Progress towards ageing cuttlefish *Sepia hierredda* from the northwestern African coast using statoliths

C. P. Raya, M. Fernández-Núñez, E. Balguerías, C. L. Hernández-González

Instituto Español de Oceanografía, Centro Oceanográfico de Canarias, Carretera San Andrés s/n, E-38120 Santa Cruz de Tenerife, Spain

**ABSTRACT:** The cephalopod fishery in Eastern Central Atlantic waters, from Cape Blanc (21°N) to Cape Bojador (26°N), is economically very important for Spain, due to the high commercial values on the international market of the main species caught: octopus *Octopus vulgaris* Cuvier, 1797, cuttlefish *Sepia hierredda* Rang, 1837 and squid *Loligo vulgaris* Lamarck, 1798. After octopus, *S. hierredda* is the most important species for this fishery. For cuttlefish stock assessment, it is necessary to elucidate a key aspect of its biology, namely growth. In 1992, in order to view growth increments, statoliths were embedded in a clear fast-drying resin, and their surfaces were ground and polished. Some were etched. Several mounting positions were analyzed, of which 2 were chosen for the study. In polished statolith surfaces, thin growth increments were observed and assumed to be daily structures, because of their regular pattern and by analogy with validated results on the families Loliginidae, Omastrephidae (order Teuthoidea) and Idiosepiidae (order Sepioidea). Thicker and more widely spaced growth rings were also observed and their periodicity is discussed. As this is the first study on the ageing of cuttlefish off the northwestern African coast using microincrements of statoliths, the results have not yet been validated. However, these hard parts seem to be very useful tools for direct age determination of cuttlefish (family Sepiidae) and of *S. hierredda* in particular, as they reveal much information concerning individual growth.

**KEY WORDS:** Statoliths · Growth · *Sepia hierredda*

**INTRODUCTION**

After octopus *Octopus vulgaris*, cuttlefish *Sepia hierredda* is the most representative species in the catches of the Spanish cephalopod fishery in central eastern Atlantic waters, from Cape Blanc (21°N) to Cape Bojador (26°N) (Saharan Bank). The last Working Group on stock assessment in the Fishery Committee for the Eastern Central Atlantic (CECAF) (FAO 1992) concluded that it was necessary to elucidate the basic biological parameters of the central eastern Atlantic cephalopods.

Growth rings in cephalopod statoliths were first observed by Clarke (1966) and were first employed by Lipinski (1978) as tools for direct ageing (Dawe 1981). Since then, the daily deposition hypothesis has been validated only for some teuthoids. Jackson (1989) seems to have been the first author to provide evidence of daily rings in a sepioid (*Idiosepius pygmaeus*). Nevertheless, no attempt has been made to study growth from statolith microstructure analysis in species of the genus *Sepia*.

The present study establishes a method which permits the viewing of microstructures related to growth of *Sepia hierredda* statoliths.

**MATERIAL AND METHODS**

**Sampling.** A total of 390 individuals of *Sepia hierredda* were sampled from the catches of the Spanish cephalopod freezer trawlers operating between 21 and 26°N off the northwestern African coast, and were analyzed for biological and morphometric data. A total
of 24 individuals (14 males and 10 females), mantle length (ML) 6 to 39 cm, were selected for statolith analysis. Their characteristics are shown in Table 1.

**Extraction, conservation and preparation.** Statoliths were extracted in a similar way to that described for *Illex illecebrosus* by Lipinski (1981) and Morris & Aldrich (1984). After extraction they were preserved in 70° ethanol (Guerra & Sánchez 1985, Rodhouse & Hatfield 1990a), since dry storage makes crystals opaque, as pointed out by Dawe et al. (1985). Statoliths were embedded into small cylindrical molds with a clear fast-drying resin. The statolith nomenclature used is the one established by Clarke (1978). The main parts of a *Sepia hierredda* right statolith are shown in Fig. 1. Several mounting positions were tried, and 2 of them were finally chosen: right statoliths were mounted in a ‘classical’ position, with the medial side facing upwards, and the left ones were mounted in a completely original way, with the medial-posterior side facing upwards. Each statolith was first ground with silicium carborundum paper, and then polished with a 1 μm diamond polishing compound (Linkowski 1991). Left statoliths were etched with 0.1 M ethylene diamine tetraacetate (EDTA) (Gauldie & Radtke 1990, Lipinski 1991) for a variable time, and the same procedure was tried on the rostrum of the right statoliths.

