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The Observation of Growth Rings in Statoliths from the Ommastrephid Squid, *Illex illecebrosus*

by

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Abstract

An examination was made of statoliths from the squid, Illex illecebrosus. Rings found in the statoliths were shown to be 'true' rings and not merely optical aberrations. The number of rings increased exponentially with the maximum statolith radius and the mantle length. Cohort analysis did not indicate that the rings could be interpreted chronologically.

Introduction

To date studies of the population biology of squid have been hampered by the lack of a reliable technique for ageing. Age determinations and growth rate calculations have been restricted to attempts at equating size frequency modes with different brood or year-classes (Mesnil, 1977; Okutani, 1977; Squires, 1967; Summers, 1971). However, complications arise even within a single year-class with overlapping size distributions due to the presence of mixed age groups possibly resulting from a protracted spawning period (Mercer, 1969).

Clarke (1965) suggested that 'age determinations from laminations of some kind would overcome these difficulties', similar to scale or otolith reading in fish. To investigate this, workers have examined the rings found in various body sturctures of squid such as beaks (Tinbergen and Vervey, 1945; Clarke, 1965) radulas (Wirz, 1963) but no method was discovered.

Evidence of rings in cephalopod statoliths has been reported by various authors (Brothers et al., 1976; Clarke, 1966; Lipinski, MS 1978; Young, 1960).

Lipinski (MS 1978) speculated that the rings in statoliths of Illex illecebrosus might be daily rings within the nucleus and monthly rings beyond this zone.

The purposes of this study were to 1) examine the microstructure of the rings found in the statoliths of Illex illecebrosus and determine if they were 'true' rings formed by differential deposition of $CaCO_3$ and organic material and not an optical aberration as was suggested by Dilly (1976), 2) describe the growth of statoliths and the relationship between the number of rings laid down and mantle length and 3) by means of back calculation determine if the rings had a chronological interpretation as originally proposed by Lipinski (MS 1978).

Materials and Methods

Statoliths from 129 squids (65 females, 64 males) were examined. Their mantle lengths ranged from 1.5 to 25.0 cm. Of these, 35 statoliths were collected from squids caught by the Soviet research vessel RTM Belogorsk in March-May 1979 during a plankton cruise in the area of the Gulf Stream off the Canadian Atlantic coast. The remaining statoliths were collected in either June 1978 from a bottom trawl survey on the southwest slope of the Grand Banks or from Newfoundland inshore commercial samples obtained on a monthly basis from 10 June to 17 November 1978.

The statoliths are paired calcareores concretions located in the ventro-posterior region of the cartilaginous skull (Fig. 1) in the anterior end of two adjacent cavities, the statocysts, in an area known as the macula princeps (Clarke, 1978; Young, 1964). By means of x-ray diffraction the composition of the statoliths was determined to be aragonite for Illex illecebrosus which was similar to that found for the composition of other cephalopod statoliths (Clarke, 1978; Dilly, 1976).

Two methods were employed for the extraction of statoliths. First by dissection using a method similar to that described by Clarke (1978). The second technique made use of the differing chemical composition of the cartilaginous skull and the aragonitic statoliths. Ordinary bleach (Na $HClO_3$) was effective in dissolving away the skull while leaving the statoliths intact. The latter method would be preferred over the former in the field as no painstaking dissection would be required.

For each squid, the sex was determined (except for squids <2.0 cm which could not be sexed) and the mantle length was measured.

The statolith was first embedded in Ward's cement and mounted on a glass microscope slide. The statolith was ground on its concave antero-lateral plane (Fig. 2) against the frosted surface of another microscope slide until concentric rings were clearly visible along a radius from the nucleus to the outer edge of the statolith. The rings which have been defined as a pair of light and dark adjacent bands (Taubert and Coble, 1977) were counted with the aid of a compound microscope at magnifications ranging from 250-550X. To examine the microstructure of the statoliths a scanning electron microscope (SEM) (Cambridge Stereoscan Mark II A) was used (Fig. 3a, b). The ground surface of each statolith was polished with 1-µm diamond paste, etched with 0.1N HCl for 3 to 4 minutes, mounted on an aluminum stub with double-sided tape and finally coated in a vacuum evaporator with 150 Å of gold.

The maximum statolith radius was measured with the aid of a 2 mm scale micrometer by determining the maximum distance outwards from the center of the nucleus (usually in a dorsal direction) to the edge of the statolith.

The back calculation procedure was adapted from Ricker and Lagler (1942) for back calculating fish lengths from a non-linear curve. The following formula was used to correct a ring count that did not correspond to the ring count for a squid of that mantle length:

$$\bar{R} = \frac{\bar{R}\ R_n}{R}$$

where $\bar{R}_n$ = adjusted ring count to an earlier sampling date

$\bar{R}$ = average ring count for an animal of the observed mantle length

R_n = estimated ring count at an earlier sampling date assuming one day

R = observed ring count on statolith

Results

Observation of rings

In this study rings were observed both under the light microscope and SEM. This confirms earlier observations by other authors (Brothers *et al*.

1976; Clarke, 1966; Lipinski, MS 1978; Young, 1960). Dilly (1976) could not find any evidence of rings in the statoliths that he examined.

Fig. 3 (a, b) shows SEM photographs of prepared statoliths. The darker band of each ring represents an area where the acid etching removed more $CaCO_3$ than in the lighter areas which may be made up of higher concentrations of organic material as in fish otoliths (Degens et al., 1969). The etching would indicate that the rings were 'true' rings and not merely thin parts of the statolith passing more transmitted light than the thicker parts, as suggested by Dilly (1976).

