Dana, vol. 1, pp. 39-51, 1980

Periodical growth rings in cephalopod statoliths

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Abstract

In this paper a direct age determination method for squid is introduced. Periodical growth rings in statoliths of the squid *Gonatus fabricii* (Lichtenstein, 1818) are described. By comparison with the previously determined growth rate of juvenile specimens and a bimodal monthly size distribution, growth rings, called first-order bands, are for the first time shown to be daily whereas bands of second and third order are fortnightly and monthly, respectively. The relationship between the pen length and both statolith length and the number of growth rings are shown. Presence of organic material in statoliths and its significance for banding are demonstrated. Periodical growth rings are also shown in statoliths of *Rossia glaucopis* (Lovén, 1845) and *Alloteuthis subulata* (Lamarck, 1798).

Introduction

The purpose of this paper is to describe observed concentric growth rings in cephalopod statoliths and relate them to time, making direct age determination possible. The reliability of this method is demonstrated by the good correspondence between growth rate found through age determined by the method, and growth rates earlier found by studying changes in the mode of sample means from month to month (Kristensen, 1977) and the difference in size distribution between two year-classes of *Gonatus fabricii* (Kristensen, in prep.).

Although Clarke (1965 and 1966) described growth lines in beaks of squids and observed concentric growth patterns in statoliths, it was impossible to relate any of them to time, and until now no direct age determination methods for squids have been known.

Moreover the existence of organic material in statoliths is demonstrated and its significance for the concentric growth rings is shown. Growth rings in fish otoliths are caused by differences in the ratio of organic to inorganic material (Panella, 1971), and though an inorganic component (aragonite, Clarke & Fitch, 1975) is known in statoliths, no organic material like that in fish otoliths has until here been reported.

Material and methods

Statoliths from 39 squids, *Gonatus fabricii* (Lichtenstein, 1818), from Disko Bugt, West greenland, were studied. Specimens were kept frozen or preserved in 70 % ethanol until dissection. It is important to avoid specimens preserved or kept in formalin because even the weakest acid from the formalin damages the statoliths.

The statoliths which are found within the cartilage of the skull were removed as described by Clarke (1978) and stored dry. Both light microscopy (LM) and scanning electron microscopy (SEM) were used.

Statoliths increase in size from a central nucleus by adding concentric increments (rings). The growth rings are best studied in the dorsal and ventral regions of the statoliths, where the rings are thickest. The growth gradient therefore appears best when the statoliths are ground on the anterior and/or posterior surfaces (Fig. 1).

Light microscopy: Each statolith was mounted in Lakeside No. 70 on a slide and ground on both sides on fine sandpaper (Dragon Fly, Water proof 600). Before mounting the statolith, a small piece of Lakeside mountant is melted on the slide by holding it over a flame. When the mountant has melted the statolith is positioned with the anterior or posterior side up. After the statolith has been ground on one side, the mountant is again melted and the statolith turned over and ground on the other side. The statoliths were ground as near the centre plane as possible and finally covered with a thin layer of Lakeside to make the grinding marks disappear. Etching with 1 % HCl was tried but did not improve results and was therefore abandoned.

Scanning electron microscopy: After being mounted on a slide in Lakeside No. 70, the statolith was ground on one side, polished with fine grain paste (1 μm aluminium oxide) and washed in fat-free soap before being etched with ethylenediaminetetraacetic acid (EDTA). Finally a small piece of glass slide with the statolith was mounted on an aluminium stub and covered with carbon and gold.

For the description of growth rings in the statoliths LM was found to be adequate and with SEM it was impossible to make the smallest rings visible over a sufficiently large field. However, this will probably be possible with an improved technique.

The preparation technique is difficult and needs much care and experience. In some statoliths it is not possible to count rings over a complete nucleus to margin radius. In such cases, measurements of rings were made at several locations along the radius, and total counts were calculated by extrapolation, or by following a rather circuitous route from one area of the statolith to another.

Results

Zones and bands in the statolith

Several recurrent concentric ring systems occur in the statoliths (Fig. 1). Also some radiating patterns caused by the radiating aragonite needles are visible (Fig. 2). However, the ring systems dominate and show four different zones.

