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Statolith Length and Increment Number for Age Determination in Squid

*Illex illecebrosus* (LeSueur, 1821) (Cephalopoda: Ommastrephidae)

by

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ABSTRACT

SPECIAL SESSION ON SQUIDS

A new method using a tracing to count increments in the statolith of the Ommastrephid *Illex illecebrosus* is described. The increments noted are true growth increments, i.e., they increase in number with statolith size, animal size, and time. However, statolith length is a more precise age indicator than is increment number. The increment-bearing portion of the statolith can be divided into three regions based on increment widths and variations of widths. These regions reflect different stages in the development of the animal.

Retention of larval characteristics of gross morphology, change in growth constant of the statolith and change in configuration of the statolith, suggest that the larval condition extends beyond the rhynchoteuthion ( 8.5 mm dorsal mantle length) to include squid of dorsal mantle lengths from 50-70 mm.

## INTRODUCTION

Recent interest in resource management of the species Illex illecebrosus (Lesueur, 1821) has stimulated efforts towards age determination. Previous attempts to age this squid have been made using the statoliths (Hurley, et al., 1979; Hurley and Beck, 1980; Lipinski, 1980; Mesnil, 1976; Squires, 1967) with varying degrees of success, limited chiefly by the unavailability of very young specimens. The current work being here presented investigates the length of statoliths and the number of growth increments, in an effort to determine age and, by extrapolation, to identify hatching dates, and the number of growth increments in a statolith.

Microscopic patterns of lamellae in the statoliths are variously referred to as "growth rings", "growth lines" or "growth increments". The definition used here parallels that of Pannella (1980) who defines a "growth ring" as a single dark lamella, a "growth increment" being a light lamella plus the immediately following dark lamella. For counting purposes, "ring" and "increment" are essentially synonymous but must be considered as different when referring to the physiology of their formation.

The nomenclature used for the identifiable parts of a statolith (Fig. 1) is that employed by Clarke (1978).

## MATERIALS AND METHODS

Squid were collected from five sites during 1981 (Fig. 2, and Table I) and frozen. These were subsequently thawed within plastic bags submerged in luke-warm water.

Upon dissection of the cartilage of the cephalic region, statocysts were cut open and the statoliths removed with fine

forceps. Adhering tissue and other remaining debris were dissolved in 5% sodium hypochlorite. The statoliths were then twice washed in distilled water and stored in 100% glycerine. By use of a Nikon S-Ke II microscope with an eyepiece micrometer measurements were taken of the glycerine-mounted specimens.

Following washing in 95% ethanol to remove glycerine, and twice rinsing in distilled water, the statoliths were then air dried and mounted. The specimens were individually mounted on their convex side in single drops of EPON 812 resin (Fisher Scientific Company) on glass slides and placed in a polymerizing oven for eight to twelve hours at 50 C. The success of observing increments over the entire counting area is greatly improved if one ensures that the orientation of the statoliths has not changed during processing, and that the dorsal dome of each statolith projects slightly downward.

To facilitate observations of increments, the specimens were ground against a glass plate covered with a mixture of 1200 grit carborundum powder and glycerine. The specimens were then stained with ninhydrin-schiff using the method as described by Humason (1976). Using a Zeiss microprojector, an image of each statolith was projected onto an aluminium surface-coated mirror and reflected onto the whitest paper available. Increments were traced onto the paper, the width of an increment represented by the width of a pencilled line, its darkness represented by the length of the line (Fig. 3). Three tracings of each statolith were made before the increments were counted. The accepted count was taken as the average of the two most similar of the three counts that differed by no more than 5%. If these conditions could not be satisfied, a fourth tracing was made.

The numbers of increments were plotted against the date of capture (Fig. 4), length of statolith (Fig. 5) and squid dorsal mantle length (DML) (Fig. 6). Statolith length was also plotted

against date of squid capture (Fig. 7) and against DML (Fig. 8). Where a non-linear relationship was apparent, attempts were made to fit a von Bertalanffy growth curve to the data. For other graphs a linear relationship was calculated.

## RESULTS

The first trials of grinding revealed an area of irregularly arranged crystals near the nucleus of the statoliths (Fig. 9). These crystals occlude underlying increments. Many such crystals but not all could be removed during grinding if the dorsal dome is properly positioned, and this is best done midway through the polymerizing process. The resin is still pliable, yet sufficiently hard to anchor the statolith.

Statoliths from male I. illecebrosus proved to be more difficult to prepare for analysis than were female specimens, as has been pointed out by Lipinski (1980). The data presented here regrettably are from female squid only, since statoliths from male specimens were effectively lost in the course of developing and perfecting the methodology here reported.

Based on analyses of the increments observed, the statolith of I. illecebrosus can be divided into three regions, plus a nucleus. The nucleus consists of an inner translucent area (the kernel) and the initially formed ring. We have called the three regions R1, R2 and R3. Region One (R1) is composed of relatively narrow and uniform increments (Fig. 9 and Table 2). The second region (R2) comprises the bulk of the increment-bearing portion of the statolith and is characterized by increments that are more variable in width and darkness, but are generally wider than are those in R1. At the inner margin of R2 the main axis of statolith growth shifts some 35° toward the dorsal midline of the animal (Fig. 9). The third region (R3) is comprised of narrow, regularly spaced increments forming the outer portion of the statolith. R3

is the last region to be formed and was found only in female squid which, upon examination, were found to display an enlarged ovary, signalling the onset of sexual maturity.

Several authors have remarked about a pattern evident in the sequence of incremental lines. We can offer the observation that throughout the statolith, noticeably darker rings often occur with a pattern of one dark ring followed by either seven, or a multiple of seven, lighter rings (Fig. 10).

Upon analysis, most of the data clearly showed, and easily fitted, linear or curvilinear relationships. However, when statolith length is plotted against DML (Fig. 8), a curvilinear relationship is evident, but not one which conforms to a von Bertalanffy growth function, and a tentative linear relationship was calculated for such.

#### DISCUSSION

The slope of the plot of increment number versus date of capture (Fig. 4) indicates an approximately daily rate of increment formation during the first few months of development. The data from statoliths collected in October, however, suggest that the rate of increment formation by that time decreased sharply. All specimens collected in October exhibited some degree of gonadal development and had statoliths with an R3 region. Since fasting has been found to be associated with sexual maturation in Octopus vulgaris (Wells and Wells, 1977), and in I. illecebrosus (personal observation), and since it is known that sexual maturation in I. illecebrosus can be induced by starvation (Rowe and Mangold, 1975), it is postulated that the closely aligned increments in R3 may be associated with decreased food intake and maturity. It is known that starvation causes a narrowing of growth increments in structures such as the shells of Mercenaria

mercenaria (Pannella and MacClintock, 1968) and the otoliths of the drum fish Lepomis cyanellus (Taubert, 1975). The depressed increment counts for the specimens captured in October may be due to either the non-recording of certain days, or difficulties encountered by the observer in resolving the smallest increments present in R3, or both.