With transmitted light the rings were also evident as pairs of dark and light bands. Fig. 4 (a, b, c, d) shows photographs of ground statoliths from animals of 1.7-22.0 cm mantle length. In general the rings in the nuclear areas (<30 rings) tended to be narrower (1.0-2.5 μm) than the peripheral rings (2.0-5.0 μm). In many statoliths in nuclear area was defined by a relatively dark ring (Fig. 4d). The fine rings within the nucleus may have corresponded to the "juvenile statolith" zone of Lipinski (MS 1978). Beyond this zone, toward the periphery of the statolith, he counted relatively fewer rings than were observed in this study (Fig. 4a, b, c, d).

Ring counts were compared statistically between readers and between individual statoliths of a pair to investigate the precision of the results. In a sample of 16 statoliths read by two readers without a priori knowledge of the other reader's count it was determined that there was no significant difference between both readers' ring counts ($t = 0.14$, at the conventional 5% probability level) (Sokal and Rohlf, 1969, p. 331). Also there was no significant statistical difference in the ring counts between individual statoliths of a pair in a sample of 40 pairs of statoliths read by the same reader ($t = 0.91$, at the conventional 5% probability level) (Sokal and Rohlf, 1969, p. 331).

Relationship between mantle length and maximum statolith radius

The maximum statolith radius was measured for 82 squid. The results of these measurements were stratified into one-cm mantle length groups and the mean radius was calculated for each length group. The animals measured ranged in mantle length from 1.5 to 25.0 cm. A curvilinear fit to the data (Fig. 5) ($y = 48314.6\ x^{2.69}$, $r^2 = 0.96$, $n = 21$) proved to be statistically significant (F-test, $P \leq 0.05$). The growth of the maximum statolith radius

(Fig. 5) was allometric particularly over the smaller mantle lengths (< 10.0 cm). At larger mantle lengths (> 10.0 cm) the statolith growth became more isometric. This is similar to the bony structure radius-body length relationship in many fish species (Casselman, 1974; Fagade, 1974; Ricker and Lagler, 1942; Taubert and Coble, 1977). Dilly (1976) stated that any direct correlation between statolith and body size was abscure, but he noticed that statoliths from large animals were generally larger than those from smaller animals.

Relationship between mantle length and ring count

The relationship between mantle length and ring count was determined and curves were fitted separately for statoliths taken from 1) males ($y = 2.5 \times 10^{-5} x^{2.68}$, $r^2 = 0.91$, $n = 64$, 2) females ($y = 4.5 \times 10^{-4} x^{2.12}$, $r^2 = 0.89$, $n = 65$), 3) both sexes combined ($y = 3.07 \times 10^{-4} x^{2.19}$, $r^2 = 0.87$, $n = 29$). Ring count was chosen as the independent variable since the curve in (Fig. 6) for both sexes combined was used later to back calculate mantle lengths. A curvilinear fit to the data also best described the probable relationship over the smaller size ranges not analyzed in this study (<2.5 cm for females; <2.7 cm for males).

Back calculation

Fig. 7 shows a plot of percent frequency of mantle lengths by sex for each of the samples collected in 1978 and gives the modal mantle length range for animals in each sample from which statoliths were examined. These length-frequency samples showed an increasing range of mantle length modes for the months June through September (males) and June through October (females). However, from September to November there was an apparent slowing down in the population's rate of growth an indicated by the mantle length modes.

The monthly mantle length modes identified in Fig. 7 could be compared with back calculated mantle lengths, similar to the cohort analysis using beak growth rings by Clarke (1965), if it was assumed that in the 1978 fishing season there was only one brood of squid that were spawned within a relatively short period of time. Squires (1957) concluded that this was the case because length distributions showed increasing montone growth throughout the season (i.e. with age).

Both maximum statolith radius ($r^2 = 0.96$) and ring count ($r^2 = 0.87$, both sexes) predicted mantle lengths equally well since an analysis of covariance on the logarithmic transformation of the two relationships showed no difference between the slopes ($F_{1, 139} = 2.08$, not significant at the conventional 5% probability level). For purposes of back calculation in this study the ring count-mantle length relationship (Fig. 6, both sexes) was employed adapting the method of Ricker and Lagler (1942) (see Materials and Methods) for back calculating fish lengths from a non-linear curve. The back calculation procedure was carried out assuming that one ring represented a time interval of one day.

In Fig. 8 the mean mantle lengths, back calculated from all successive samples, had much lower values (Δ10.6 - Δ11.2 cm) that the expected values (found on the equisector, Fig. 8) had. Since tagging results from inshore Newfoundland have shown that individual Illex can remain in the same location for at least one month even in late season, it was thought useful to derive back calculated mean mantle lengths only from statoliths examined from the immediately successive sampling date and compare those values with the expected values. These back calculated mean mantle lengths were also lower (Δ1.4 - Δ10.2 cm) and their confidence limits did not overlap the expected mean mantle lengths but were much closer to the expected values than were the mean mantle lengths back calculated from all successive sample data.

Discussion

Possible chronological interpretation of rings

There was little evidence from back calculation in this study that the rings could be interpreted as chronological marks. The correlation of back calculated mean mantle lengths with expected mantle length modes improved when an attempt was made to eliminate the possible biasing of late season migration (Squires, 1957). This apparent lack of agreement may have been due to the presence of mixed age-groups within a single year-class spawned over a protracted season hypothesized by Mercer (MS 1975) who observed bimodal and trimodal length distributions in some years. The presence of mixed age-groups would have made it impossible to take statolith samples from a single monthly mantle length mode and would have complicated the validation of ageing by back calculation.