Zone 1, the nucleus, is a core of rather consistent diameter. In all statoliths studied the diameter of the nucleus measured about 160 μm. Outside the nucleus is zone 2, which is light and rather uniform. Next comes zone 3, which is quite dark and obviously divided into periodical increments. Beyond zone 3 is zone 4, of less obvious periodicity but with clearly deposited increments. Possibly the zones are related to different maturation stages. Likewise, growth of the statoliths is cor-

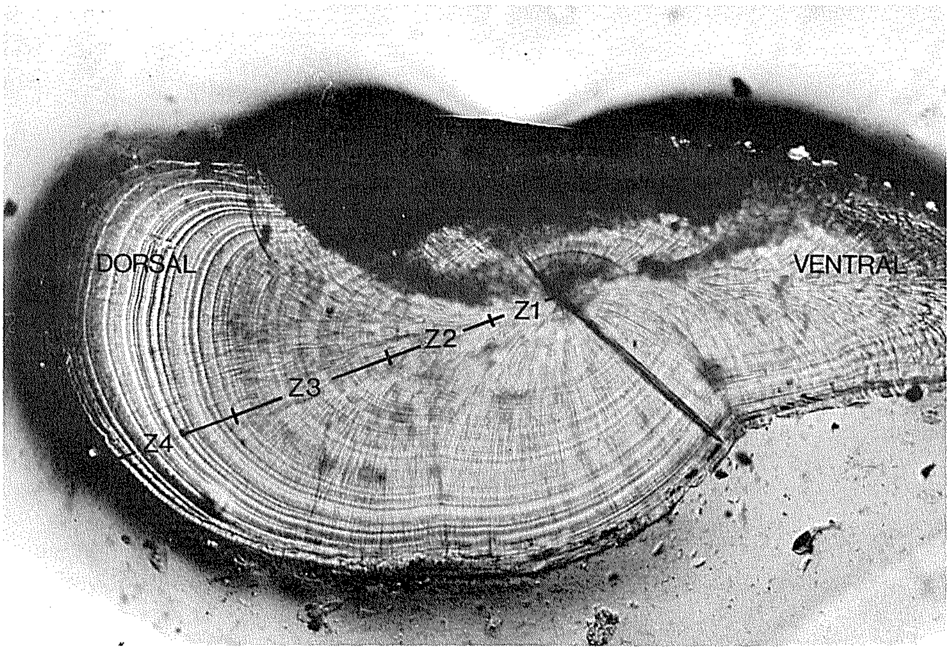

Fig. 1. Statolith, grounded on the anterior surface, from a specimen of *Gonatus fabricii* with pen length (PL) of 9.3 cm. Greatest width of the dorsal lobe from centre of nucleus to the border is 0.56 mm. Z = zone.

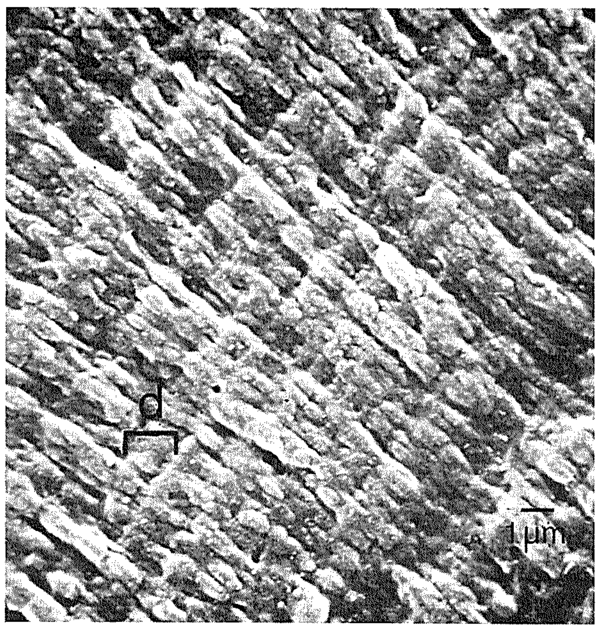

Fig. 2. Scanning electron micrograph of first-order bands. d = first-order band or daily ring.

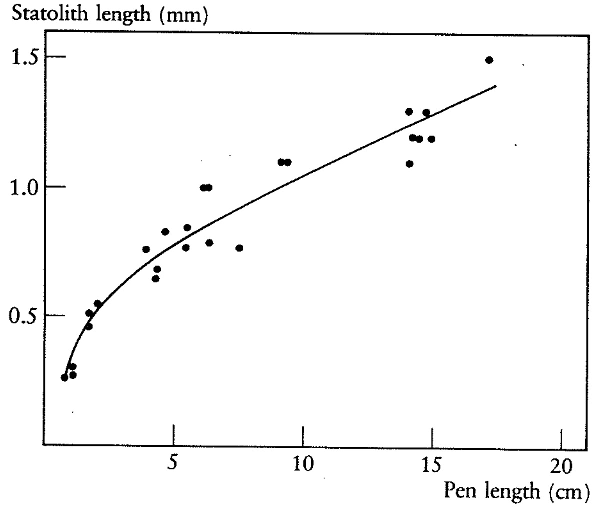

Fig. 3. Correlation between pen length and statolith length, based on specimens of 0.8-17.1 cm pen length.

related to the length of the pen. In early life, statoliths have a rapid growth which becomes slower and approximately linear above a pen length (PL) of about 5 cm (Fig. 3).