Statolith length and number of increments are linearly related with a y-intercept very near zero, as expected (Fig. 5). However, increment number versus DML (Fig. 6) and statolith length versus DML (Fig. 8) have y-intercepts that deviate greatly from the expected value of zero. These indicate a very rapid increase in size of statolith and number of increments relative to DML in early post-hatching development.

Although the data in Figure 8 clearly indicates a curvilinear relationship between DML and statolith length, a von Bertalanffy growth curve could not be fitted to these data due to a discontinuous relationship between the growth of the mantle and the statolith, i.e., a change in the growth constant (K) of the von Bertalanffy equation. This change apparently occurs between 50 and 70 mm DML. This is the approximate DML coincident with a radical change in the basic shape of the statolith (Morris and Aldrich, 1984) and presumably associated with a change in the locomotive habits of the animal. Similar findings of change in growth rate of the statolith relative to DML at similar mantle lengths have been noted in the Arctic squid Gonatus fabricii by Kristensen (1980) and Wiborg, Gjosaeter and Beck (1984).

Change in growth constant is frequently used to distinguish larval and juvenile stages of fish (Nesis, 1979). Our data suggest that the larval stage of the Ommastrephidae may extend beyond the presently accepted rhynchoteuthion stage which ends at 7.5-8.5 mm DML (Roper and Lu, 1978; Vecchione, 1978). Other characteristics of post-rhynchoteuthid I. illecebrosus that are considered larval in other cephalopods, i.e., having suckers along

more than half the tentacular length, and relative arm lengths not matching those of the adult (Nesis, 1979), have been also noted by the authors in the course of this investigation.

The statolith must be of sufficient size at hatching to be functional. Statoliths of laboratory hatched Loligo vulgaris and Alloteuthis subulata measure 100-130 um while a pair from a hatchling of Sepia officinalis measured 490 um (Morris, unpublished data). It is thus almost certain that in view of the small size of the nucleus in statoliths of I. illecebrosus, evidence of incremental growth is already present at hatching.

Regression of the plot of number of increments versus date of capture (Fig. 4) indicates a date of initial increment formation in mid-December. If we assume that the pre-hatching increments are the approximately 38 found in R1, then the hatching date predicted from Figure 4 is incorrect and should therefore be advanced by 38 "days" to a hatching date then occurring in late January. Such a date for hatching closely agrees with that which may be predicted on the basis of statolith length (Fig. 7), and by regression of mantle length (Squires, 1967).

Region two (R2) thus begins to form at hatching. The hatchling squid, once released from the protection of the egg mass, is more exposed to environmental variations affecting formation of increments. This fact is reflected in the greater variation in both increment width and ring darkness found in R2 than is the case in R1.

The appearance of a single dark ring after seven, or a multiple of seven, lighter rings cannot yet, in our opinion, be given a final biological interpretation. Other, presently unidentifiable biological events can produce dark rings which may be interspersed amongst this periodicity, thus making it impossible to fully and accurately interpret the record to be read in the statolith.

The length of statolith varies less than the number of increments in any given sample. Until better techniques are developed to examine growth increments in I. illecebrosus, the length of statolith may prove to be a more reliable indicator of age than is any method involving the counting of incremental lines.

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TABLE I. Capture data for specimens of Illex illecebrosus used in the study of statoliths.

| Date of Capture (1981) | Location (Lat., Long.)                             | Location (General)            | Gear                     | Depth (Meters) |
|------------------------|--|-------------------------------|--------------------------|----------------|
| Feb. 27                | 38 <sup>0</sup> 24.7' N<br>56 <sup>0</sup> 00.0' W | Gulf Stream                   | Engel Midwater Trawl     | 100            |
| Mar. 4                 | 41 <sup>0</sup> 14.9' N<br>53 <sup>0</sup> 00.0' W | Gulf Stream                   | Engel Midwater Trawl     | 100            |
| May 25                 | 45 <sup>0</sup> 00.1' N<br>45 <sup>0</sup> 30.0' W | Carson Canyon                 | Engel Midwater Trawl     | 500            |
| June 20                | 43 <sup>0</sup> 36.5' N<br>51 <sup>0</sup> 54.8' W | Grand Banks                   | Engel Midwater Trawl     | 124            |
| Oct. 13                | 47 <sup>0</sup> 45.1' N<br>54 <sup>0</sup> 01.2' W | Refinery Pier, Come By Chance | Japanese Jigging Machine | 10             |

TABLE 2. Mean measurements and ranges of increment widths and regional widths from seven statoliths from seven female specimens of *Illex illecebrosus* captured in October, 1981. All statoliths from which these data arise exhibited growth typical of region three (R3). (See Text)

| Region  | Mean Increment Width (um) | Range of Increment Width (um) | Mean Region Width (um) | Standard Deviation of Region Width (um) | Range of Region Width (um) | Mean Number of Increments | Standard Deviation of Number of Increments | Range of Number of Increments |
|---------|---------------------------|-------------------------------|------------------------|---|----------------------------|---------------------------|--|-------------------------------|
| Nucleus | -                         | -                             | 17.8                   | 1.5                                     | 15-21                      | -                         | -  | -                             |
| R 1     | 3.0                       | 2.5-3.5                       | 122.0                  | 9.5                                     | 109-139                    | 40.1                      | 7.8  | 30-51                         |
| R 2     | 3.4                       | 1.5-5.0                       | 403.0                  | 22.5                                    | 378-431                    | 127.1                     | 7.9  | 97-140                        |
| R 3     | 2.3                       | 2.0-2.6                       | 112.0                  | 10.5                                    | 98-122                     | 50.6                      | 8.7  | 40-65                         |