There has been very little published material on growth rates of squid from known age specimens. However the inferred growth rate of Illex illecebrosus (Fig. 8) is quite similar to the measured growth rate of laboratory-raised Loligo opalescens if a growth ring is assumed to represent a time interval of one day, (Won Tack Yang, Marine Biomedical Inst., Texas, pers. comm). Although this is a comparison of growth rates between two different species of squid the similarity of the curves suggests that a chronological interpretation of the rings cannot be discounted.

It is possible that the rings observed in the statoliths were simply growth rings and not chronological marks. Dilly (1976) felt that any diurnal rhythm is probably too rapid to affect stataconial crystal growth. Growth rings in freshwater fishes were laid down on a daily basis according to a 24-hour, light-dark cycle which entrained an internal, diurnal clock assuming that a supply of food was available, (Taubert and Coble, 1977; Ottaway, 1978). The chronological interpretation of rings may be more difficult for a marine animal that is an opportunistic feeder such as Illex illecebrosus (Ennis and Collins, 1979) which may spend several days without feeding. During periods of starvation, Bilton (1974) showed that rings did not form in fish scales. The percentage of empty stomachs and high rate of cannibalism (Squires, 1957; Mercer, MS 1965) among squid sampled in inshore Newfoundland waters may indicate temporary scarcity of food supply and probable sporadic feeding. If food availability is a limiting factor in the formation of daily rings then perhaps statoliths should be examined from Illex illecebrosus found in Northwest Atlantic offshore waters where they have been shown to feed on a 24-hour cycle (Amaratunga et al., MS 1979).

Summary

Rings found in statoliths from Illex illecebrosus were found to be more numerous than previously reported. The rings were shown to be 'true' rings and not merely optical aberrations.

The number of rings increased with mantle length but did not represent chronological marks based on cohort analysis for samples from inshore Newfoundland. However, age validation may have been complicated by the presence of mixed age-groups.

Other age validation techniques should be tried in the future such as examining statoliths from squid of known age or putting a 'time' mark on statoliths using an antibiotic such as tetracyline (Weber and Ridgeway, 1967).

Acknowledgements

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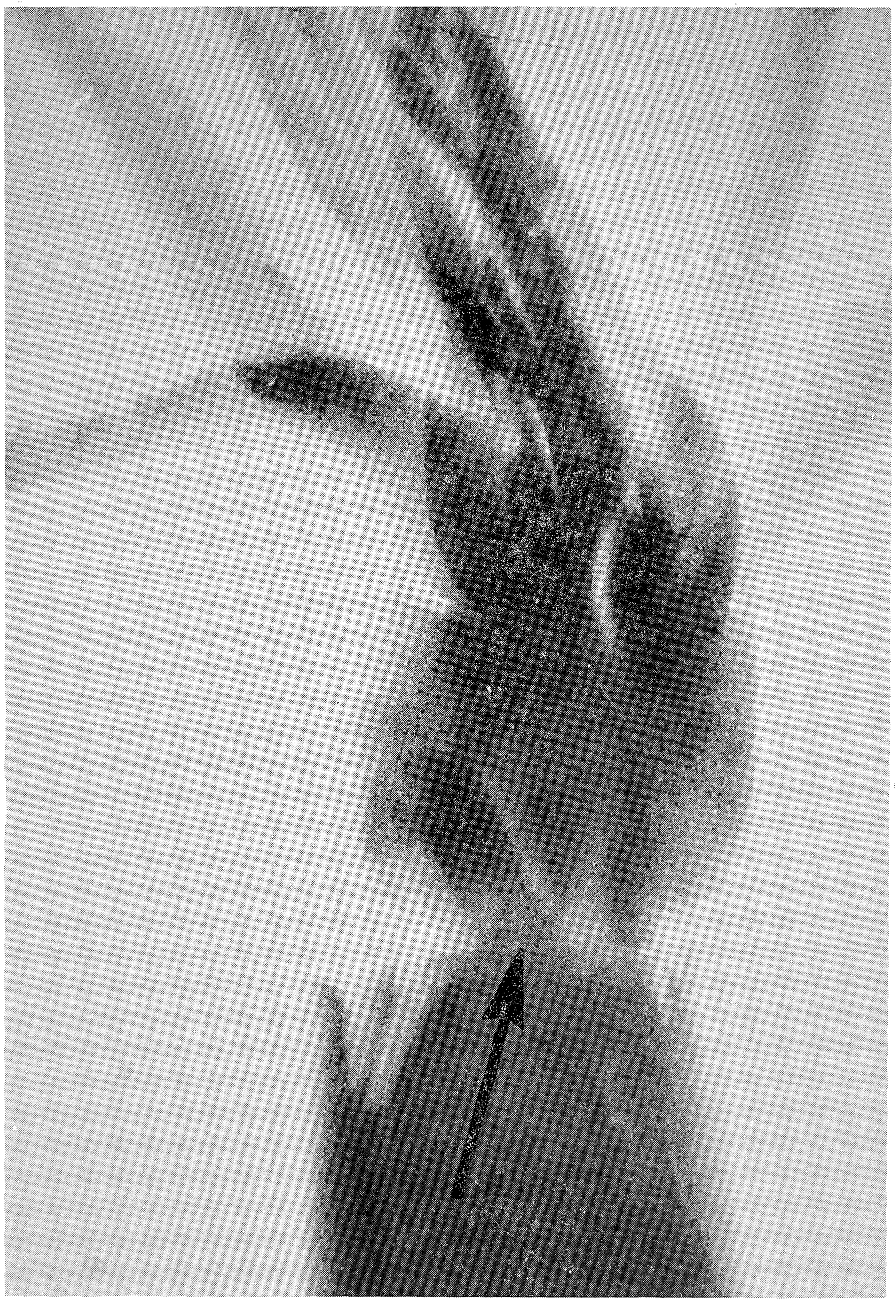

Fig. 1. X-ray of head region of *Illex illecebrosus*. Arrow points to area of head where statoliths are found.