The smallest periodical patterns seen in the statoliths are increments 1-4 μm wide (Figs 2 and 4). These increments, or first-order bands (d), consist of one black band and one light band. First order bands are always best recognizable in zones 2 and 3.

In zone 3, groups of about 14 first-order bands constitute two kinds of second-order bands (Fig. 5): rather dark second-order bands (f_1) composed of broad first-order bands alternating with lighter second-order bands (f_2) composed of narrower and less sharply defined first-order bands. Together these two kinds of bands comprise a third-order band (m).

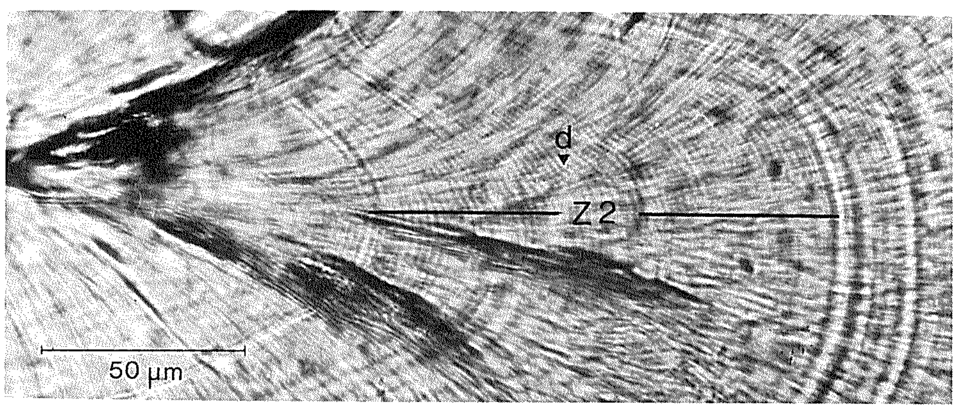

Fig. 4. First-order bands in zone 2. d = first-order band or daily ring. Z = zone.

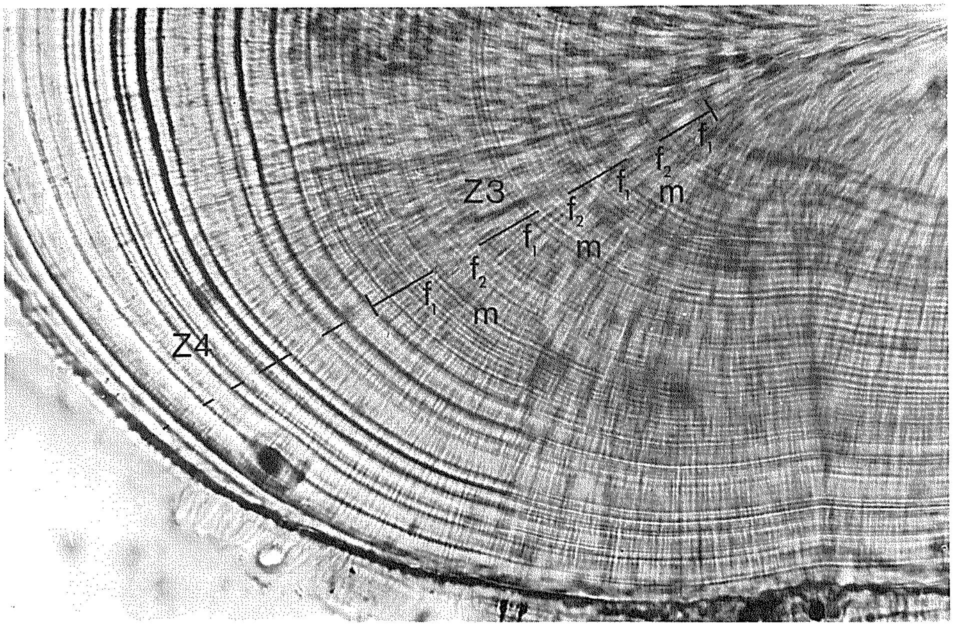

Fig. 5. Periodical growth increments in zones 3 and 4 of the specimen shown in Fig. 1 (PL 9.3 cm). In zone 3 rather dark second-order or fortnightly bands (f_1) composed of broad first-order bands alternate with lighter second-order or fortnightly bands (f_2) composed of narrower and less sharply defined first order bands. Together they compose one monthly band (m). In zone 4 the alternation between dark and light second-order bands is indicated as in zone 3; the broad concentric black bands in zone 4 are caused by unfocused first-order bands, as seen in the lower right part of the figure.