**Table 1. Sepia hierredda.** Data collected from each individual studied. ML: mantle length; Rmin: minimum radius; NI: number of increments

<table>
<thead>
<tr>
<th>ML (cm)</th>
<th>Total weight (g)</th>
<th>Sex</th>
<th>Rmin (μm)</th>
<th>NI</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2</td>
<td>38.7</td>
<td>M</td>
<td>244</td>
<td>42</td>
</tr>
<tr>
<td>9.4</td>
<td>117.9</td>
<td>M</td>
<td>314</td>
<td>59</td>
</tr>
<tr>
<td>11.8</td>
<td>207.7</td>
<td>M</td>
<td>366</td>
<td>66</td>
</tr>
<tr>
<td>12.0</td>
<td>243.5</td>
<td>M</td>
<td>356</td>
<td>52</td>
</tr>
<tr>
<td>14.0</td>
<td>308.9</td>
<td>M</td>
<td>354</td>
<td>61</td>
</tr>
<tr>
<td>15.1</td>
<td>361.3</td>
<td>M</td>
<td>354</td>
<td>63</td>
</tr>
<tr>
<td>16.8</td>
<td>532.2</td>
<td>M</td>
<td>354</td>
<td>63</td>
</tr>
<tr>
<td>18.0</td>
<td>707.3</td>
<td>M</td>
<td>340</td>
<td>63</td>
</tr>
<tr>
<td>20.5</td>
<td>945.2</td>
<td>M</td>
<td>352</td>
<td>67</td>
</tr>
<tr>
<td>20.5</td>
<td>983.2</td>
<td>M</td>
<td>432</td>
<td>66</td>
</tr>
<tr>
<td>23.9</td>
<td>1741.9</td>
<td>M</td>
<td>452</td>
<td>73</td>
</tr>
<tr>
<td>27.0</td>
<td>2469.0</td>
<td>M</td>
<td>452</td>
<td>72</td>
</tr>
<tr>
<td>32.0</td>
<td>3538.0</td>
<td>M</td>
<td>520</td>
<td>77</td>
</tr>
<tr>
<td>38.5</td>
<td>6085.0</td>
<td>M</td>
<td>460</td>
<td>82</td>
</tr>
<tr>
<td>8.6</td>
<td>106.1</td>
<td>F</td>
<td>362</td>
<td>53</td>
</tr>
<tr>
<td>10.0</td>
<td>132.6</td>
<td>F</td>
<td>370</td>
<td>48</td>
</tr>
<tr>
<td>13.3</td>
<td>319.9</td>
<td>F</td>
<td>342</td>
<td>52</td>
</tr>
<tr>
<td>15.0</td>
<td>412.2</td>
<td>F</td>
<td>368</td>
<td>62</td>
</tr>
<tr>
<td>17.4</td>
<td>562.1</td>
<td>F</td>
<td>364</td>
<td>68</td>
</tr>
<tr>
<td>19.3</td>
<td>749.5</td>
<td>F</td>
<td>330</td>
<td>52</td>
</tr>
<tr>
<td>21.2</td>
<td>994.5</td>
<td>F</td>
<td>418</td>
<td>71</td>
</tr>
<tr>
<td>22.5</td>
<td>1466.5</td>
<td>F</td>
<td>432</td>
<td>69</td>
</tr>
<tr>
<td>30.0</td>
<td>3439.3</td>
<td>F</td>
<td>492</td>
<td>64</td>
</tr>
<tr>
<td>30.0</td>
<td>3116.0</td>
<td>F</td>
<td>484</td>
<td>72</td>
</tr>
</tbody>
</table>

**Fig. 1. Sepia hierredda.** Electron micrograph of the right statolith from a cuttlefish of 10.7 cm mantle length (ML). Anterior view (Clarke 1978). DD: dorsal dome; LD: lateral dome, RA: rostral angle; R: rostrum; S: spur

**Analysis of preparations.** Viewing and counting of rings was accomplished from images produced by a video camera coupled to a compound microscope (128×). The images were digitized and filtered with 2 Macintosh image analysis programs: ‘Image 1.37’ (Apple Computer), useful for previous treatment and filtering images, and ‘Bony Parts’ (Brittnacher & Botsford 1988), specially developed for analysis of hard parts of marine organisms. The latter permits the creation of numerical files containing the values of minimum radius (μm), number of increments detected, and distances (also in μm) between 2 successive increments.

