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Statoliths as a Possible Tool for Squid Age Determination

by

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Summary

A new method for squid statolith extraction and preparation is described. Close correlation of the statolith ring number (R) and statolith total length (TSL) with squid dorsal mantle length (DML) was found. This correlation suggests that statoliths could be used for squid age determination. The validity of the hypothesis "one ring-one day" is discussed.

Introduction

Summers (1971), Ikeda and Kawahara (MS 1975) and Mesnil (1977) analyzed size distributions to study growth and problems related to age in squids. Clarke (1965) expressed the opinion that the methods utilizing the distribution of size groups in the population and the change of these size groups with time are not generally very convenient. Indeed, as was shown by Squires (1957) and Sato (1974), squid migrate in characteristic "waves" which result in variable length frequencies over short periods of time (Lipinski, MS 1979a). Sometimes, however, the population seems to be relatively stable over several months (Mercer, MS 1975; and pers. comm.).

Clarke (1965, 1966), LaRoe (1971) and Summers (1974) searched for a "direct" method of ageing, i.e. utilizing natural time marks on beaks, statoliths and pens.

The most recent efforts concerning this method concentrated on the examination of statoliths (Spratt, 1978; Lipinski, MS 1978; Wiberg, 1979; Kristensen, 1980). Spratt (1978) found growth rings inside the statoliths of *Loligo opalescens* (Berry, 1911). Lipinski (MS 1978) found and illustrated growth rings in the statoliths of *Illex illecebrosus* (Lesueur, 1821). Hurley *et al.* (MS 1979), Hurley and Beck (MS 1980) attempted to validate age readings from the statoliths of *Illex illecebrosus*, suggesting that all growth rings in the statoliths were daily marks. Simultaneously, a similar opinion was expressed by Lipinski (MS 1979b).

Materials and Methods

The specimens used for this study were collected by means of Japanese jiggers in Conception Bay, Newfoundland, in 1976, pelagic trawls and jiggers on board the M/T *Pletwal* in 1977, also M/T *Szczytne* and R/V *Profesor Siedlecki* in 1978. The bulk of the material consisted of fresh and thawed specimens of *Illex illecebrosus* from the Northwest Atlantic. Other species examined were *Illex argentinus* (Castellanos, 1960, *Martialia hyadesi* Roch. et Mab., 1887, *Todarodes angolensis* Adam, 1962, *Ommastrephes bartrami* (Lesueur, 1821), *Sthenoteuthis pteropus* (Steenstrup, 1855), *Histioteuthis macrohista* N. Voss, 1969, *H. eltaninae* N. Voss, 1969, *Taningia danae* Joubin, 1931 and *Loligo pealei* Lesueur, 1821.

Using a method similar to that described by Clarke (1978), the statoliths were dissected very easily, even from squid with mantle length as small as 6 cm, contrary to the observation of Summers (1974) and Hurley *et al.* (MS 1979). The method of dissection is briefly described as follows:

a) Cut off the head of the squid between the muchal cartilage and dorsal proximal V-ridge (Fig. 1b). It is important that the liver is not damaged.

b) Lay open the proximal skull hole and cut the skull in the frontal plane across the hole (Fig. 2a).

c) Remove the ventral part of the pleurovisceral ganglion from the ventral plate (Fig. 2b). It is possible to see the small whitish particles (statoliths) inside the tissue through the semitranslucent skull.

d) Cut the ventral plate in the sagittal plane in each of its two corners and find the statoliths within the fluid-filled cavity with the use of very sharp tweezers (Rontax, Junkers SA are probably the best).

This method is reliable for thawed, as well as fresh material. It is difficult to find statoliths in specimens kept long in alcohol and impossible to find them in specimens kept in formalin. A magnifying glass was used for the extraction of statoliths from small squids.

After dissection, the statoliths were put into small paper bags and allowed to dry, or in small vials in 70% ethyl alcohol. The whole statoliths were cleared and mounted on glass slides in the laboratory with the concave side upward (Fig. 4a, Table 1).

Selected statoliths were mounted on microscopic slides in evaporated Canada balsam, after which they were ground and polished, first with a water-silicon carbide (1,000 and/or 1,200 grit) and with the use of glycerine and a frosted glass to the mid-frontal and/or mid-transverse plane. Both sides of the statoliths were polished until the nucleus was exposed.

The total length (as defined by Clarke, 1978) of 266 pairs of statoliths from *Illex illecebrosus* were measured with the use of a Zeiss micrometric microscope. Counts of the growth rings were made with the use of a PZO MB-30 microscope. The least squares method was used for fitting appropriate curves. Photographs were made by means of a Zeiss photo-microscope on ORWO film. The terminology for describing the statolith structure and measurements were adopted from Clarke (1978).

Table 1. Methods used in preparing the slides of *Illex illecebrosus* statoliths.