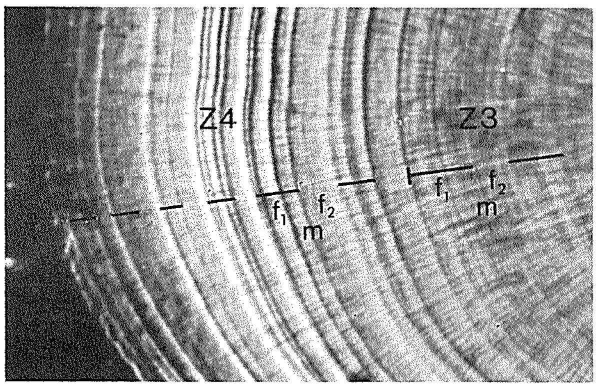

Fig. 6. Second-order or fortnightly bands in zone 4 of the statolith seen in Fig. 5. Thick-striped bands corresponding to darker fortnightly bands (f_1 and black bars) in zone 3 (Fig. 5) alternating with uniform bands corresponding to lighter fortnightly bands (f_2). Together they compose one monthly band (m).

First-order bands are not easily recognizable in most of zone 4. Groups of darker bands alternating with lighter, less sharply defined bands, representing the second-order bands seen in zone 3, are also found here (Fig. 5). However, in this zone the dark bands are seen as thickstriped (f_1), and the light bands are seen as nearly uniform bands (f_2) (Fig. 6). The width of the second-order bands decreases a little towards the border of the statolith.