As established for bony fish, a growth increment consists of 2 lamellae: 1 light and 1 dark (Campagna & Neilson 1985). Each dark lamella is termed a ‘growth ring’, and an ‘increment’ consists of 1 light lamella and the immediately following dark lamella (Pannella 1980). The image analysis program ‘Bony Parts’ identifies and counts the increments by counting its dark lamella. In this paper growth units will be termed without distinction as ‘growth rings’, ‘bands’ or ‘increments’.
RESULTS

After grinding and polishing from the medial side, in the ‘classical’ position (Clarke 1978), microstructures were observed in 2 areas: the lateral dome and the rostrum. In the lateral dome (Figs. 2 & 3), a very regular series of concentrical rings was observed near the nucleus, while thicker and more widely spaced growth bands were observed in outer areas. Counting rings in the lateral dome is laborious, because they do not appear in the same focal plane (Dawe et al. 1985, Dawe & Natsukari 1991). Growth rings were most clearly seen in the lateral dome region near the rostral angle (Fig. 1), and this, also noted by Jackson (1989) on Idiosepius pygmaeus, might be due to the lesser opacity of this area, which simplifies observations under transmitted light.

In the rostrum, etching the polished surface revealed a series of rings arranged regularly but only across certain areas of the anterior side of the rostrum (Fig. 4). When visible, they are positioned almost parallel to the outer margin of the statolith. Natsukari et al. (1988) observed that, after grinding the statolith of Photololigo edulis from the anterior side, similar rings appeared.

Fig. 2. Sepia hierredda. Ground and polished surface (medial side up) of the right statolith from a cuttlefish of 4.6 cm ML showing the increments of the lateral dome, near the rostral angle. Small regularly spaced bands

Fig. 3. Sepia hierredda. Ground and polished surface (medial side up) of the right statolith from a male of 13.8 cm ML. The pattern of increments shows a number of darker, more strongly emphasized bands.
However, grinding the concave zone (anterior side) of the Sepia hierredda statolith (Fig. 1) did not reveal any comparable structure. Counting rostrum rings was too difficult, because there was no axis from the nucleus to the outer margin of the rostrum along which growth microstructures were continually deposited.

The grinding and polishing of left statoliths from the medial-posterior plane (Fig. 5) permitted the viewing of a distinctive ‘tongue-shaped’ structure. This structure (not visible in Fig. 1 because internal) is located in the area between the nucleus and the spur. The number of clearly marked bands in the tongue-shaped structure is always greater than the number of growth increments counted in the lateral dome, e.g. the program detected 70 increments in the lateral dome of a
Mantle length (cm)

Fig. 6. Sepia hierredda. Relationship between ML and minimum radius, sexes pooled

Back-calculation of ML was performed in order to retrace the growth history of cuttlefish studied. Back-calculation is a technique that uses a set of measurements made on a fish at one time to infer its length at an earlier time or times (Francis 1990). The back-calculation was possible because a significant correlation between ML and Rmin of the statolith existed. It was accomplished according to the 4 stage process described by Francis (1990), and using Rmin values for each growth increment (Ri). These Ri are calculated by accumulating distances between the 2 dark lamellae of 2 successive growth increments, which were measured by 'Bony Parts' when detecting and counting bands. For each Ri value, regression of ML on Rmin enables the calculation of a MLi, assumed to be the ML of a specimen hav-

\[
ML = 0.000025 \times Rmin^{2.26}
\]

\(r^2 = 0.85, n = 24, p = 0.05\)

The statistically significant correlation obtained between ML and Rmin fits a power function:

\[
ML = 0.000833 \times NI^{2.4043}
\]

\(r^2 = 0.71, n = 24, p = 0.05\)