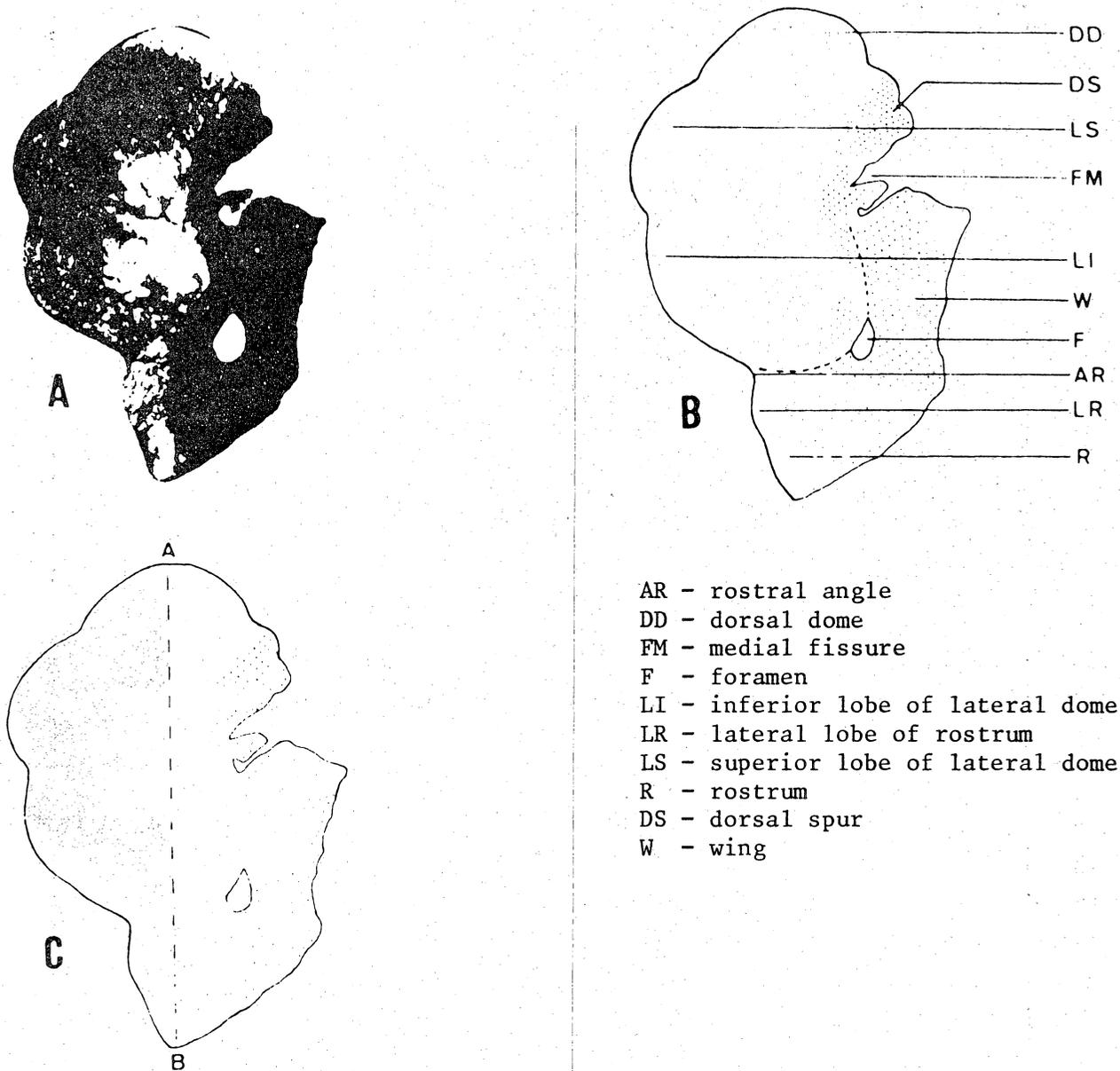


Fig. 1. Posterior aspects of the statolith of *Illex illecebrosus* (Lesueur) showing visible structures and measurements used in this study.

- A. Light micrograph. Notice opaque region toward median side, composed of irregularly arranged crystals. (Magnifications: 65x).
- B. Diagram. Dotted line indicates position of posterior dome indentation. Stippled area indicates region of irregularly arranged crystals.
- C. Diagram showing axis along which total length (AB) of a statolith was measured.

(Modified from Morris, 1980. Nomenclature as from Clark, 1978)

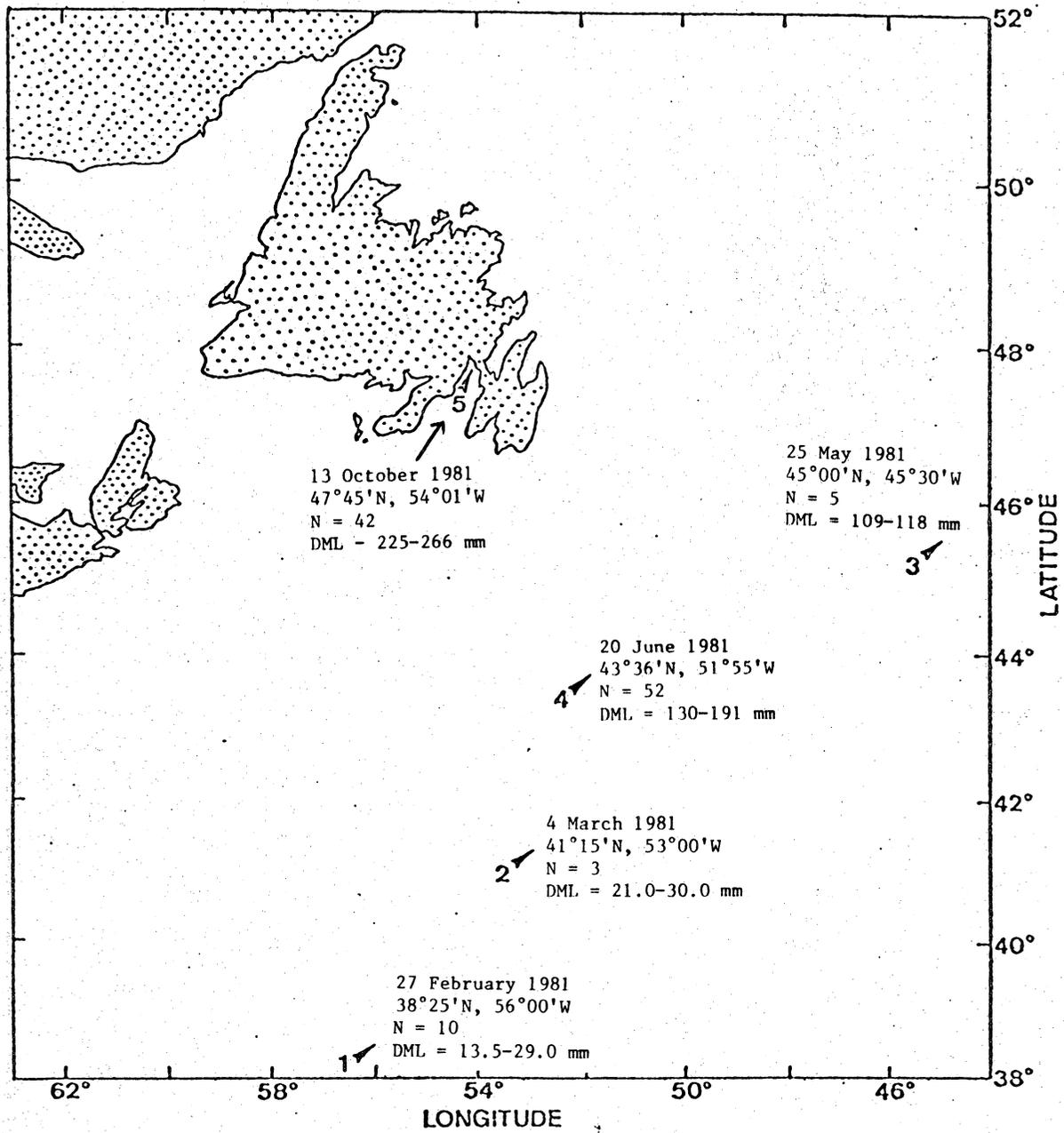


Fig. 2. Map of northern Gulf Stream region and insular waters of Newfoundland showing sites of capture of *Illex illecebrosus* (Lesueur) used in this study of statoliths.