Biological data			Number of statolith pairs	Clearing agent and time (hrs)	Mountant
DML	Sex	Maturity[1]			
238-277	F	III	4	Karboxylene (72)	Eukitt[2]
191 225	M F	II II	2	Glycerine (528)	Canada balsam
242	M	IV	1	Creosote (48)	Canada balsam
190 240	M M	II III	2	Lactic acid (48)	Canada balsam
202	M	II	1	Euparal (48)	Euparal
185-200	M	II	3	Burning	Eukitt
220-245	M	III-IV	3	10% KOH (2, 4 and 16)	Eukitt
60-340	M,F	I-V	115	Eukitt (48-196)	Eukitt

[1] Maturity scale according to Lipinski (MS 1979c)
[2] O. Kindler, 78 Freiburg i. Br., Silberbachstrasse 25, Federal Republic of Germany.

Results

The method used to extract the statoliths enabled the removal of 50 pairs of statoliths per hour; this method was quick and simple and the necessary training time was no longer than 2-3 days. No difference was found in the readability of statoliths dried or preserved in alcohol.

A comparison between different clearing agents and mountants showed that the Eukitt and Euparal treatments were the best. However, good readability was achieved for approximately 30% of the statoliths. Generally, the statoliths from the younger squid were more readable. The results of clearing in Eukitt are illustrated in Fig. 3. The polished statoliths were very seldom fully readable. Usually there were substantial strata, where growth rings were not exposed. Typical results of polishing are illustrated in Fig. 4.

Different species of squids have different readability of their statoliths. Those of *Illex illecebrosus*, *I. argentinus*, and *Martialia hyadesi* are most easily readable, followed by *Todarodes angolonsis*. *Ommastrephes bartrami* and *Sthenoteuthis pteropus* stratoliths were hardly readable, the growth rings being present but mostly obscured. *Loligo pealei* had statoliths with narrow stratum only near the edge with very fine growth rings. No growth rings were found in the statoliths of *Histioteuthis macrohista*, *H. eltaninae* and *Taningia danae*.

The statoliths of the same species (and even specimens) often have quite different forms, particularly as regards wing development. The form of the statoliths of the same species change with growth (Fig. 5, a-d), whereas the statoliths of different species (adults) have rather different shapes (Fig. 5e).

The hypothesis that the mean length of the left statoliths is equal to the mean length of the right statoliths was tested using the paired Student-t test, the result indicating that there was no reason to reject the null hypothesis. The relationship between dorsal mantle length (DML) and the total statolith length (TLS) for *Illex illecebrosus* is given in the following equation:

$$TSL = 0.072\ DML^{0.505}$$

with correlation coefficient, $r = 0.946$. The results suggest that there are no substantial differences between the curve for different areas, sexes and years (Fig. 6).

The relationship between DML and the number of growth rings (R) of *Illex illecebrosus* females can be expressed by the equation:

(A) $R = 2.9\ DML^{0.8}$ for $N = 22$, with correlation coefficient $r = 0.96$

or (B) $R = 0.463\ DML^{1.197}$ for $N = 9$, with correlation coefficient $r = 0.95$

(Fig. 7). Only the results for females were presented as the number of readable male statoliths were few.

Discussion

Two methods of extraction of the statoliths are known at present. The first is described here in detail; a similar method was described by Clarke (1978). The other method was described by Hurley *et al.* (MS 1979) and Hurley and Beck (MS 1980). They dissolved the skull of the squid in sodium hypochlcrite ($NaHCLO_3$) and pepsin.

Both methods are very effective in field condition and even very small squids (0.8 cm - Kristensen, 1980) can be handled easily.

Grinding and clearing methods, making the statoliths readable, are now in use. A grinding method was described by Spratt (1978), Lipinski (MS 1978), Hurley *et al.* (MS 1979), Hurley and Beck (MS 1980) and Kristensen (1980). A clearing method was described by Lipinski (MS 1978), and in the present work. Both methods are sufficient for young squids, i.e. when the whole statolith is readable. Neither is reliable, however, nor statoliths of fully grown squids.

Different readability of statoliths of ten different squid species has been noted here for the first time. Generally the statoliths of ommastrephid squids were easiest to read. Previously good readability of the *Illex illecebrosus*, *Gonatus fabricii*, *Rossia glaucopis*, *Alloteuthis subulata* and *Loligo opalescens* was described (Spratt, 1978; Lipinski, MS 1978; Kristensen, 1980).

Variation in shape between statoliths within one species was first mentioned by Clarke, (1978). Changes in shape during the growth of *Illex illecebrosus* statoliths have been described here for the first time.

The present paper supports the hypothesis that growth rings, observed on statoliths of cephalopods, are the real time marks (Spratt, 1978; Lipinski, MS 1978; Kristensen, 1980). This hypothesis is supported by the high correlation between statolith total length (TSL) and dorsal mantle length (DML) of *Illex illecebrosus*, based on the measurements of 266 pairs of statoliths. A high correlation between ring numbers (R) and DML was also found. The relation between R and DML was a power function, as was shown in two different ways:

(A) for juvenile and fully grown females not very legible statoliths of older individuals (67-325 m DML, N = 22);

(B) for juvenile females only; completely readable statoliths (67-144 DML, N = 9).