The relationship between ML and number of increments (NI) (Fig. 7) was also established. The 'count bands' function of 'Bony Parts' permits an automated count of the bands detected over the Rmin of the lateral dome of the 24 right statoliths studied. The NI considered for the analysis corresponds to the average of 2 successive counts over the same radius. A statistically significant correlation was obtained between the ML of a specimen and the NI counted over its statolith, by fitting points to a power function:

\[
ML = 0.000833 \times NI^{2.4043}
\]

\(r^2 = 0.71, n = 24, p = 0.05\)

Fig. 7. Sepia hierredda. Relationship between ML and number of increments, sexes pooled

statolith from a specimen of 21 cm ML, while 103 bands were detected on the tongue-shaped structure. Unfortunately, after careful observation of this structure for all the left statoliths prepared, it seems that its form changes with growth, because it was easily observed only for cuttlefish of ML ranging from 15 to 22 cm. In the sample considered for this study, only a few specimens showed this particular structure with the growth increments clearly marked, and this is why no quantitative analyses were carried out for left statoliths.

The relationship between ML and statolith minimum radius (Rmin) (Fig. 6) was established after measuring Rmin with the aid of the 'scan length' function of 'Bony Parts', by determining the minimum distance (in μm) outwards from the nucleus center to the edge of the 24 right statoliths prepared for this study. Measurements were carried out in the lateral dome area, near the rostral angle which was identified as being the most suitable area for counting bands. The statistically significant correlation obtained between ML and Rmin fits a power function:
Fig. 8. *Sepia hierredda*. Relationship between back-calculated number of increments and back-calculated ML. (A) Males; (B) females.

Fig. 9. *Sepia hierredda*. Mantle length increase per band, calculated for back-calculated ML classes. (A) Males; (B) females.

The complex crystalline structure of cuttlefish statoliths has been a major obstacle for observing growth increments (Morris 1988, Natsukari & Tashiro 1991). However, the present study proposes a methodology which permits the viewing of a series of regularly disposed bands (one light and another dark). By analogy with the aspect and disposition of the growth microstructures described for bony fish otoliths and other cephalopod statoliths, the bands observed in *Sepia hierredda* statoliths were assumed to be growth increments. As pointed out for squid by Rodhouse & Hatfield (1990a), the existence of positive relationships between measurements of body size (ML) and statolith size (Rmin), and between the ring count (NI) for each individual and its size (ML), seems to support the view that the statolith grows in concert with the rest of the animal and that increments are laid down regularly during growth. The relationship between ML and Rmin (Fig. 6) can be interpreted regarding both the ML increase or the growth of the statolith. One possibility is that the statolith radius has a rapid growth rate until ML = 10 to 15 cm. Guerra & Sánchez (1985) pointed out that the growth of *S. officinalis* statolith is slower after sexual maturity starts. They proposed a metabolic explanation: when cuttlefish come to maturity, mucopolysaccharides are preferentially employed for gonad development to
the detriment of statolith, which contains mucoprotein layers, as demonstrated by Dilly (1976) and Radtke (1983). Morris & Aldrich (1985), Lipinski (1986) and Arkhipkin & Scherbich (1991) also observed a slower growth of the statoliths of Illex illecebrosus, Alloteuthis subulata and L. argentinus, respectively. The other possibility is that small cuttlefish grow slowly, while above ML = 10 to 15 cm, the relationship becomes more isometric. This is the most probable explanation, as it is in agreement with the first slower growth stage inferred from Fig. 8, and as no evidence of statolith increment width decrease with age has been found. Even if only 3 specimens of less than 10 cm ML have been studied, this size could correspond to a critical growth phase for S. hierredda populations on the Saharan Bank, probably related to the beginning of sexual maturity.