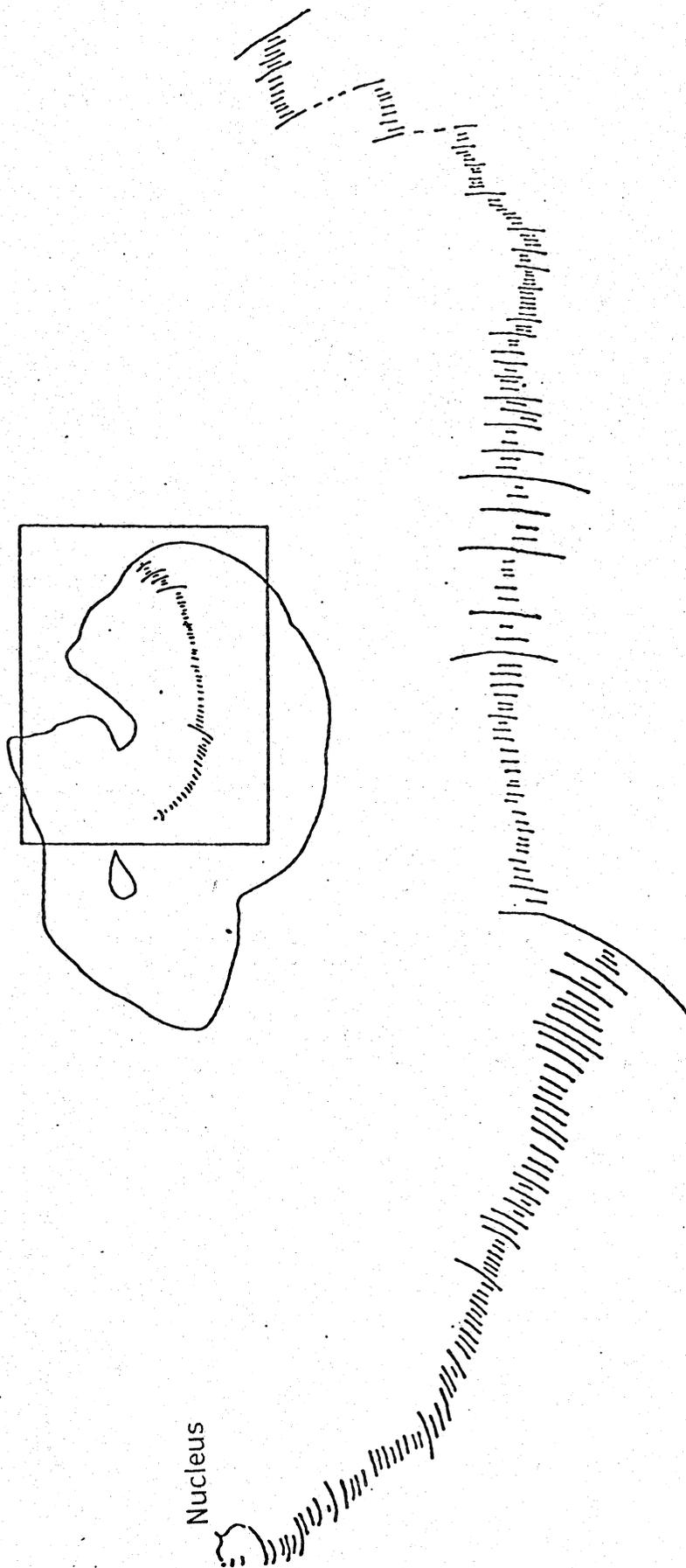


Fig. 3. Tracing of rings observed in ground and stained specimen of *Illex illecebrosus* (Lesueur). The length of line representing one ring indicates relative darkness while width of same line indicates width of ring being traced.