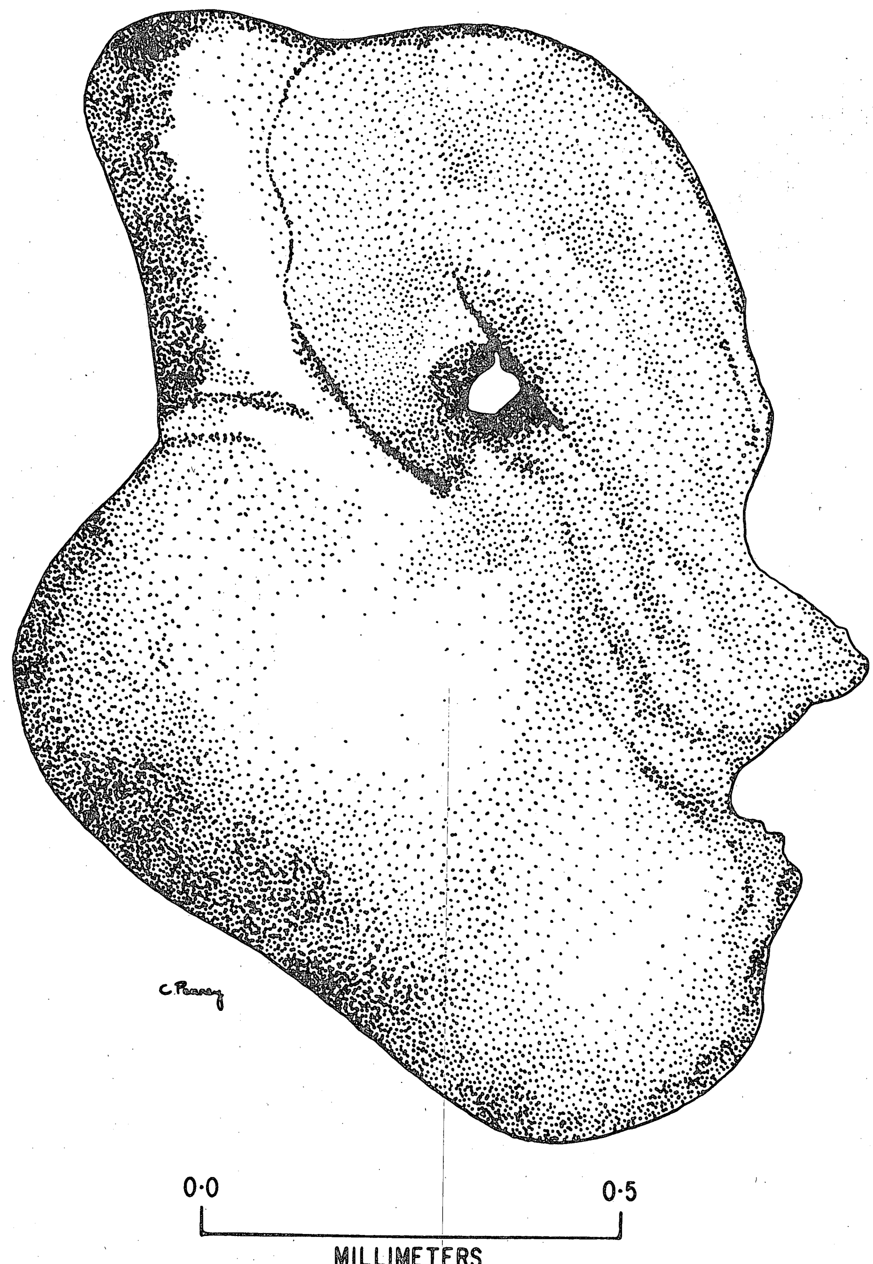

Fig. 2. Illustration of a statolith from *Illex illecebrosus* showing grinding surface (antero-lateral view). Top of photograph is the ventral side of the statolith.