For each sex, the relationship between back-calculated ML and the number of increments estimated corresponding to each ML, is shown in Fig. 8. Taking into account the error introduced by back-calculation by neglecting individual growth variation (back-calculated values are always an average value of the dependent variable, assumed to be a single point based on the independent variable) and considering that the sample ML range does not cover the smallest and the largest sizes observed for Sepia hierredda, this figure suggests 2 things. First, a growth stage where ML increase for males and females is slower than the growth during a second stage, beginning at about ML = 10 cm. As stated before, this size could be related to sexual maturity. For S. officinalis officinalis off the northwestern African coast, first maturity is 12 to 14 cm ML, for males and 14 cm ML for females (Hatanaka 1979); females of the same species from northwestern coasts of Spain mature at 7 to 8 cm ML (Guerra & Sánchez 1985), and females of S. hierredda from Senegalese waters reach first maturity at 13.5 cm ML (Bakhayokho 1983). Second, for the same size the statoliths of females showed less increments than did statoliths of males; this seems to indicate a faster growth for females. A differential growth of this kind was observed for Idiosepius pygmaeus (Jackson 1989, Jackson & Choat 1992), Illex argentinus (Rodhouse & Hatfield 1990b) and Sepia australis (Sánchez & Villanueva 1991).

The values for ML increment associated with the formation of an increment in the statolith (Fig. 9) show that differences between the values of average growth per band for males and females are observed only from ML = 10 cm onwards, the female average being slightly higher from this size; this observation also seems to support the hypothesis of the existence of a physiological change probably connected with the beginning of sexual maturity. However, this idea should be confirmed by further increment counts on specimens smaller than 10 cm ML. As cuttlefish size increases, the ML increase associated with the deposition of a band also increases (Fig. 9). This might result from underestimating the number of increments (because some are not detected by the analyzer), and it could explain the differences in ring counting found between right statolith lateral domes and the tongue-shaped structures of some left statoliths. The finding of more bands in the tongue-shaped structure than in the lateral dome is particularly important: age based on counts from the dome area would be underestimated (e.g., if the tongue-shaped structure forms earlier in ontogeny than the lateral dome) or age based on the tongue-shaped counts would be overestimated (if bands on this structure are not formed daily, e.g., feeding events). The ML increase associated with the deposition of a band could increase due to the fact that the time elapsed between formation of 2 successive increments increases. A third possibility exists: if periodicity of increment formation does not change, then growth is faster for older individuals. This would contradict observations for Gonatus fabricii (Kristensen 1980), Abralia trigonura (Bigelow 1992), Todarodes senegalensis (Villanueva 1992) and 2 species of Alloteuthis of the Saharan Bank (Arkhipkin & Nekludova 1993).

By analogy with validated results on Loliginidae, Ommastrephidae and Idiosepiidae, and with regard to the fact that the physiology of many cephalopods is influenced by a circadian rhythm, it is possible that increments in the statoliths of Sepia hierredda are produced on a daily basis. Due to their regularity, the rings observed in the statolith near the nucleus (Figs. 2 & 3) could be produced daily, a ring then corresponding to 1 d. However, some rings, analogous to those described near the nucleus, were also observed between the thicker and more broadly spaced rings of the outer zones of the lateral dome. The analyzer did not make any qualitative distinction between rings, considering all increments as equivalents; but, if all rings detected are considered as daily, a 38.5 cm ML cuttlefish would only be 82 d old. This age seems unrealistic, since Bakhayokho (1983) estimated the life span for this species in Senegalese waters as more than 2 yr, and the results of Le Goff & Daguzan (1991) for S. officinalis in South Brittany pointed to a life span of at least 1 yr. These considerations support the view that chronological interpretation of increment formation could be similar to the one proposed by Kristensen (1980) for Gonatus fabricii, and considers the existence of a circadian rhythm for increments near the nucleus, while another rhythm (lunar, seasonal) would explain the formation of thicker and more widely spaced rings.
CONCLUSION

Cuttlefish statoliths prepared as described in this study permitted the viewing of microincrements related to growth. However, improving some aspects of this technique (currently being undertaken) should facilitate the viewing of those increments. Having identified statoliths as possible tools for cuttlefish ageing, future work will be done with data based on actual observations and large number of samples covering the size range of the species. It is hoped that this study has improved our knowledge on the growth of Sepia hierredda, and future studies should aim to validate the chronological hypothesis proposed for increment formation.

Acknowledgements. We thank G. D. Jackson and M. T. G. Santamaría for comments and constructive suggestions on the manuscript.

LITERATURE CITED

Brittacher, J., Botsford, L. (1986). Bony Parts, an image analysis program. University of California, Davis
Rodhouse, P. G., Hatfield, E. M. C. (1990b). Dynamics of...

*This article was submitted to the editor*