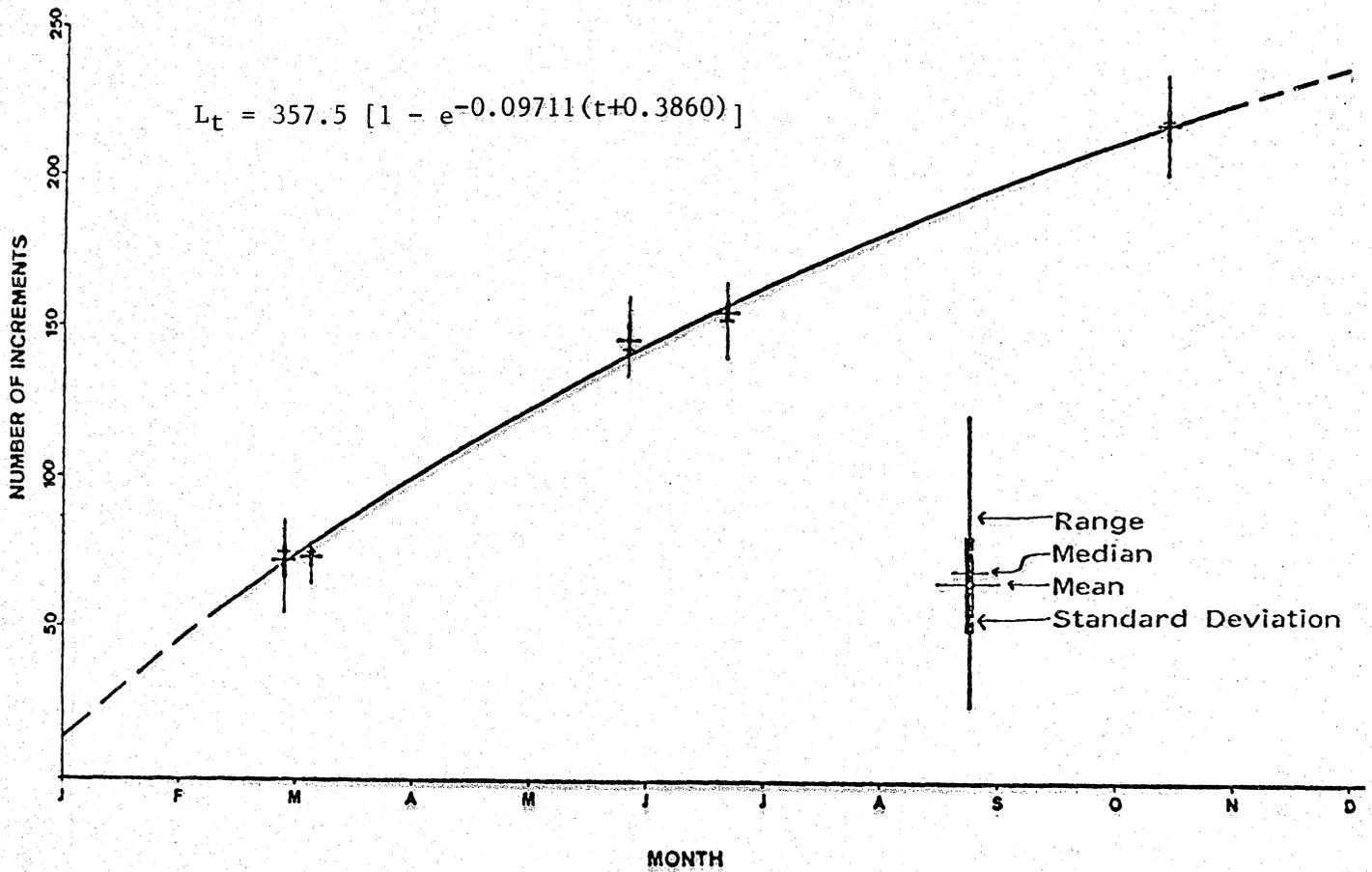


Fig. 4. Graph of von Bertalanffy growth relationship between date of capture and number of increments in the statoliths of *Illex illecebrosus* (Lesueur).

Model is  $L_t = L[1 - e^{-k(t-t_0)}]$ , where  $L_t$  = number of increments at time ( $t$ );  $L$  = number of increments at  $t_\infty$ ;  $e$  = base of natural logarithms;  $k$  = growth constant; and  $t_0$  = time at which increments begin to form.

(Calculations and symbolism as from Ricker, 1975.)

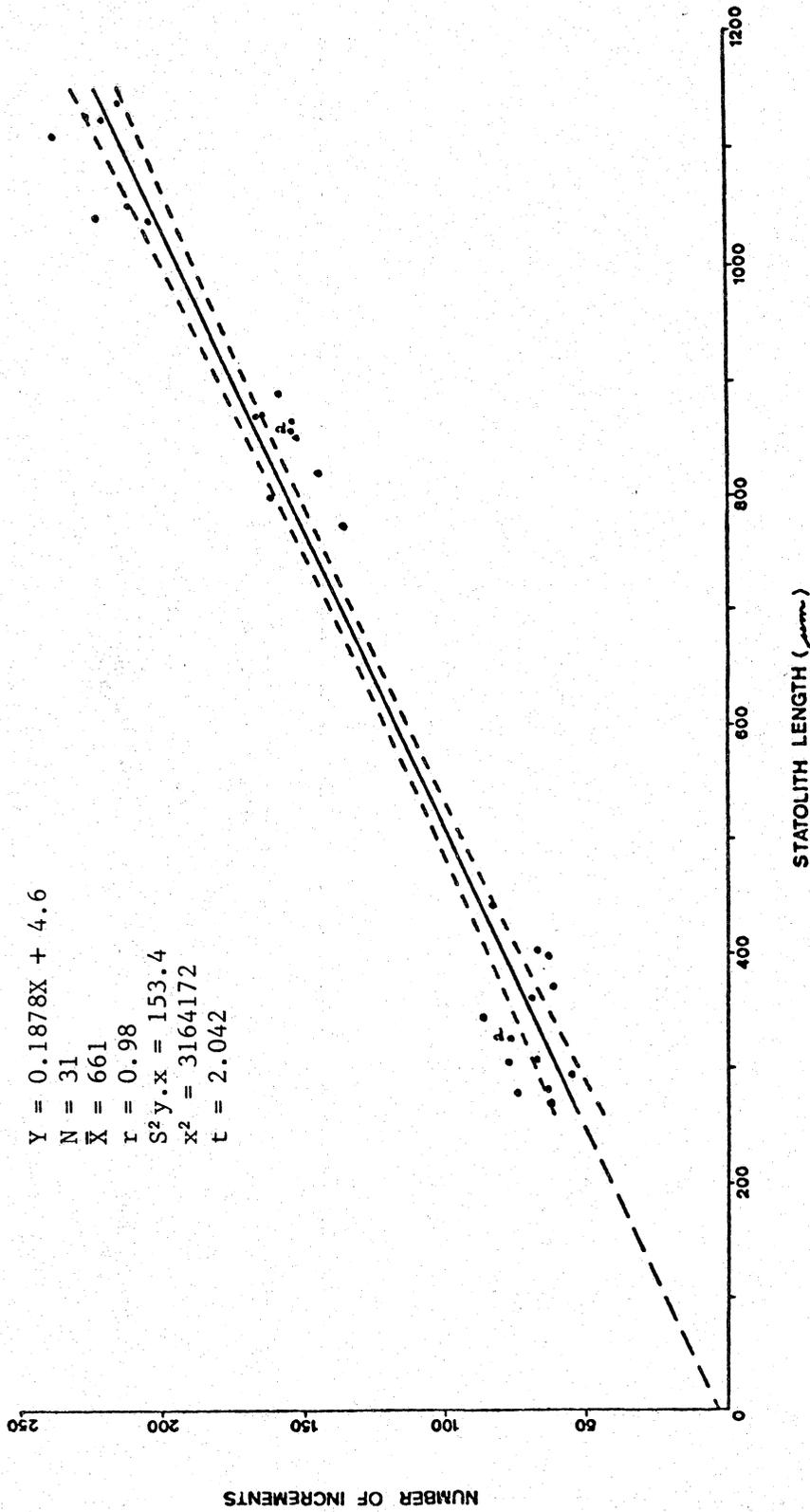


Fig. 5. Relationship (with 95% confidence limits) between statolith length and number of increments in statoliths of *Illex illecebrosus* (Lesueur). (Calculations and symbolism as from Zar, 1974.)