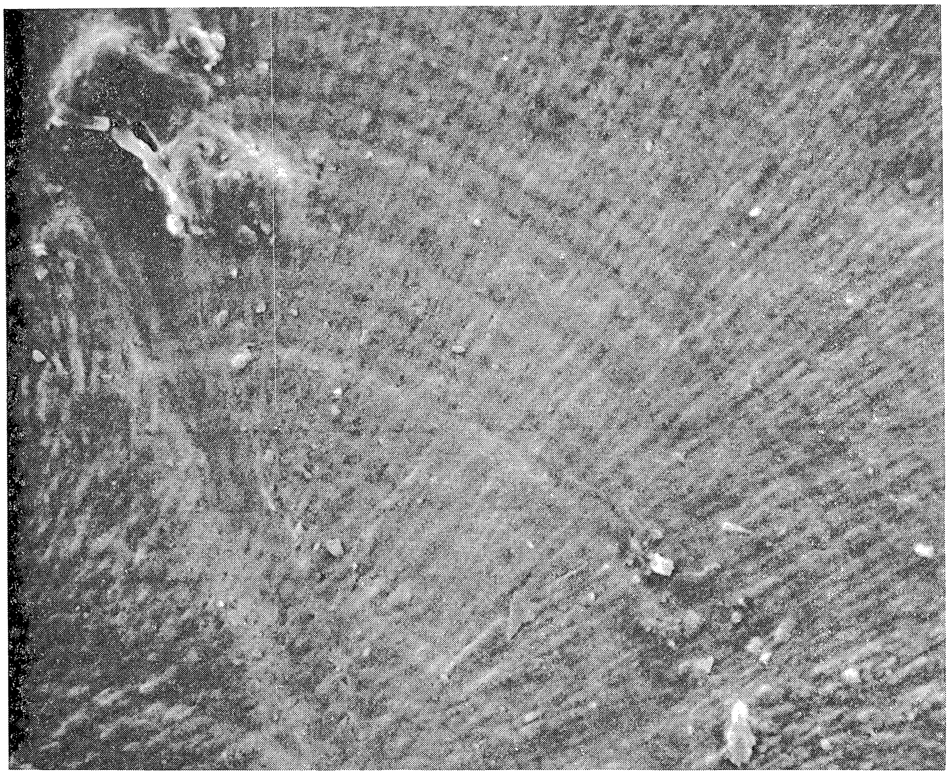

Fig. 3a. Scanning E.M. photograph (1500X) of the ground and etched surfaces of a statolith showing numerous rings.

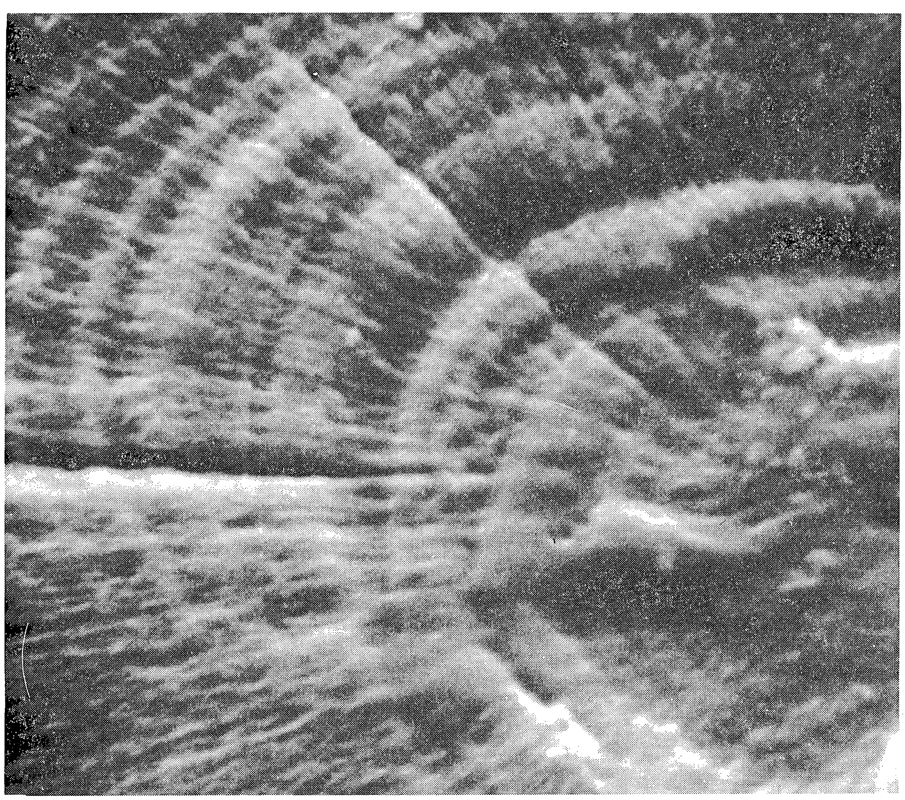

Fig. 3b. Scanning E.M. photograph (7300X) of etched rings surrounding the nucleus. The nucleus itself may consist of mucopolysaccaride and mucoprotein (Lim, 1973).

