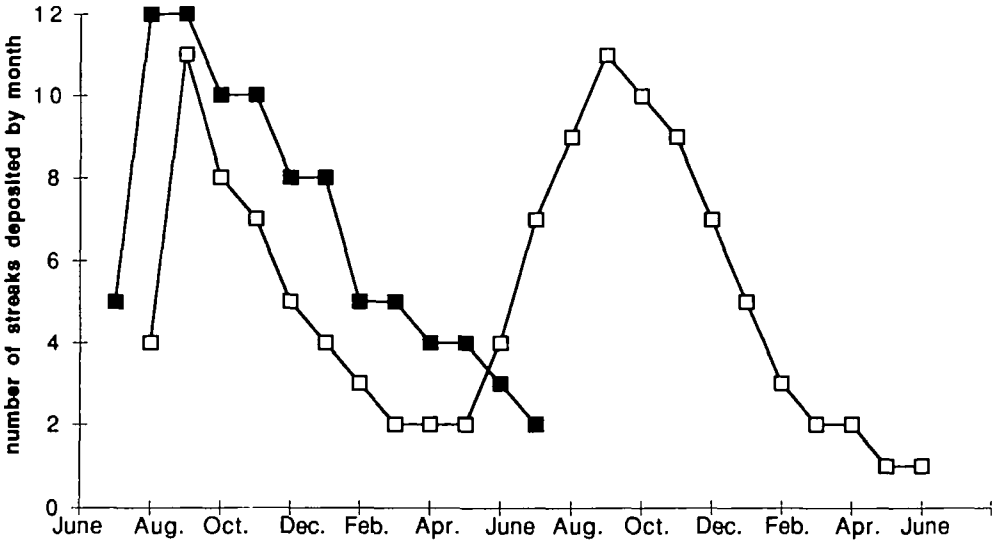

Figure 4. Rhythm of streak formation for cuttlebones of group I (RGI) and II (RGII) breeders from the northern part of the Bay of Biscay.
—■— RGI (number of streaks)
—□— RGII (number of streaks)

Table 1. Mean, minimum and maximum of the length (mm) of the interstreaks measured on the ostracum of the cuttlebone of the RGII captured during their breeding season in Morbihan Bay.

Streak	Minimum length of the interstreak	Median length of the interstreak	Maximum length of the interstreak	Number of cuttlebone analysed
1	0.75	1.00	1.40	52
3	0.55	0.80	1.05	52
5	0.55	0.80	1.00	52
8	0.30	0.55	0.80	52
10	0.55	0.85	1.10	52
20	0.70	1.00	1.80	52
25	0.70	1.46	2.30	52
35	1.00	1.56	2.30	52
45	0.90	1.34	1.90	52
55	0.90	1.29	1.80	52
65	1.00	1.64	2.30	52
75	1.50	1.89	2.60	52
85	1.50	2.08	2.60	52
95	1.60	2.05	2.50	52
105	1.10	2.03	2.60	52
115	0.90	1.65	2.10	48
120	0.60	1.50	1.90	36
125	0.50	0.80	0.90	18
130	0.60	0.70	0.80	7

mean distance of 2 mm, with a maximum observed value of 2.6 mm. The first minimum occurs at the 9th streak, just after hatching. This minimum corresponds to the slow growth of the newly-hatched when they leave their endogenous feeding (yolk reserves absorption) and change to exogenous feeding requiring capture of prey (Richard, 1971). The second minimum appears approximately between the 45th and the 60th streak. This area was named 'Z1 strip' by Denton and Gilpin Brown (1961a). Lastly, from about the 120th streak (between the 115th and the 120th for breeders) appears the last minimum, named 'Z2 strip' by