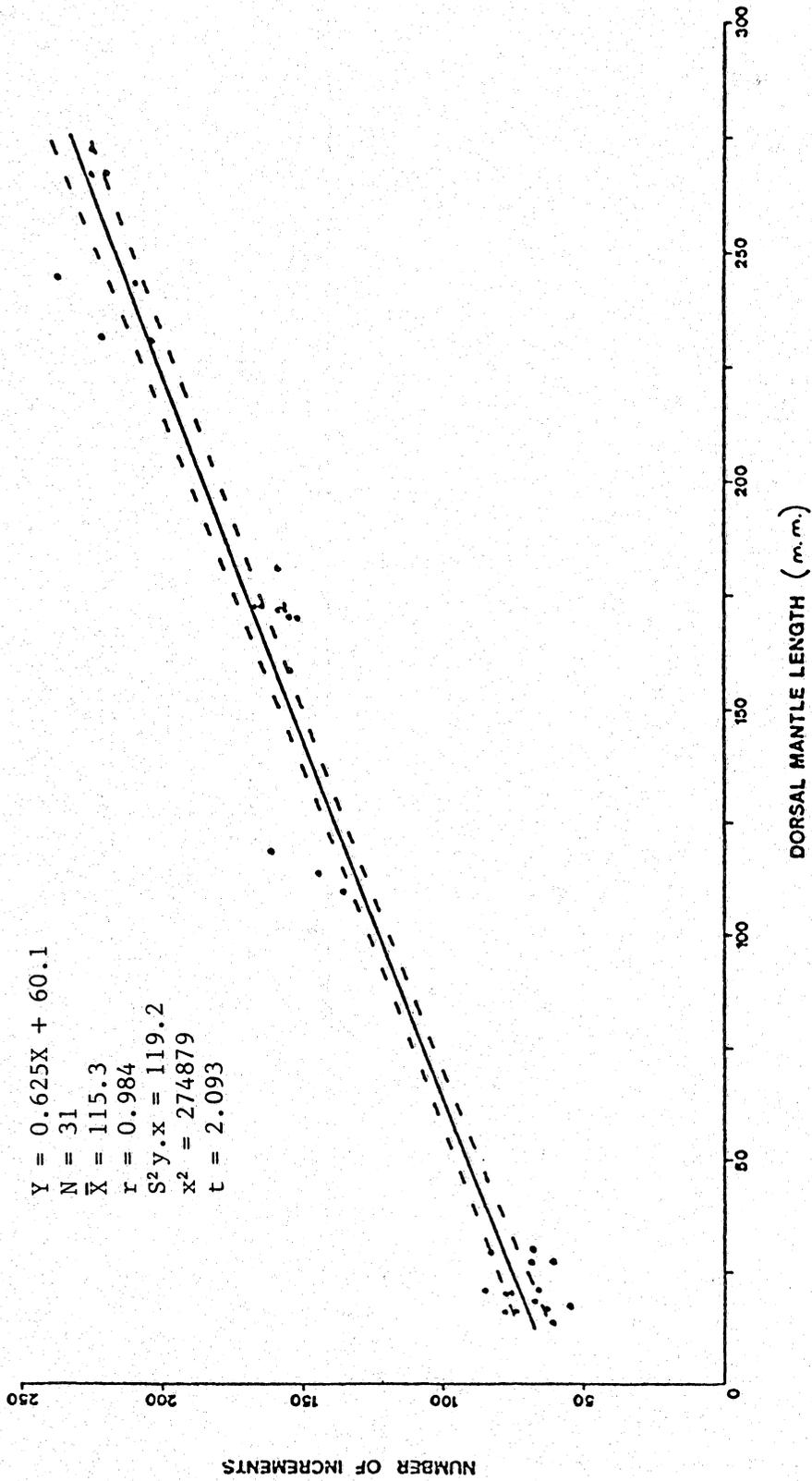


Fig. 6. Relationship (with 95% confidence limits) between dorsal mantle length and number of increments in the statolith of *Illex illecebrosus* (Lesueur). (Calculations and symbolism as from Zar, 1974.)

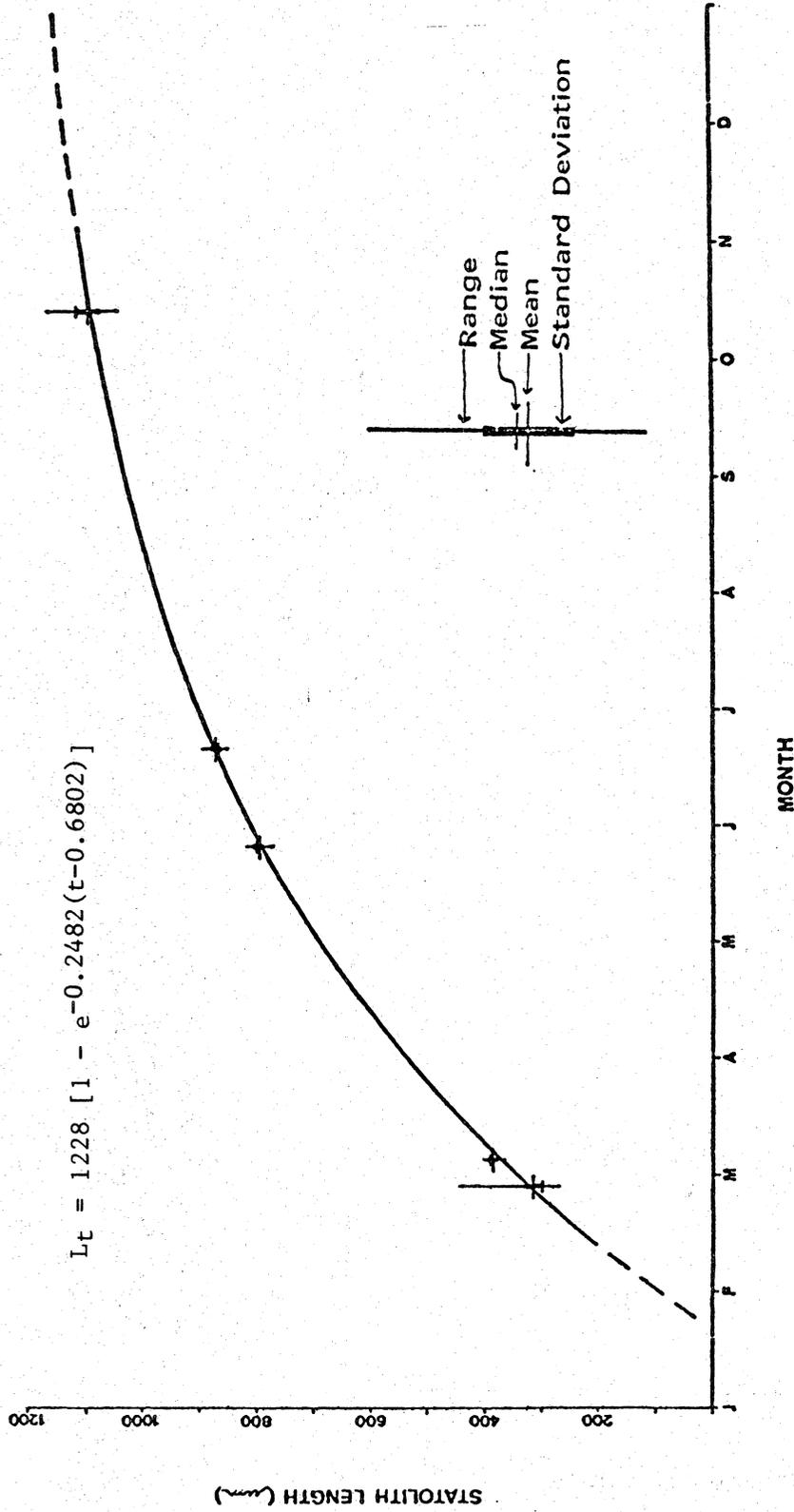


Fig. 7. Graph on von Bertalanffy growth relationship between date of capture and statolith length of *Illex illecebrosus* (Lesueur).