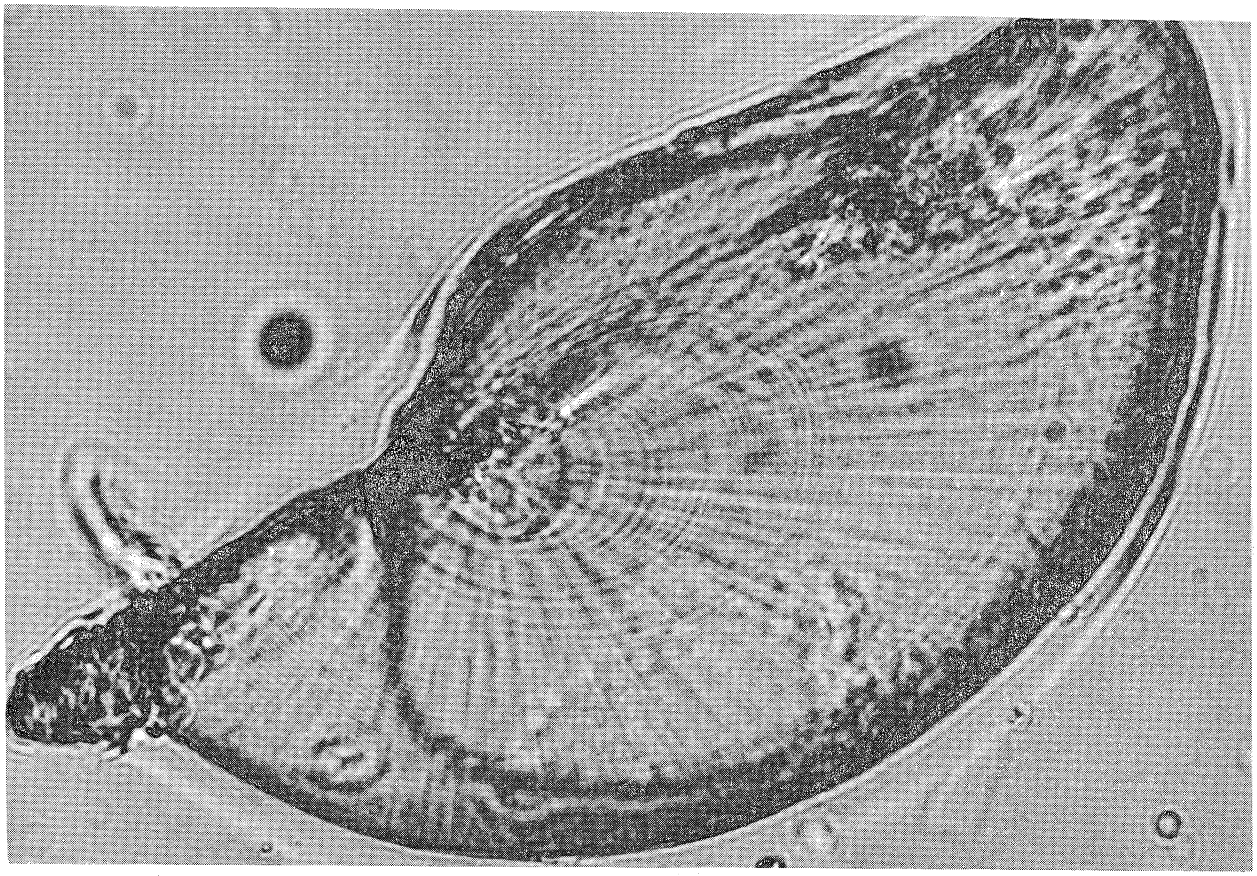

Fig. 4a. Photographs of ground statoliths (using transmitted light) from squid of various sizes. Mantle length = 1.7 cm juvenile; magnification = 525X.

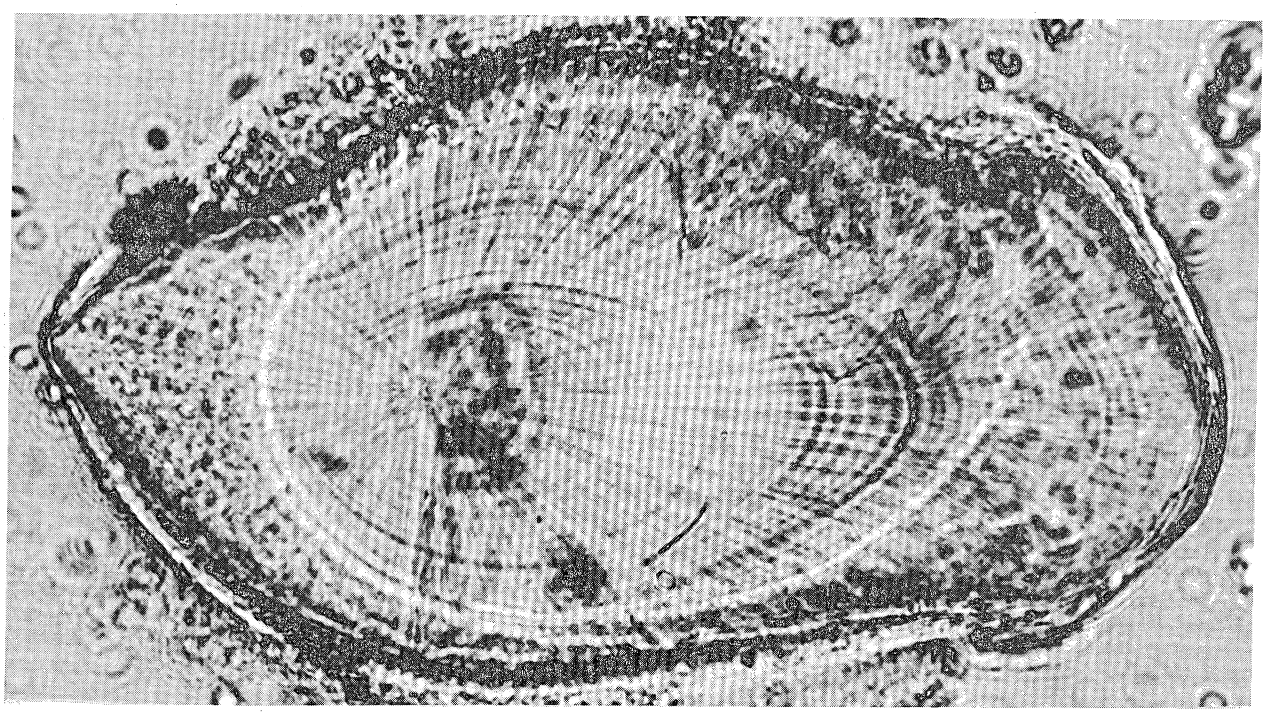

Fig. 4b. Photographs of ground statoliths (using transmitted light) from squid of various sizes. Mantle length = 4.2 cm female; magnification = 350X.