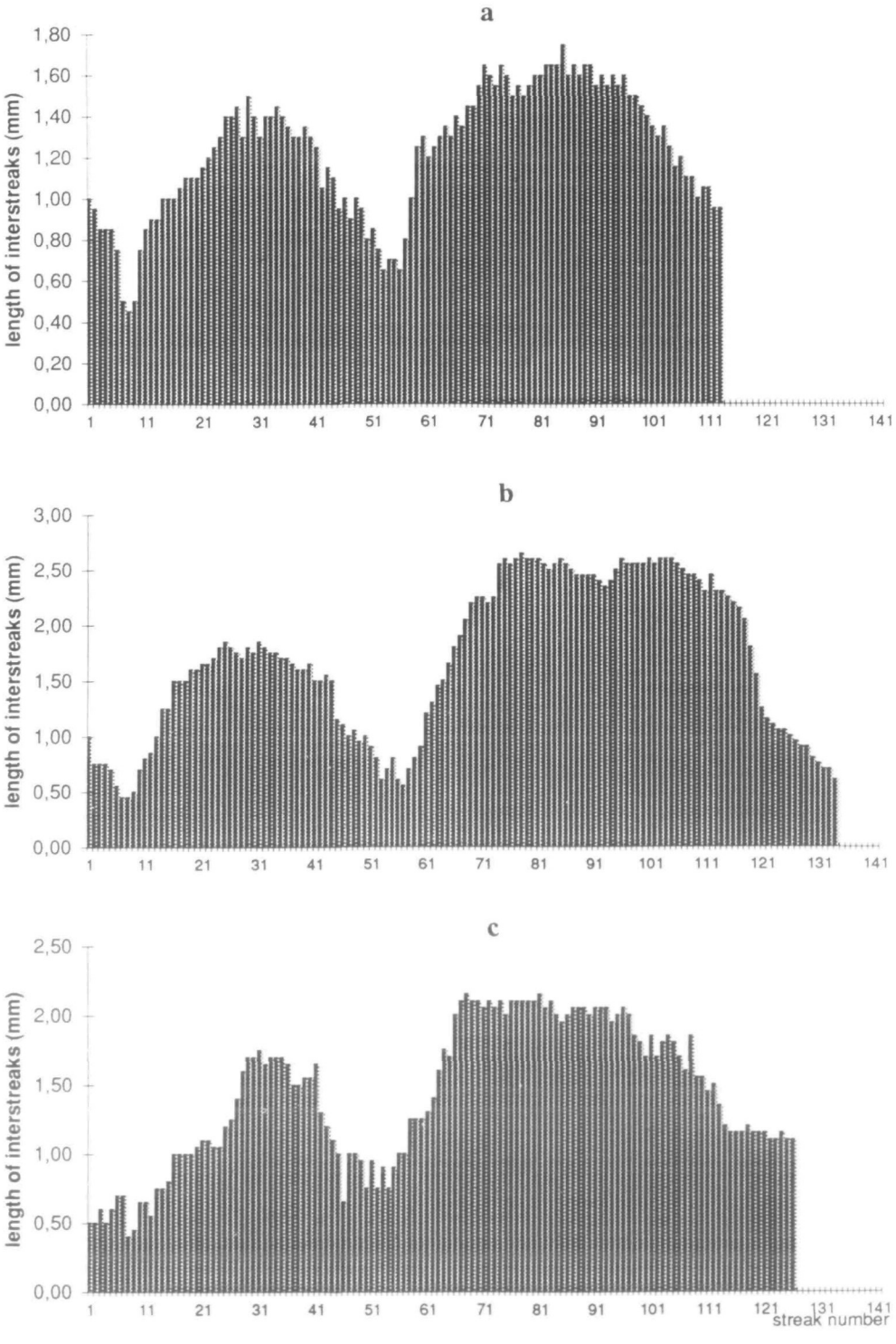

Figure 5. Length of the interstreaks measured on three cuttlebones of group II breeders (RGII) captured by cuttletrap during the breeding season in the Morbihan Bay. **a,** female of 16.5 cm of ML; **b,** male of 28.5 cm of ML; **c,** female of 19.4 cm of ML.

Richard (1971). Whereas, the first minimum appears at the 9th streak for all individuals sampled, the second and the third minimum positions depend on the individuals (Fig. 5). However, the three diagrams are very similar. Between a 19.4 cm (DML) female and a 28.5 cm male (Fig. 5 b and c), the only striking difference appearing in the shell is the lamellar thickness. Both have a 'Z1 strip' and a similar streak number. On Fig. 4c, the 'Z2 strip' appears clearly. This strip is formed during the breeding period on the cuttlebones of individuals which are caught latterly in the cuttletrap fishing season (June). A Z1 strip is made of a small number of the narrowest streaks. The position and the number of streaks which compose it are varied. On the other hand, it contains a particular streak, the narrowest (called 'Z1'), whose identification is most often the easiest (Fig. 6).

For the RGI, the profile of the inter-streak distance shows in every case, 2 minima and 2 maxima. For the first 9 streaks, including, the first maximum and the first minimum, the inter-streak distances are the same as those for RGII. The second maximum is located between the 25th and the 60th streak. The streak number which composes this second maximum is greater than that observed on RGII cuttlebones. From the 60th streak, the inter-streak decreases to a minimum (Z1 strip) around the 80th streak. This strip also appears during the breeding period, near the end of life, when the animals are nearly one year old.