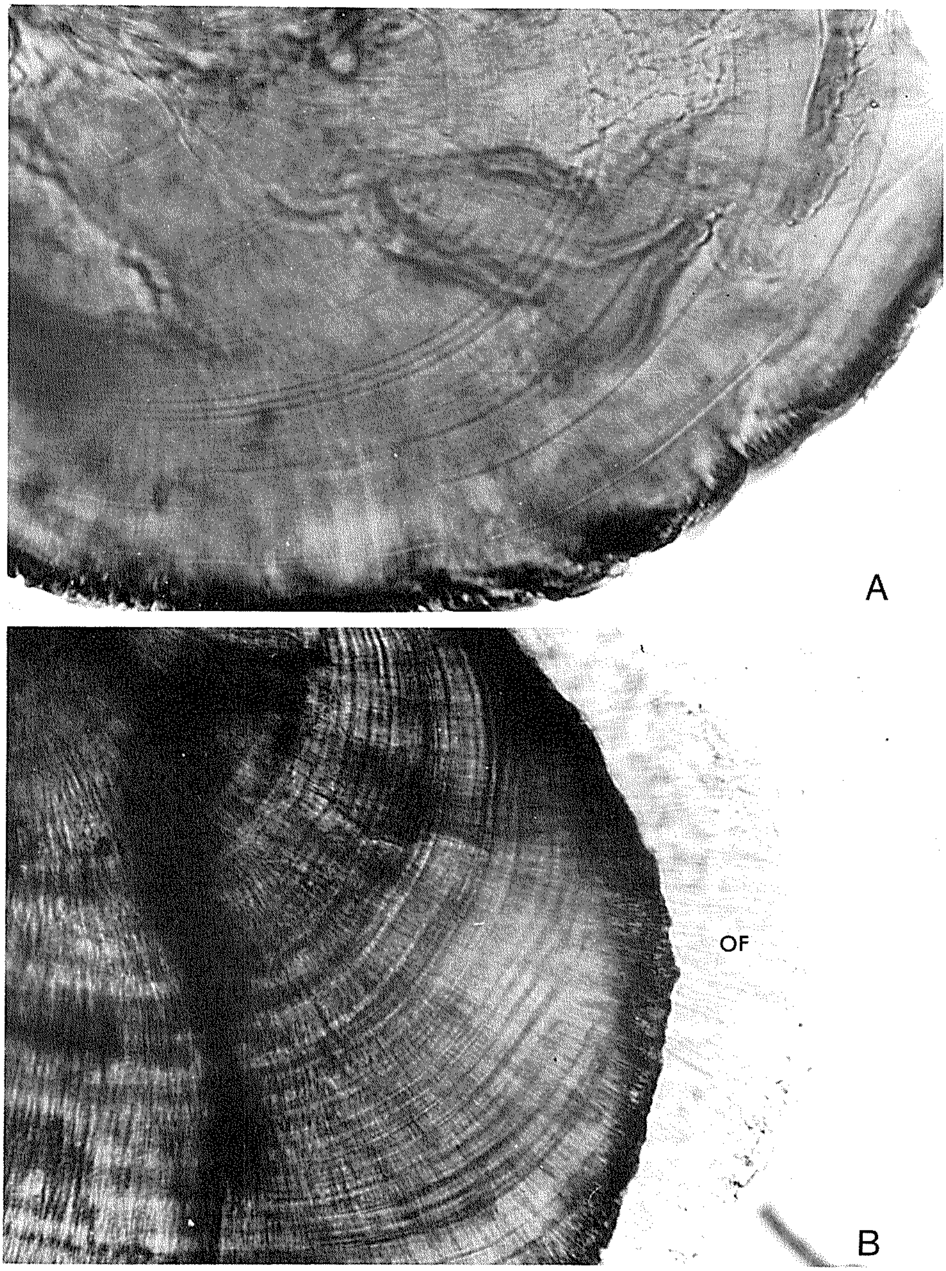

Fig. 7A. Statolith kept in EDTA for 60 hours, which removes all inorganic material. Concentric organic rings are visible. The dark splotches in the middle of the figure are stained organic material on the surface of the statolith.

B. Ground statolith kept in NaOH for one night. All organic material has been dissolved in the outer part of the statolith. OF = Organic-free zone.

Chemical components of the statolith

After 60 hours in EDTA, which removes all the inorganic material (Fig. 7A), a statolith showed rings. After staining with van Gieson Hansen stain the statolith was red. Thus organic material exists in the statolith and is collagenous, as in fish otoliths (Degens *et al.*, 1969).

After treatment with weak NaOH solution for one night, a ground statolith showed that all organic material had been dissolved in the outer part (OF) of the statolith (Fig. 7B). Here the density has been diminished and the ring formations has disappeared in the treated and thus organicfree zone. Organic material is therefore of importance for the dark striation.

Thus, as in otoliths, statoliths are composed of an inorganic and an organic component, a composition responsible for the band formation.

Interpretation of bands

The first-order bands described here have the same appearences and dimensions as the growth rings shown to be daily in fish otoliths by Panella (1971 and 1974), Lim (1974), Brothers *et al.* (1976) and Struhsaker & Uchiyama (1976). As also the constituent parts are the same in otoliths and statoliths, it seems likely that a similar interpretation is correct for squid statoliths. Choe (1963) found daily growth increments in the shell of species belonging to the cephalopod family Sepiidae, which indicates that the physiology of cephalopods might be affected by a diurnal rhythm.

In the second-order bands seen in Fig. 5 about 14 first-order bands, now interpreted as daily growth rings, occur. In order to show that this number of daily rings not is a single observation in one statolith only, daily rings were counted in several second-order bands (Fig. 8). The result of the counting shows a mean of

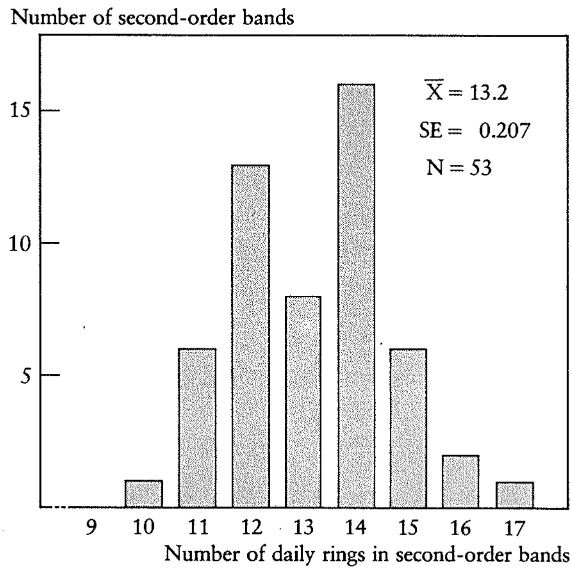

Fig. 8. Histogram showing number of daily growth rings in 53 (N) second-order or fortnightly bands. $\bar{X}$ = mean; SE = standard error.

13.2 daily rings per second-order band. Thus it seems reasonable to interpret second-order bands as fortnightly formations. Like this two fortnightly bands, one dark (f_1) and one light (f_2), constitute a monthly band (m). This is an interpretation which correspond to what is found in fish otoliths (Panella, 1971 and 1974).

In zone 4, a change between dark and light bands interpreted as fortnightly bands exists as changes between thick-striped bands and uniform bands, and the chronological order of these bands found in zone 3 is maintained (Fig. 6). The fortnightly bands become more compressed towards the surface of the statolith. Therefore the daily rings are difficult to recognize in this zone. The same pattern is seen in fish otoliths (Panella, 1971 and 1974). Like this, Brothers *et al.* (1976) found that in fish otoliths principal growth rings are most distinctly deposited in the young stages. Probably this is caused by less deposited material per surface area at increasing statolith size, which also is supported by the relation between statolith length and pen length (Fig. 3).

Age determination

Once a way of interpreting the growth rings has been found it is necessary to decide where in the centre of the statolith counting should start. This means finding out how much of the statolith exists at hatching.