The (A) curve, i.e. $R = 2.9\ DML^{0.8}$ was similar to that of Hurley and Beck (MS 1980), i.e. $R = 37.9\ DML^{0.47}$ (DML in cm.). If the hypothesis "one ring = one day" by Hurley *et al.* (MS 1979), Lipinski (MS 1979b) and Kristensen (1980) is correct, that curve would imply that older squids grow faster than the younger ones. This conclusion is not consistent with data obtained for squids kept in the laboratory (LaRoe, 1971). The poor readability of the bigger squids' statoliths could be a possible explanation of the shape of the curve (A).

Curve (B) was based upon the completely readable statoliths only. The curve (B) was used for comparison of theoretically derived ring numbers (as a "days") with the data concerning the progression of modal length groups throughout time, published by Mercer (MS 1975). According to his data, modal lengths between 6 July and 10 November (127 days) changed from 180 mm to 260 mm. The number of rings between these lengths, calculated from curve (B) was 126; this seems to be a strong support of the validity of curve (B) and hypothesis "one ring = one day".

The hypothesis "one ring = one day" was also supported by Kristensen's data (1980). He analysed the pen length of *Gonatus fabricii* (Licht., 1818) as a function of R. This function was however linear; this may be characteristic for the slow-growing species, which not achieve large size.

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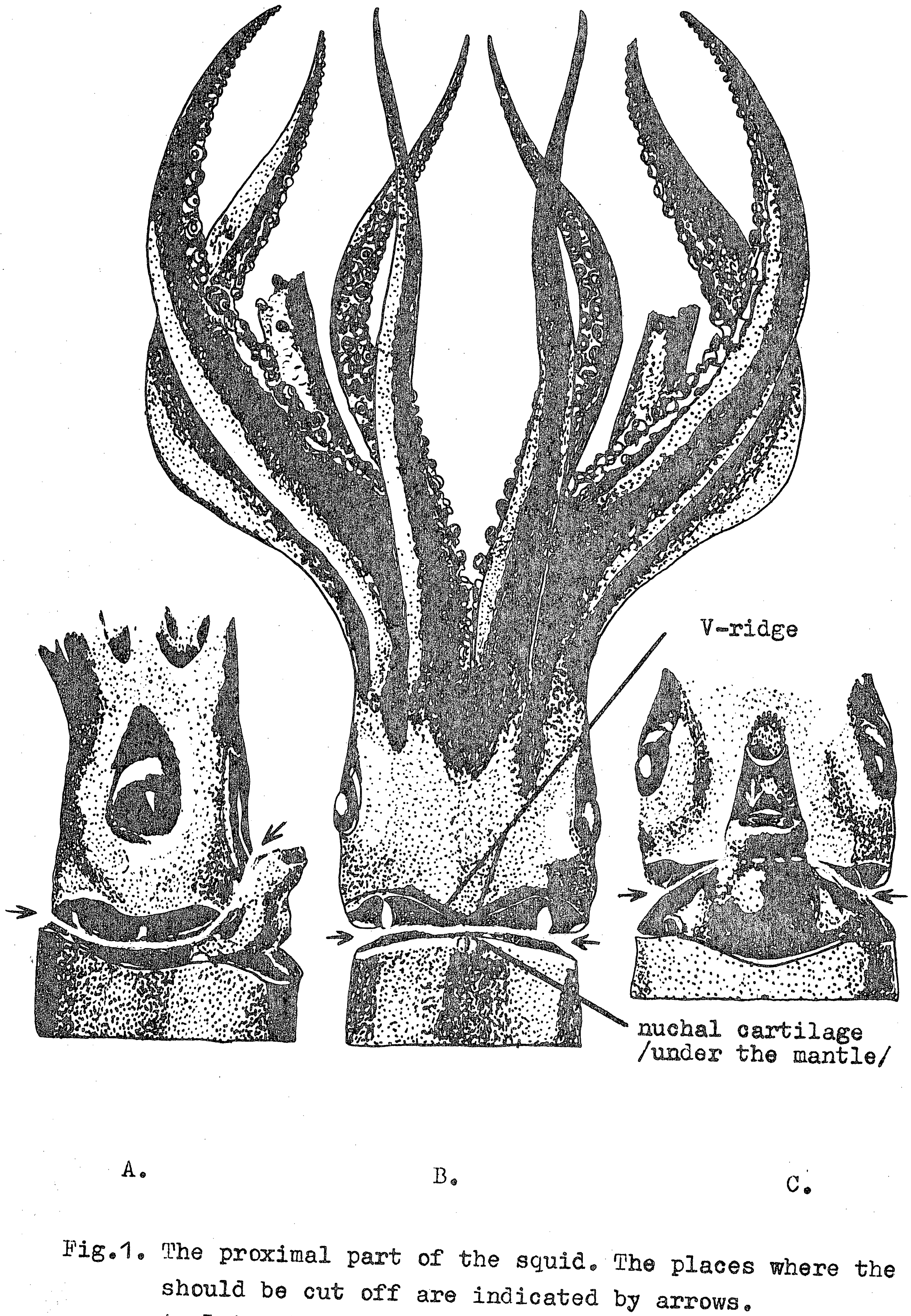

Fig.1. The proximal part of the squid. The places where the head should be cut off are indicated by arrows.
A. Lateral view. B. Dorsal view. C. Ventral view.