We may conclude that during the breeding period, all the cuttlefish showing a cuttlebone of the type 1 are RGI and those having a cuttlebone of type 2 belong to the second year class, and therefore are RGII (Fig. 7). The presence or absence of 2 strips (Z1 and Z2) appears very often directly on the siphuncular surface. Figure 8 is a diagrammatic representation of the shell of a RGII belonging to the second year class during the breeding period. When the streaks are very distant on the siphuncular surface the cuttlebone grows in thickness and

Figure 6. Variation of the interstreak length at the ZI strip level and determination of the ZI streak.

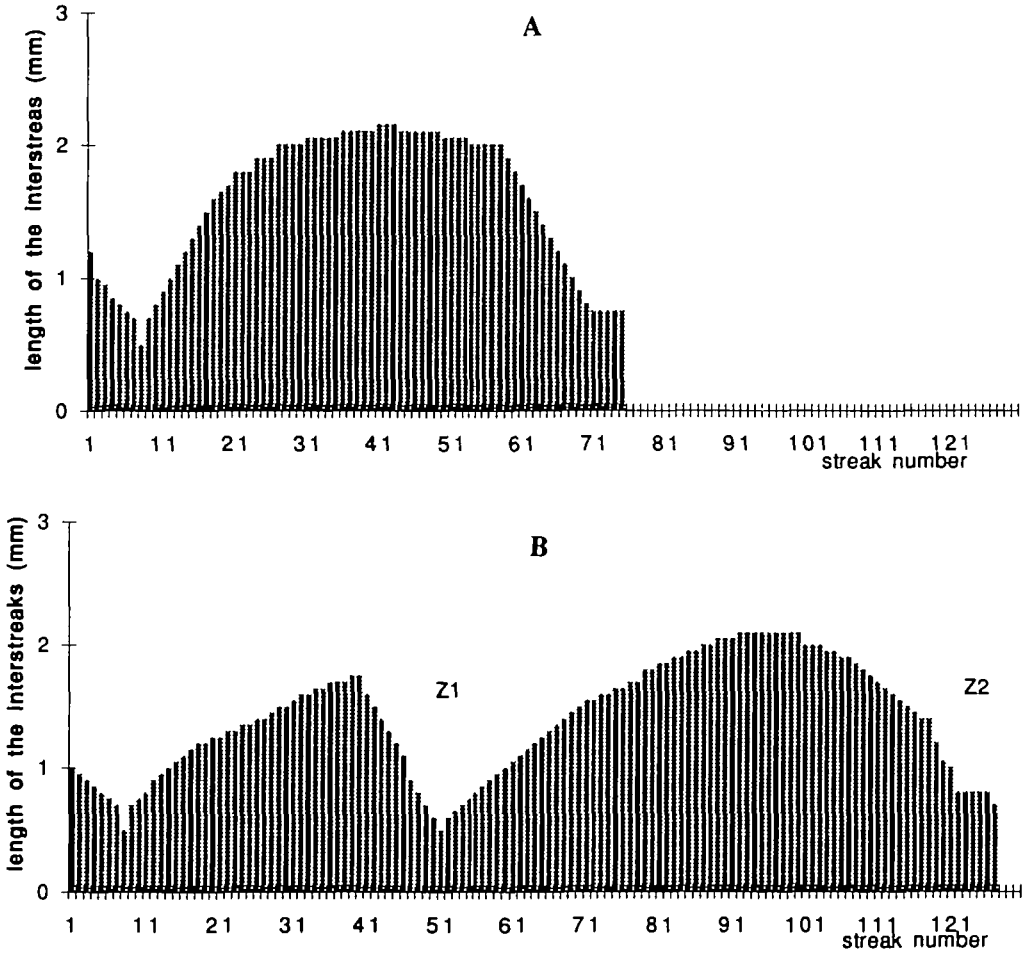

Figure 7. Representation of the cuttlebones of group I (RGI) and II (RGII) breeders during the breeding season in the north of the Bay of Biscay. A: RGI cuttlebone (type I); B: RGII cuttlebone (type II).

very rapidly in length. The Z1 and Z2 strips are also visible on the siphuncular surface (narrowing zones B and C). More remarkable is the existence of an inflexion point appearing on the siphuncular surface.