Model is  $L_t = L[1 - e^{-k(t-t_0)}]$ , where  $L_t$  = number of increments at time  $(t)$ ;  $L$  = number of increments at  $t_\infty$ ;  $e$  = base of natural logarithms;  $k$  = growth constant; and  $t_0$  = time at which increments begin to form.

(Calculations and symbolism as from Ricker, 1975.)

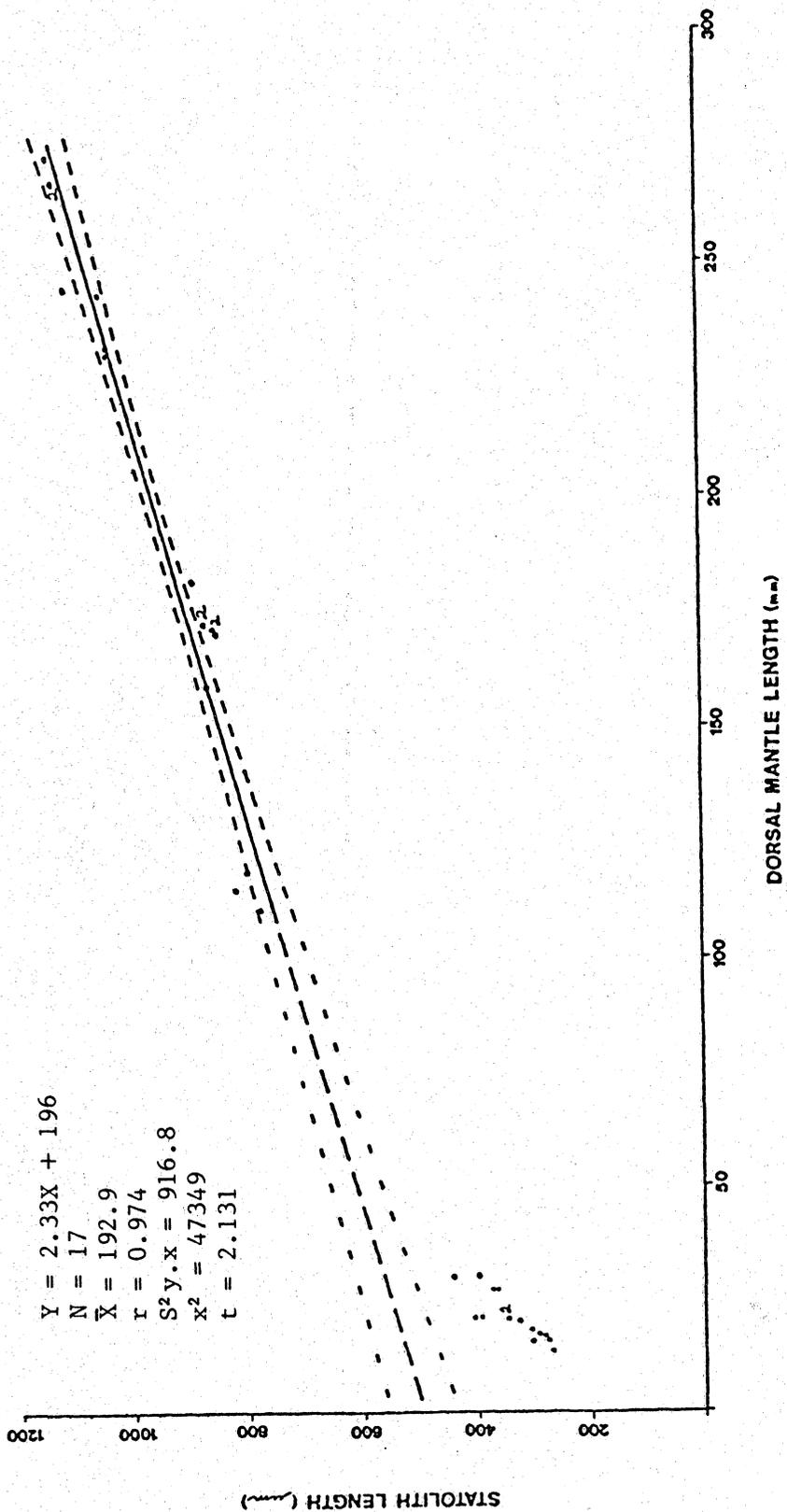


Fig. 8. Relationship (with 95% confidence limits) and statolith length in *Illex illecebrosus* (Lesueur). (Regression calculated for data from adult female specimens only. Note that the data from larval specimens lie outside the 95% confidence interval of the model.) (Calculations and symbolism as from Zar, 1974.)