Hatching size of *Gonatus fabricii* is about 3 mm PL (Kristensen, 1977). However, the smallest specimen available was 8 mm PL. The ground statolith of this specimen shows one second-order band plus about five first-order bands outside the nucleus (Fig. 9). Assuming that first-order bands are daily rings, this corresponds to an age of about 19 days.

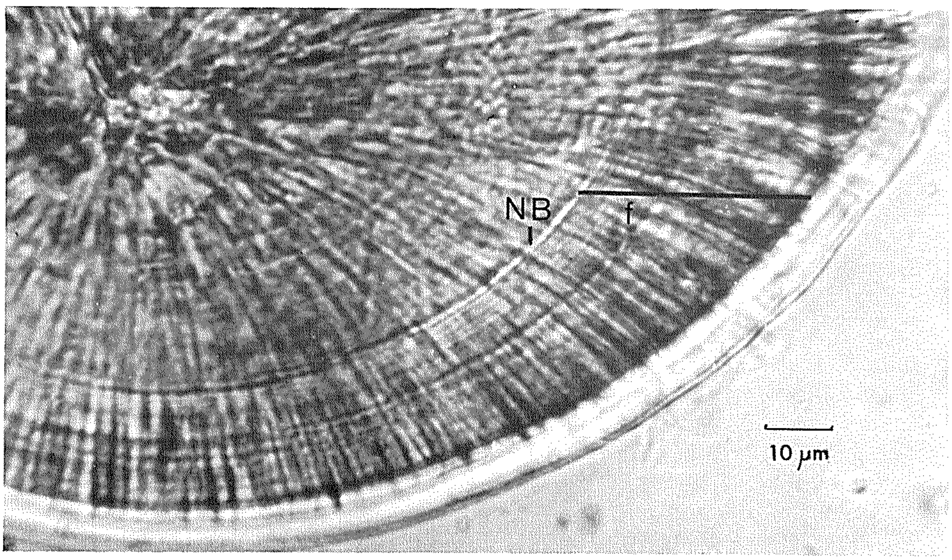

Fig. 9. Ground statolith from a specimen with a pen length of 0.8 cm. First-order bands and one second-order band or fortnightly band (f) can be seen. NB = nucleus border.

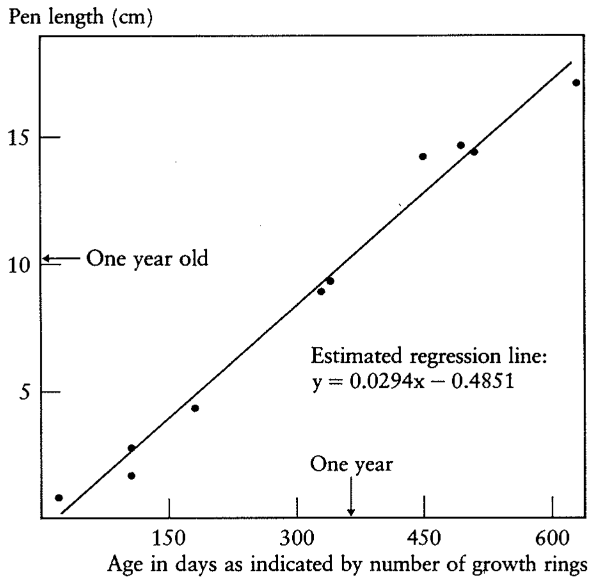

Fig. 10. Number of growth rings in relation to pen length (PL). The estimated regression line is shown. The pen length of a specimen one year (365 days) old is indicated by an arrow.

According to the growth rate found by Kristensen (1977), specimens of 8 mm are about 20 days old, thus, the nucleus most probably exists at hatching and the post-hatching growth rings are deposited outside the nucleus. This is supported by the great consistency which is found in the diameter of the nuclei, as mentioned earlier.

The rings were interpreted as mentioned above and counted on 10 statoliths. After development of the present technique these were the only statoliths left, where it was possible to count rings from nucleus to border. The counts are plotted against the pen length in Fig. 10.

A high correlation exists between age determined as counted growth rings and the pen length (correlation coefficient: 0.9923). The regression line indicates a pen length of about 10 cm for specimens one year old.

Considering the hatching size of 3 mm found in Kristensen (1977), the line seems to be too steep, giving a negative hatching size. Probably this is caused by a tendency to underestimate the age of old specimens where the growth rings in the outer part of the statolith are very close. Thus if counts of statoliths from the largest specimens in Fig. 10 had been larger, the regression line would be less steep and thus in better correspondence with the known hatching size.