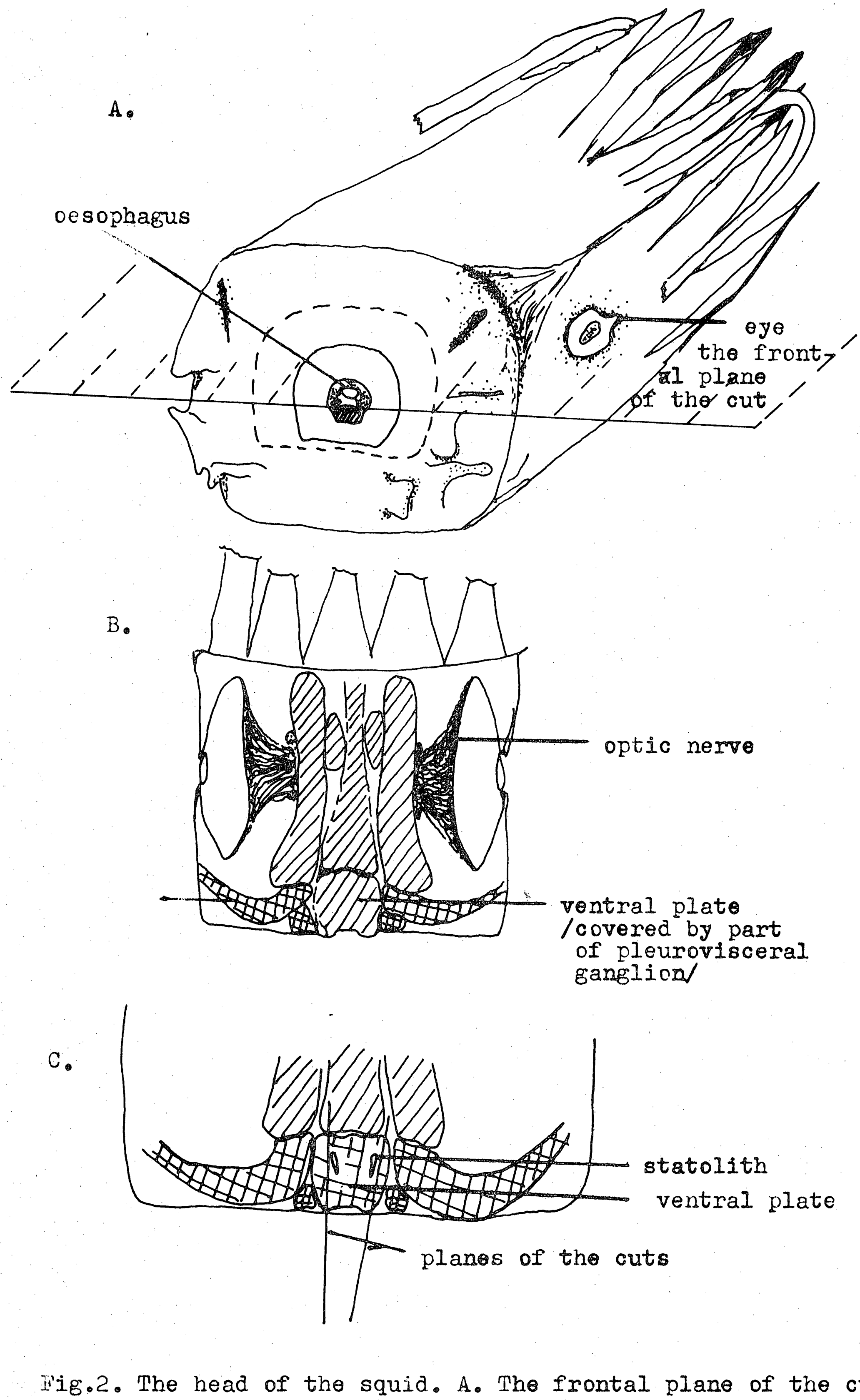

Fig.2. The head of the squid. A. The frontal plane of the cut.
B. The ventral part of the head after the cut.
C. The sagittal planes of the cuts across the ventral plate.