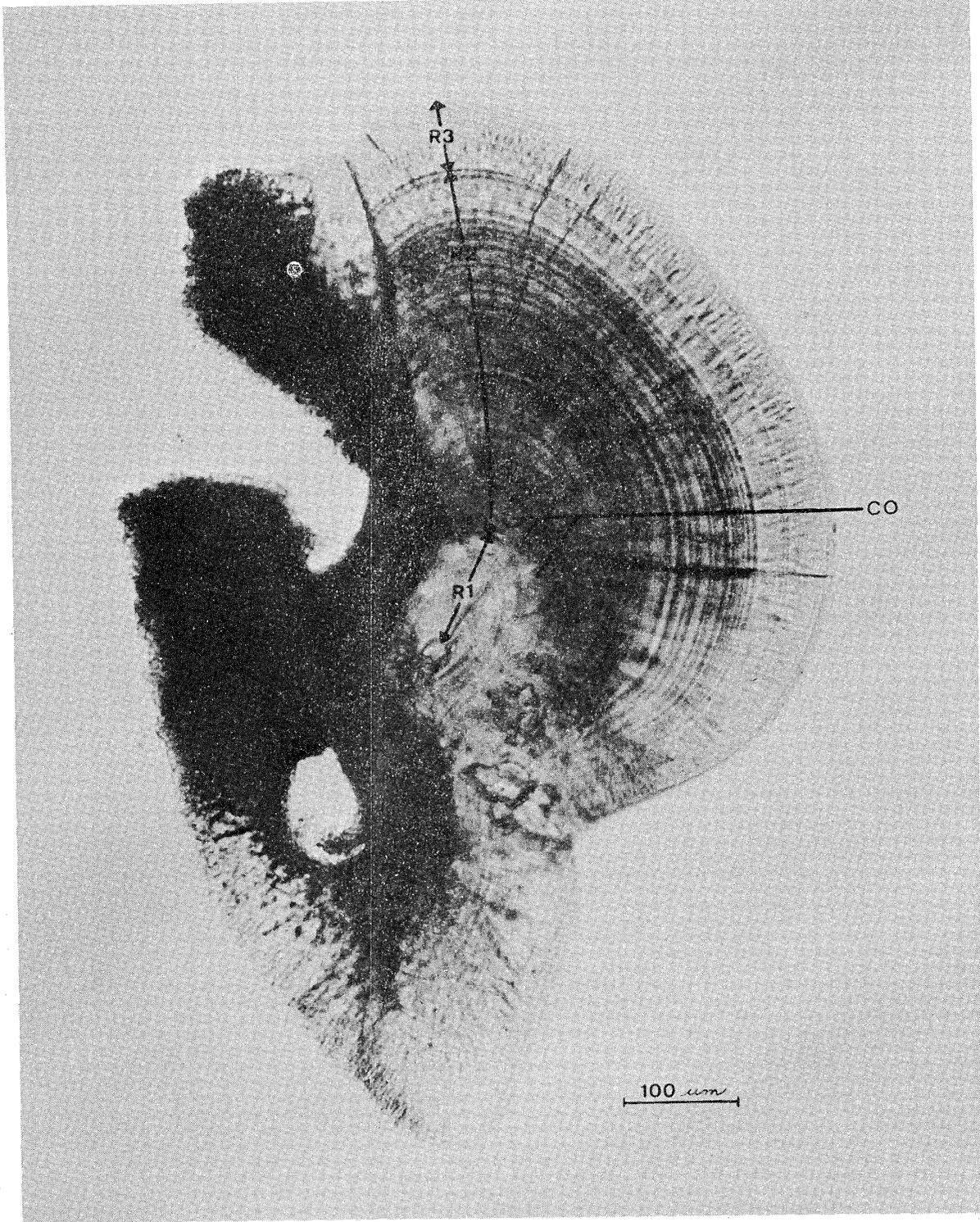


Fig. 9. Ground anterior surface of an Epon 812 embedded statolith of *Illex illecebrosus* (Lesueur) to show the three regions of increment formation and the position of the occulting crystals. Taken from a male specimen of 168 mm dorsal mantle length. (CO = occulting crystals; R1 = region one; R2 = region two; and R3 = region three.)

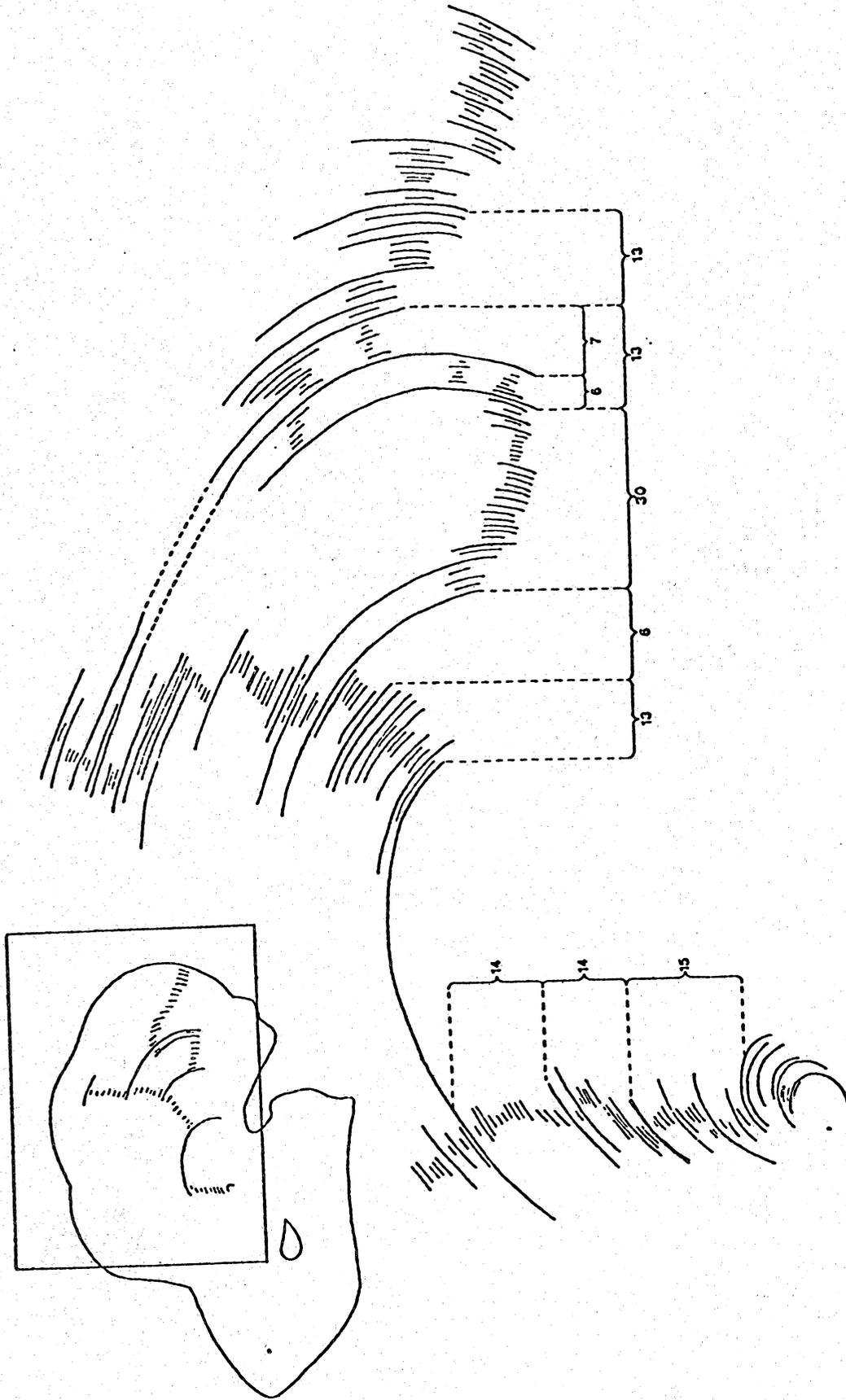


Fig. 10. Diagrammatic representation of tracing of growth rings in a statolith (length: 997  $\mu$ m) of a male *Illex illecebrosus* (Lesueur) (DML: 212 mm).