The yearly increase in pen length found here corresponds very well to the growth rate of 8 mm per month based on juvenile material (Kristensen, 1977), which amounts to 9.6 cm per year. It also corresponds very well to the differences between size distribution of two year-classes found in *G. fabricii* caught in West Greenland waters (Kristensen, in prep.) (Fig. 11). The size distribution of specimens caught from July to October over several years shows two year-classes representing the major part of the material and a third and possibly fourth repre-

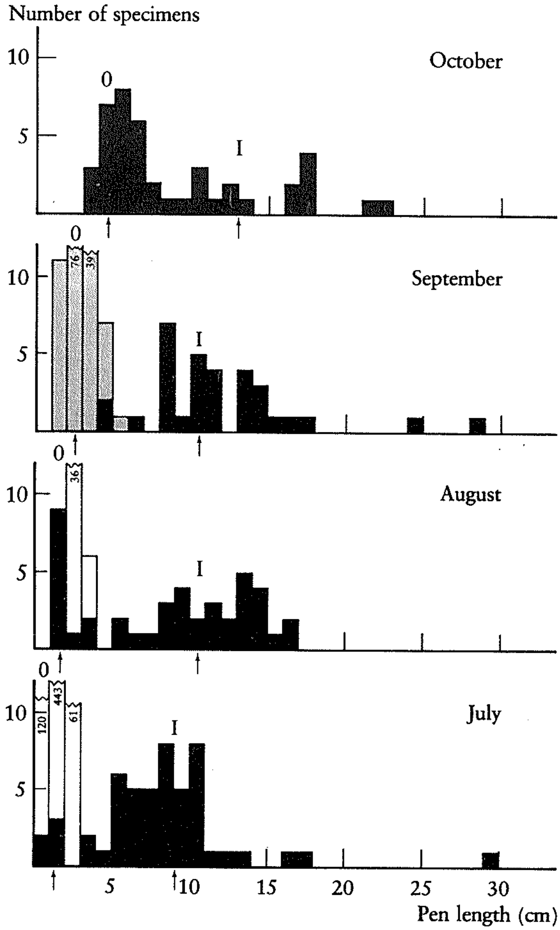

Fig. 11. Pen-length frequency distribution of *Gonatus fabricii* from West Greenland waters from July to October, (Kristensen, in prep.). Two year-classes (0) and (I) are presented (a third and fourth appear too but are not referred to in this work). Mean pen lengths of year-classes 0 and I found by the method of Cassie (1954) are shown by arrows. White bars indicate samples caught in a 2 m stramin net, obliquely hatched bars samples caught in a fine-meshed floating trawl, and black bars samples caught in a shrimp trawl.

sented by few specimens. Here only the two youngest year-classes are considered. Though material from three different types of gear is included in the first year-class (0), the good continuity and apparent growth make it reasonable to accept the bimodal distribution. Means of each year-class (arrows) were found by the method of Cassie (1954). The mean of the second year-class (I) is for each month 8-9 cm larger than the mean of the first year-class (0), indicating a yearly growth of this size. Considering the uncertainties in this method of finding growth, such an increase in growth corresponds well to the yearly increase in pen length of about 10 cm found by counting growth rings.

Periodical growth rings of the same kind as described in *Gonatus fabricii* were also found in *Rossia glaucopis* Lovén, 1845 (Fig. 12A), from the Davis Strait and *Alloteuthis subulata* (Lamarck, 1798) (Fig. 12B) from Danish waters. These specimens were not included in the main analysis but were only a supplement to show that the periodical growth increments are not limited to species of gonatids living in cold water.