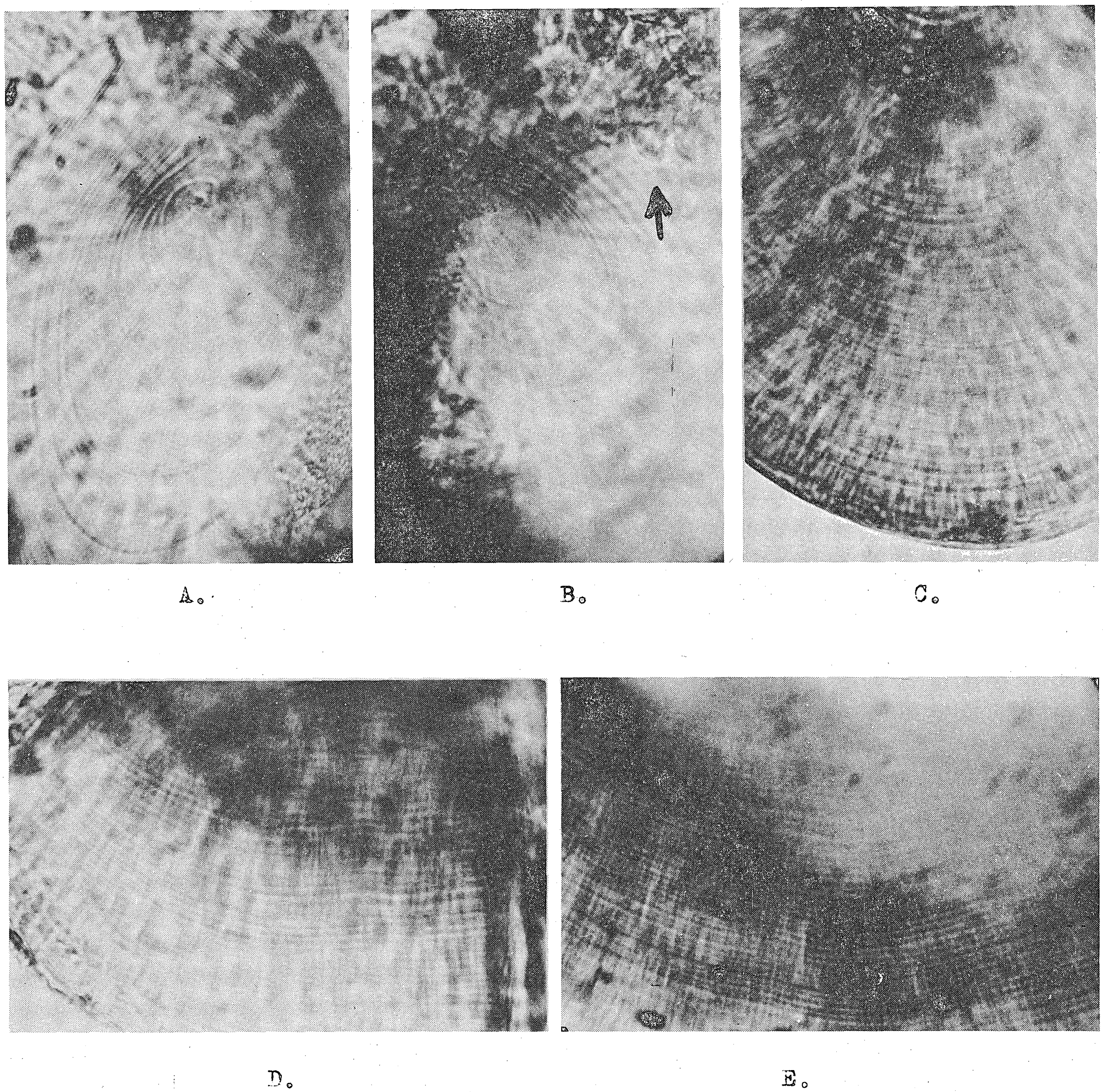

Fig. 3. The statoliths cleared in Eukitt, 450X.

A. Statolith nucleus of a young *Illex illecebrosus*, DML = 88 mm, Female I, Middle Atlantic Bight (Div. 6B).
B. Statolith nucleus of an adult *I. illecebrosus*, DML = 209 mm, Female II, Georges Bank (Div. 5Ze). The arrow indicates the "nodules" obscuring growth rings.
C. Growth rings in the statolith of young squid (same as in A).
D. Growth rings in the statolith of adult squid (same as in B).
E. Growth rings in the statolith of *Martialia hyadesi*, DML = 342 mm, Female III, Argentinian Basin.

A.

B.

C.