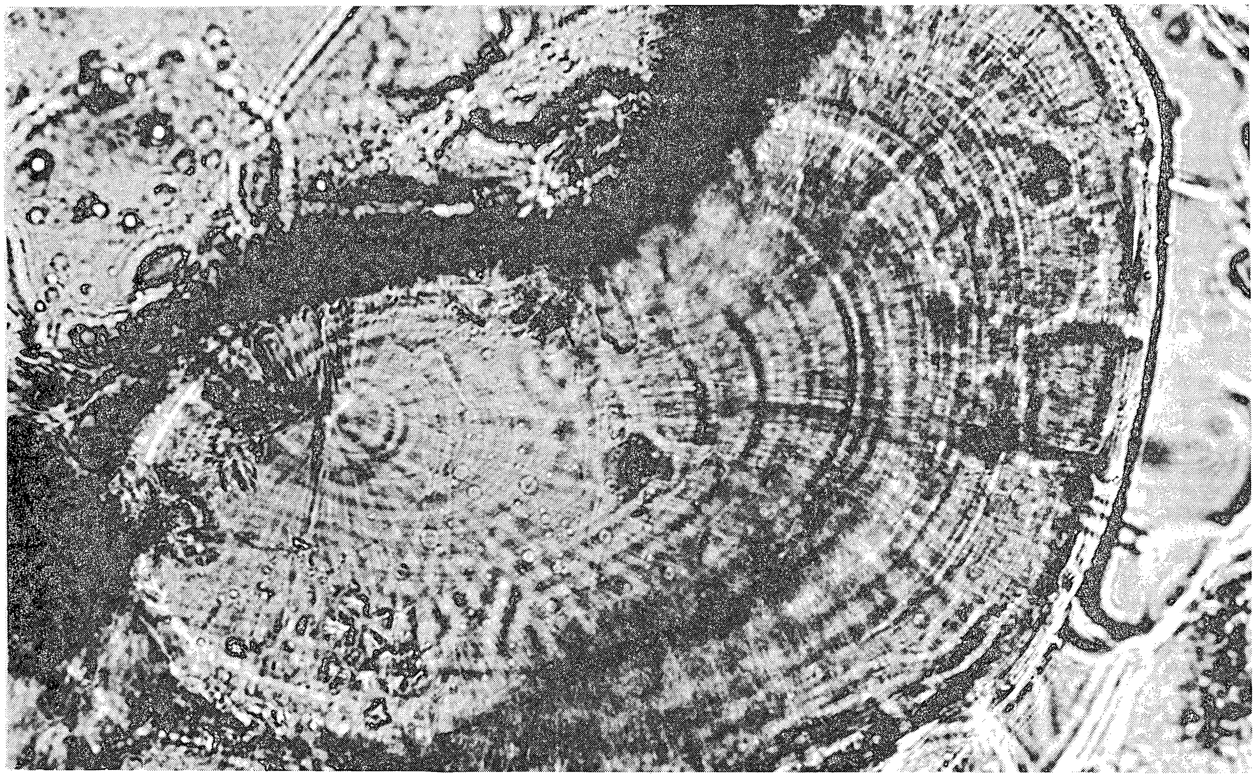

Fig. 4c. Photographs of ground statoliths (using transmitted light) from squid of various sizes. Mantle length = 11.2 cm female; magnification = 275X.

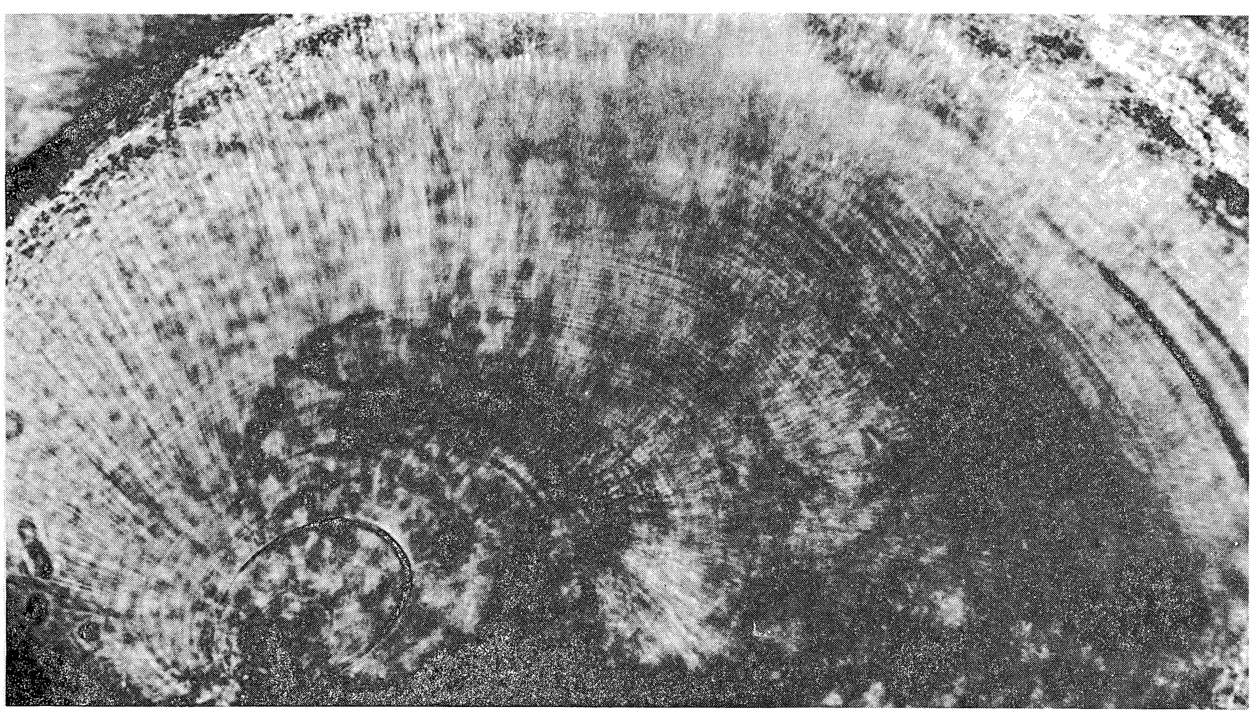

Fig. 4d. Photographs of ground statoliths (using transmitted light) from squid of various sizes. Mantle length = 22.0 cm female; magnification = 250X.