Inter-streak evolution was studied every month, by measuring the thickness of the three last chambers formed (Fig. 9). The RGI and RGII groups were processed separately from December. The animals were studied from hatching until death at the end of breeding period. For the RGII, the inter-streak distance increased rapidly during September to reach a constant level from October to January-February. Cuttlefish collected from April to June in the Morbraz area and shallow waters, show an inter-streak distance decreasing greatly (Z1 strip formation) and reaching a minimum in May. During this period we observed an increase in the rhythm of streak formation. Then, the inter-streak distance increases until September and stays quite constant until December. A new minimum takes place in March-April, (Z1 strip).

Mediterranean shells

Shells of Mediterranean cuttlefish seem to be very different from those collected in South Brittany (Table 2). Four RGII out of five in reproduction have more than 135 streaks, which corresponds to the higher streak number

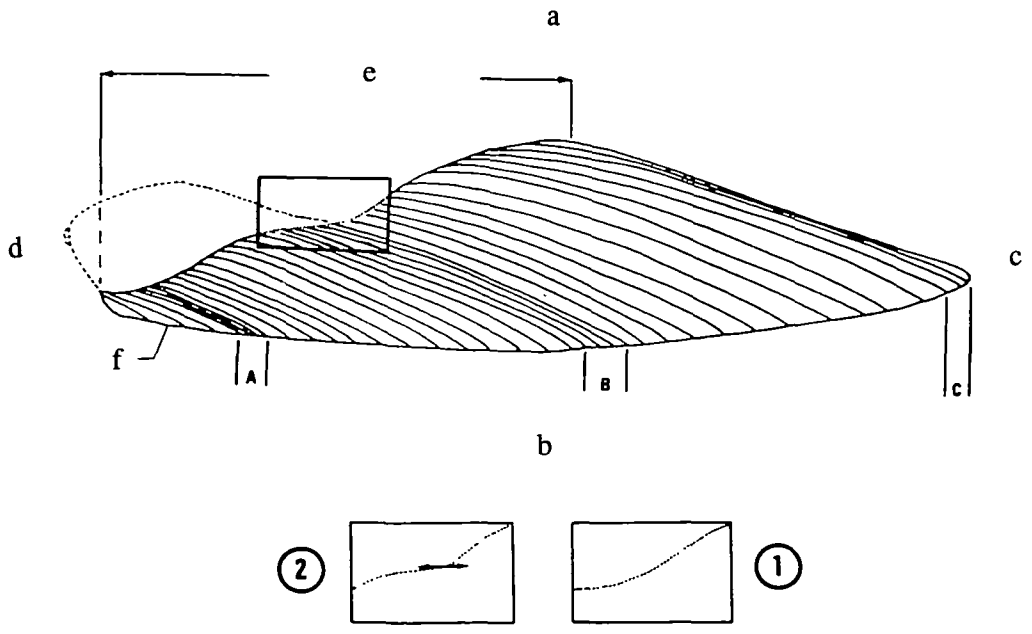

Figure 8. Diagrammatic representation of the shell striation in the group II breeders during reproduction. **A, B, C:** the three principal series of narrow lamellae visible on the hypostracum surface. The second series of narrow lamellae (B) is directly visible on the siphuncular surface (2) which allows an easy separation between group 1 (1) and group 2 (2) breeders.

Abbreviations: a, ventral region; b, dorsal region; c, anterior region; d, posterior region; e, siphuncular zone; f, hypostracum.

Table 2. Analysis of some cuttlebones from the Mediterranean sea.

	Age group	Day of the death	Length of the cuttlebone (cm)	Sex	Number of streaks	Remarks
	II	March	24.8	male	151	in reproduction
	II	January	21.8	female	151	in reproduction
	II	March	20.3	male	142	in reproduction
RGII	II	06/03/89	18.6	female	131	laying during 100 days
	II	April	18.1	female	135	laying during 1 month
	I	25/01/79	8.5	male	53	immature
	I	15/04/70	9.1	male	65	immature
	I	27/05/81	13	female	75	laying
	I	25/03/70	12.6	female	82	laying
RGI	I	04/05/70	13	female	86	laying
	I	29/01/70	11.5	male	72	end of maturation
	I	02/01/85	10.5	female	70	end of maturation

The separation of RGI and RGII was made by analogy with results from South Brittany. The cuttlebones come from Dr Bokezky.

observed on the shells from Southern Brittany. No significant difference appears with RGI (mean streak number deposited on Mediterranean shells: 77 ± 8; on Southern Brittany: 82 ± 7). The Mediterranean shells did not show the Z1 and Z2 strips. This result should be regarded with caution because of the small sample size.