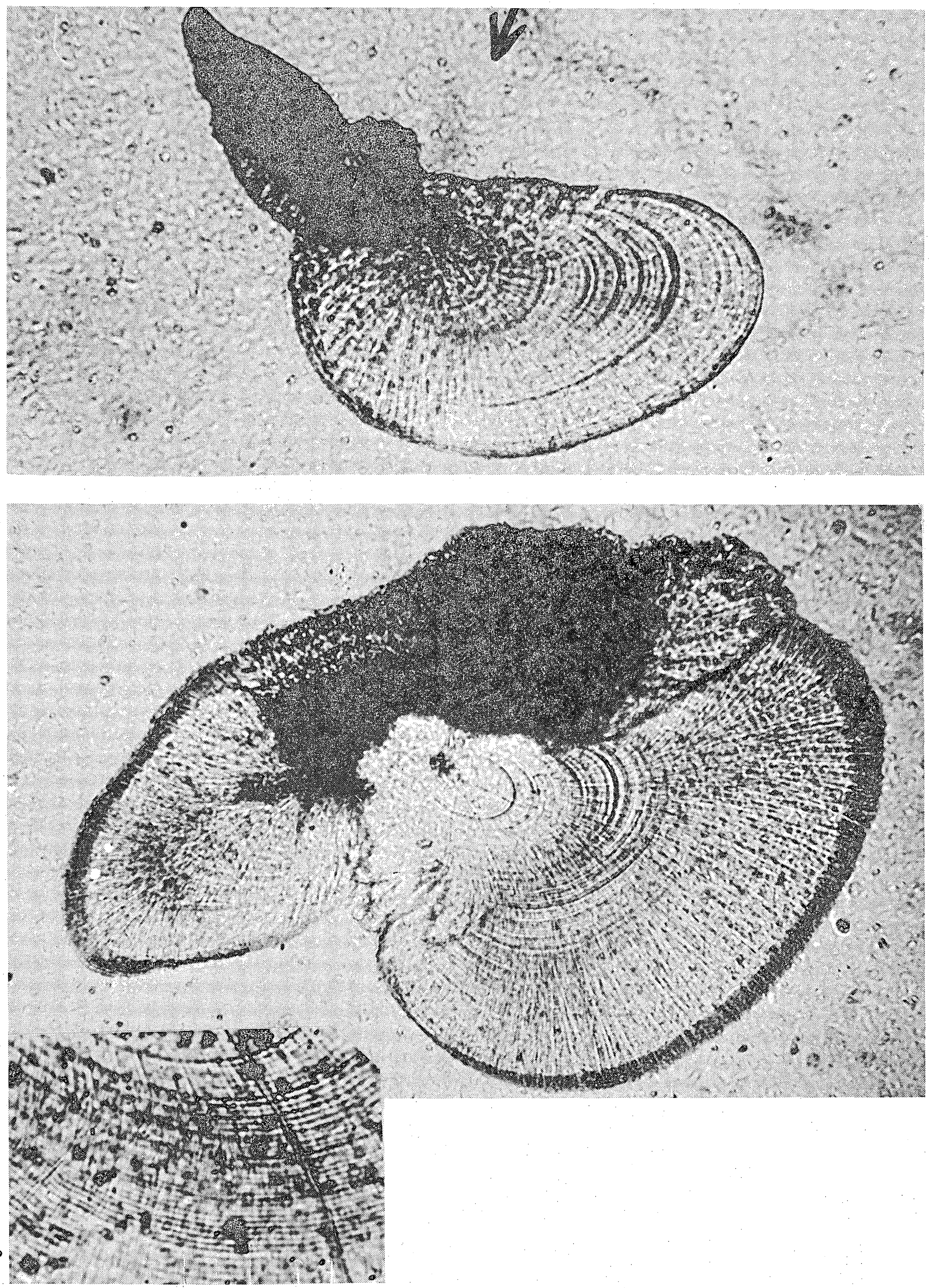

Fig. 4. Polished statoliths of *Illex illecebrosus*. A. Mid-transverse plane, 150X, Male II, DML = 220 mm, Scotian Shelf, concave side indicated by arrow. B. Mid-frontal plane, 150X (same as in A). C. The same as in B, 450X.