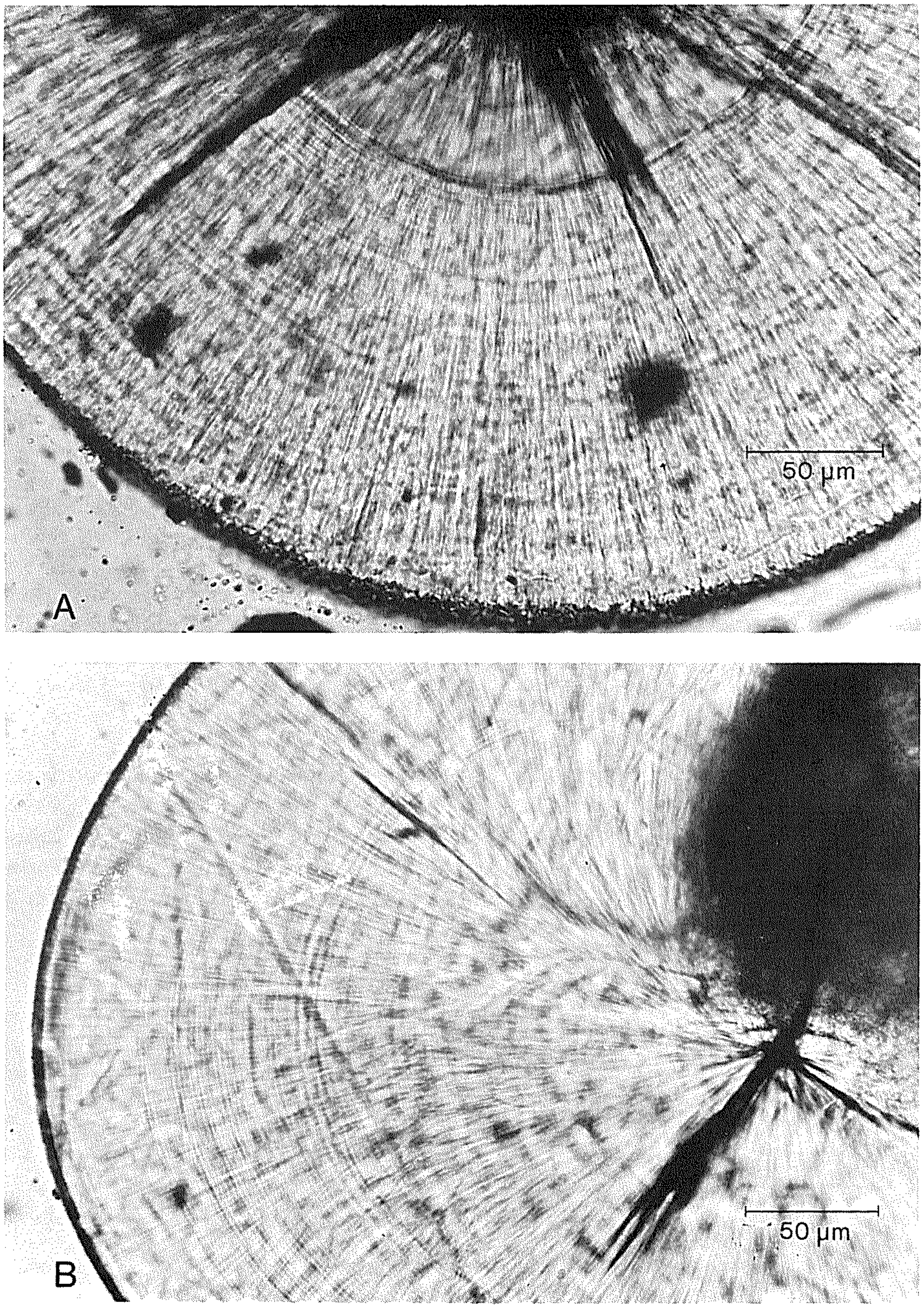

Fig. 12. A. Ground statolith from *Rossia glaucopis* from the Davis Strait. B. *Alloteuthis subulata* from the Danish waters. In both statoliths daily growth rings are seen.

Discussion

The age found by counting the growth rings corresponds so well to the known growth rate of juveniles and the size distribution of the year-classes, that the present interpretation of the growth rings seems very reasonable, and counting of the bands can really be used for age determination.

Choe (1963) showed that the deposition of daily increments is related to temperature and salinity of the water and to nutritive conditions.

The specific cause or causes of the daily cycle of calcium deposition in *G. fabricii* appear to be related only to nutritive conditions in the form of circadian rhythms in behavior, metabolism and physiology and not to the water temperature and salinity, which are rather constant throughout the year at Disko Bugt (Petersen, 1964). The stable hydrography throughout the year might also be responsible for the remarkable fact that no yearly growth pattern, as known from fish otoliths, were found in the statoliths, though several specimens were more than one year old.

If the second and third-order bands are fortnightly and monthly bands, as proposed here, the deposition of growth increments follows a lunarcycle, a relationship also found in nectonic fish (Panella, 1971).

Clarke (1966) observed concentric growth lines in ommastrephids. Now they are described from *Gonatus fabricii, Rossia glaucopis* and *Alloteuthis subulata.* Probably they exist in all statoliths composed of the same material as those presented here. Although *Alloteuthis subulata* was included in Dilly's (1976) study of cephalopod statoliths, he did not find any rings, which might be caused by formalin fixation of his material.

The described growth rings should be of great help in much future work on squids. However, the interpretations of the periodical growth increments ought to be confirmed by experiments with squids of known age reared in cultures. Likewise, improvements of the technique would be of value. In any case, the present results were found to be of sufficient interest to warrant publication although a number of uncertainties still exists.

Acknowledgements

The author wishes to express his gratitude to Dr Malcolm R. Clarke, Dr Margit Jensen and Dr Høpner Petersen for helpful discussion and criticism. The Zoological Museum and the Danish Natural Science Research Counsil are thanked for their support.

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