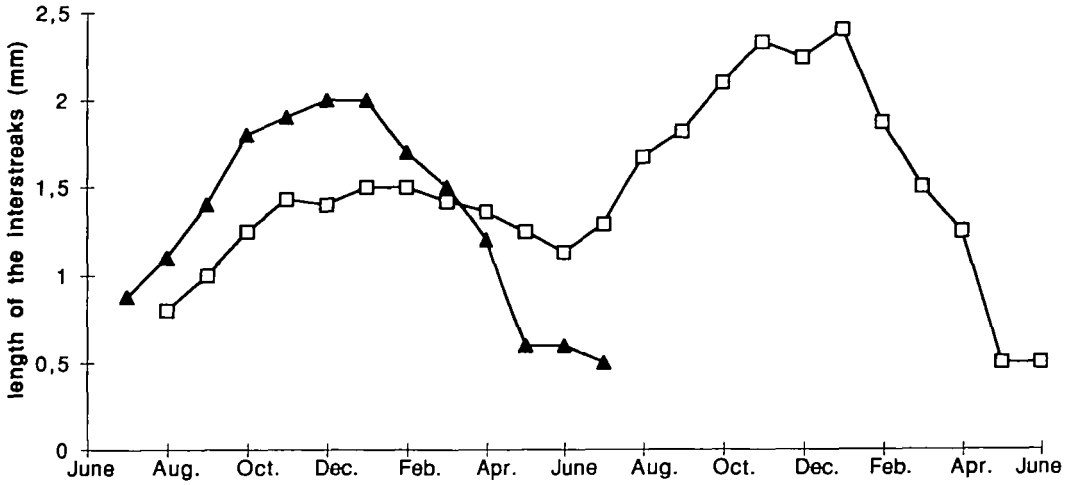

Figure 9. Monthly variation of the interstreak length for the cuttlebones of group I (RGI) and II (RGII) breeders from the Bay of Biscap.
—▲— RGI
—□— RGII

DISCUSSION

The rhythm of streak formation was not constant in Southern Brittany. For the RGII, it was quite comparable with those observed by Gi Jeon (1982) in the central area of the Bay of Biscay. The maximal streak deposition takes place in July, August and September, when the coastal waters are warmest and the minimal deposition occurs from January to June when the waters in which the cuttlefish live are the coldest. Richard (1967, 1969) showed by rearing cuttlefish at different controlled temperatures that the rhythm of the streak formation depends directly on temperature. Our results show that a streak is formed each 1.6 days (18.75 streak per month) at around 25°C. The rhythm is 5 times lower at 13°C (3.75 streaks a month). In other species of Sepiidae, such as *Sepia esculenta, Sepia subaculeata* and *Sepiella maindroni*, Choe (1963) indicates that the rhythm of formation is still constant at temperatures between 19 and 30°C. As far as we know, no study has been done concerning the total streak number deposited on a RGI shell. Comparisons with previous work are possible only for the RGII. Our results are quite comparable with those observed by Gi Jeon (1982) on animals from the central area of the Bay of Biscay, but seem to be different from those of Mediterranean cuttlefish. It seems that the number of lamellae on the Mediterranean cuttlebone is higher than in Southern Brittany. This could be explained by a more precocious laying period (from January according to Mangold, 1966) and by higher temperatures mainly in winter. Bottom temperature is constant at around 13°C in the Mediterranean Sea, compared with only 10°C (from 60–80 m depth in January–March (Vincent & Kurc, 1969; Vincent; 1973) in Southern Brittany. This could also explain the absence of the Z1 strip, as it has already been noted by Adam (1941); Mangold (1966) and Richard (1972). In cuttlefish living in the English Channel, Denton & Gilpin-Brown (1961b) found only about one hundred streaks in March and April (i.e. one month before reproduction according to Medhioub, 1986) and the Z1 strip appears very clearly in sexually mature individuals. It seems that the streak number found during the life of *Sepia officinalis*, varies with the latitude. This would be linked with both temperature and breeding period which takes place later from the south to the north of the distribution range of this species. The mainly narrow chambers seen up to the Z1 strip in RGII remain, as yet, difficult to understand. Among factors liable to influence its appearance in nature, we accept the role of temperature (Richard, 1972; Boletzky, 1974b) and feeding (Choe, 1963; Richard. 1972; Boletzky, 1974a). The third narrow strip (Z2 strip) of the RGII appears during reproduction, at a time when growth has almost stopped (Richard, 1971) as has food

intake (Pinczon du Sel & Daguzen, 1992). The narrow lamellae up to the Z1 strip in RGI occurring during reproduction, could be considered as the analogue of the Z2 strip of the RGII and not as the Z1 strip.

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