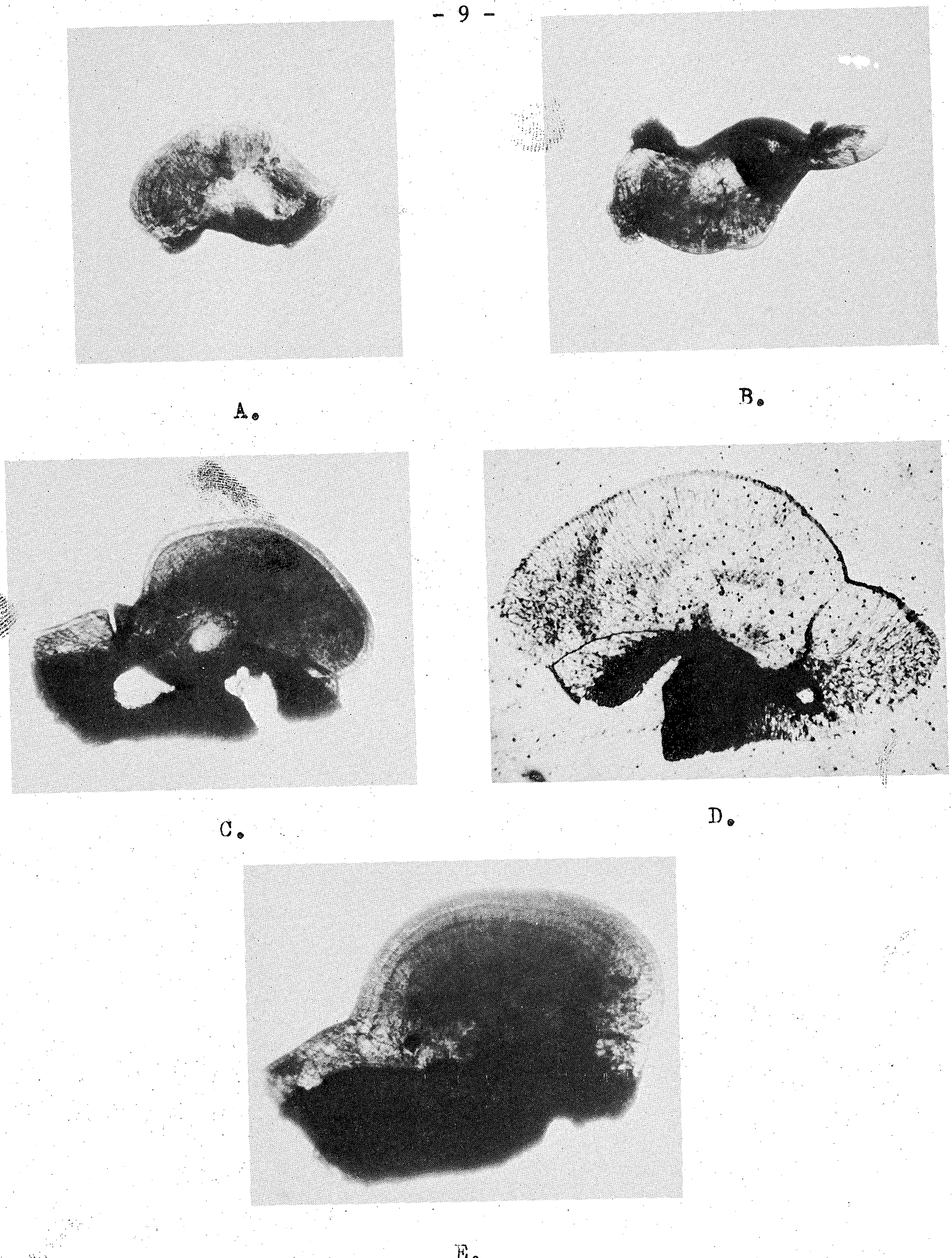

Fig. 5. Growth sequences of statoliths, 70X.

A. *Illex illecebrosus*, DML = 88 mm, Female I, Middle Atlantic Bight (Div. 6B)
B. *Illex illecebrosus*, DML = 145 mm, Female II, Georges Bank (Div. 5Ze)
C. *Illex illecebrosus*, DML = 188 mm, Female II, Georges Bank (Div. 5Ze)
D. *Illex illecebrosus*, DML = 292 mm, Female II, Georges Bank (Div. 5Ze)
E. *Martiala hyadesi*, DML = 342 mm, Female III, Argentinian Basin.

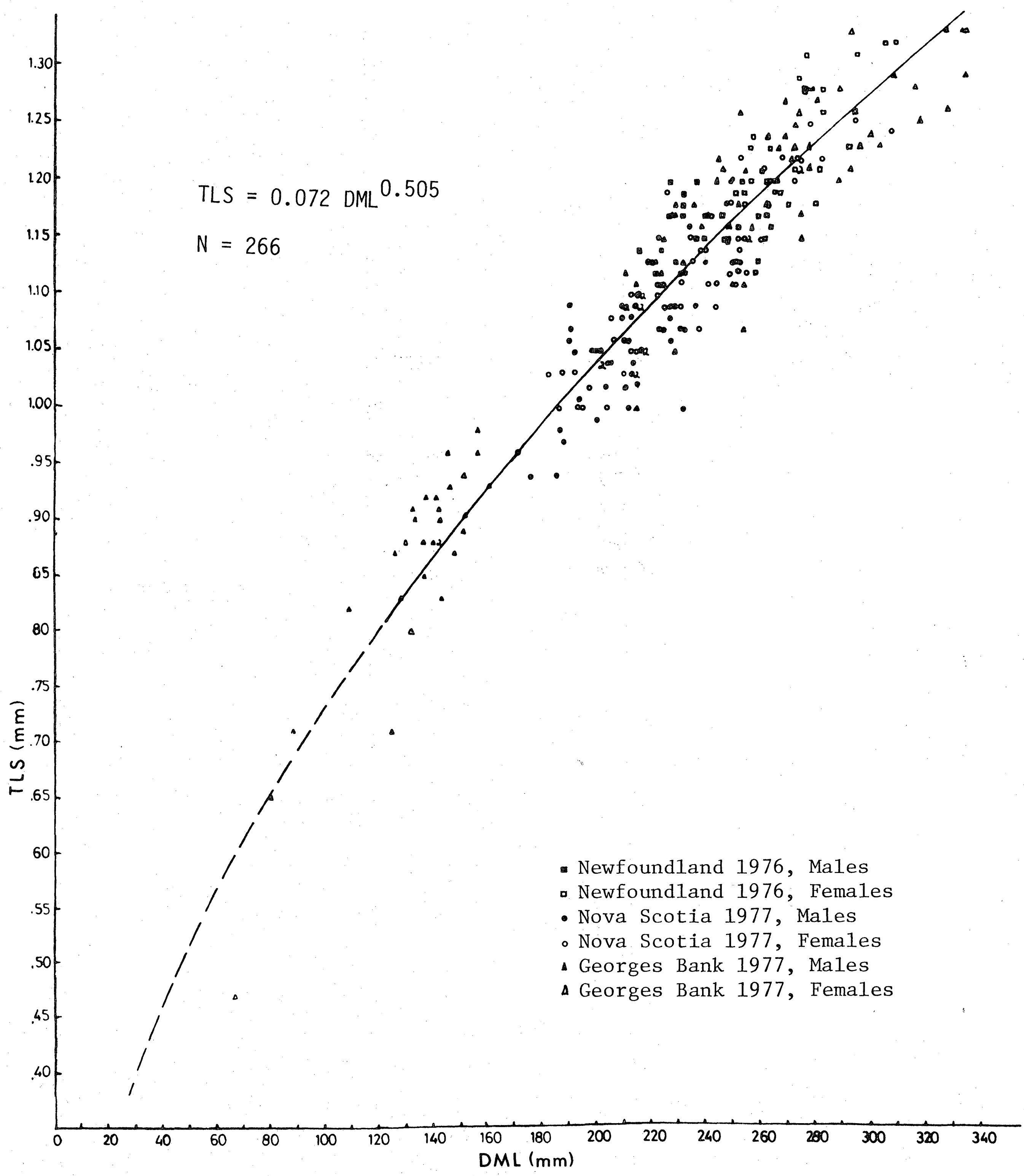

Fig. 6. The relationship between total length of the statolith (TLS) and dorsal mantle length (DML) of the squid *Illex illecebrosus*.

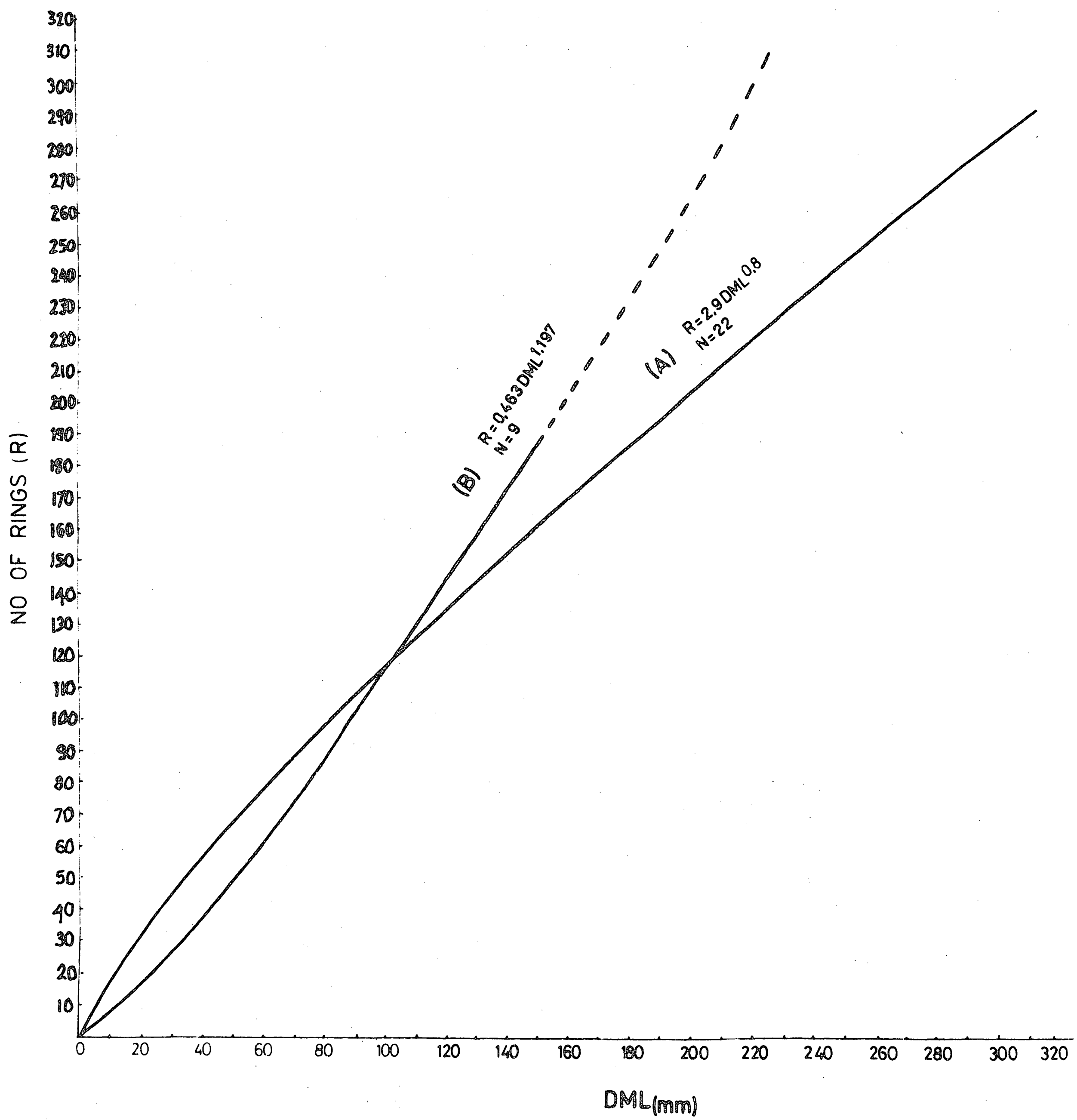

Fig. 7. The relationship between the number of growth rings (R) and dorsal mantle length (DML) of the squid *Illex illecebrosus* (Nova Scotia, 1977, females).

Curve A. - full DML range, but statoliths with poor readability included.
Curve B. - 67-146 mm DML range, completely readable statoliths only.