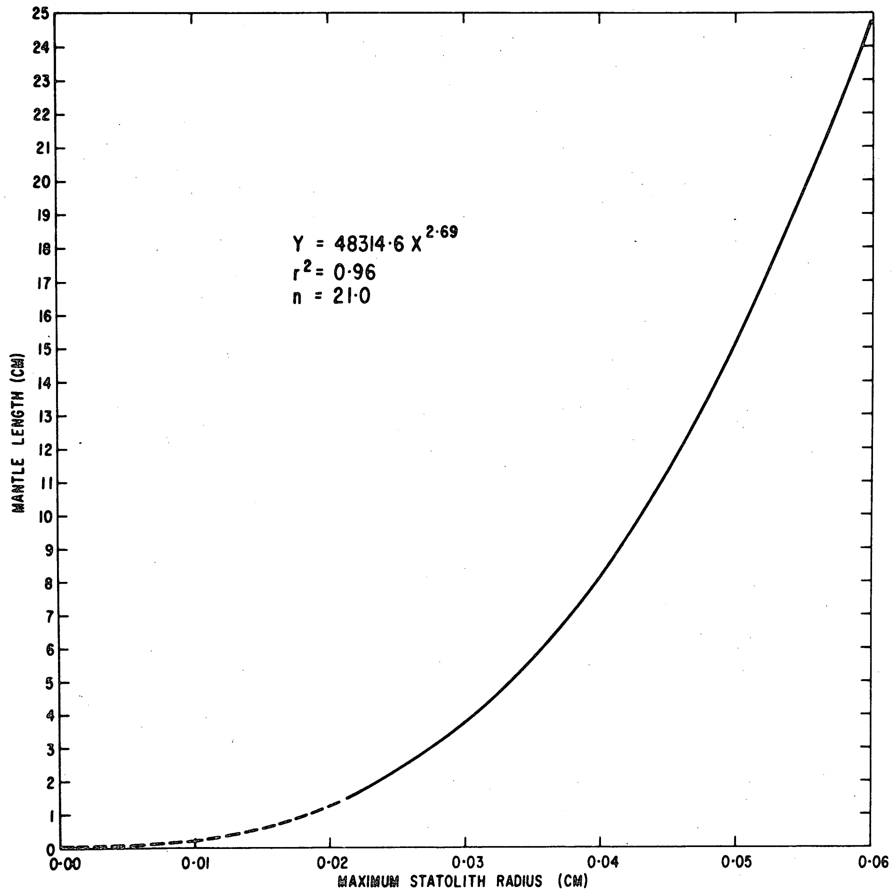

Fig. 5. Maximum statolith radius (X) - mantle length (Y) relationship. ($Y = 48314.6 X^{1.69}$. Solid line represents the range of observed values. Dashed line represents extrapolated value from the fitted curve.

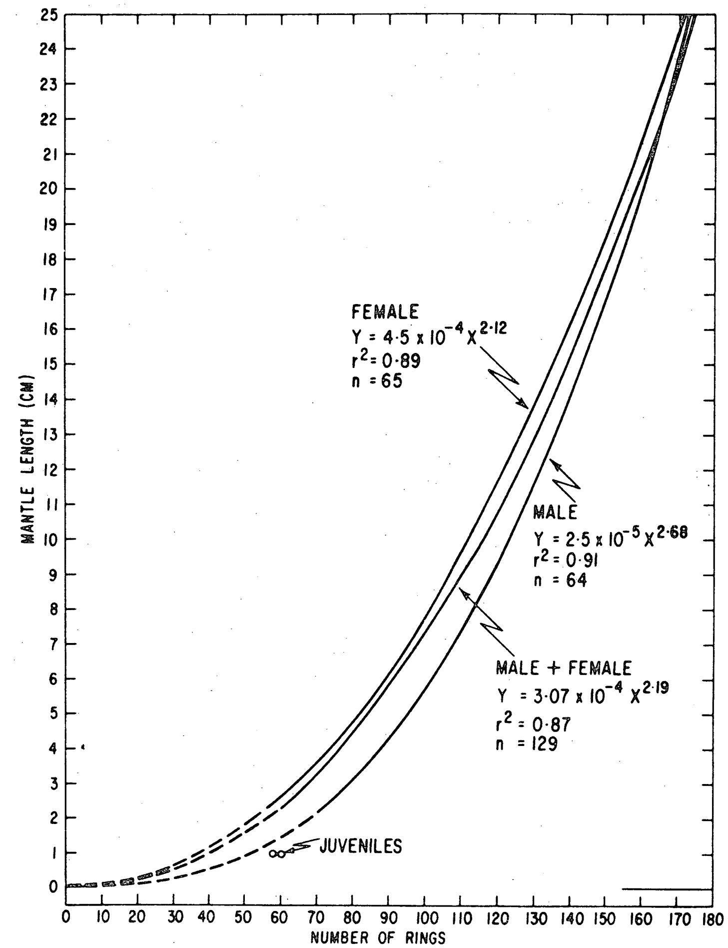

Fig. 6. Ring count (X) - mantle length (Y) relationship. Curves were fitted to values for males ($Y = 0.000025 X^{2.68}$) for females ($Y = 0.00045 X^{2.12}$), and for both sexes combined ($Y = 0.000307 X^{2.19}$) from 1978 and 1979 data. Solid line represents the range of observed values. Dashed line represents extrapolated values from the fitted curve.

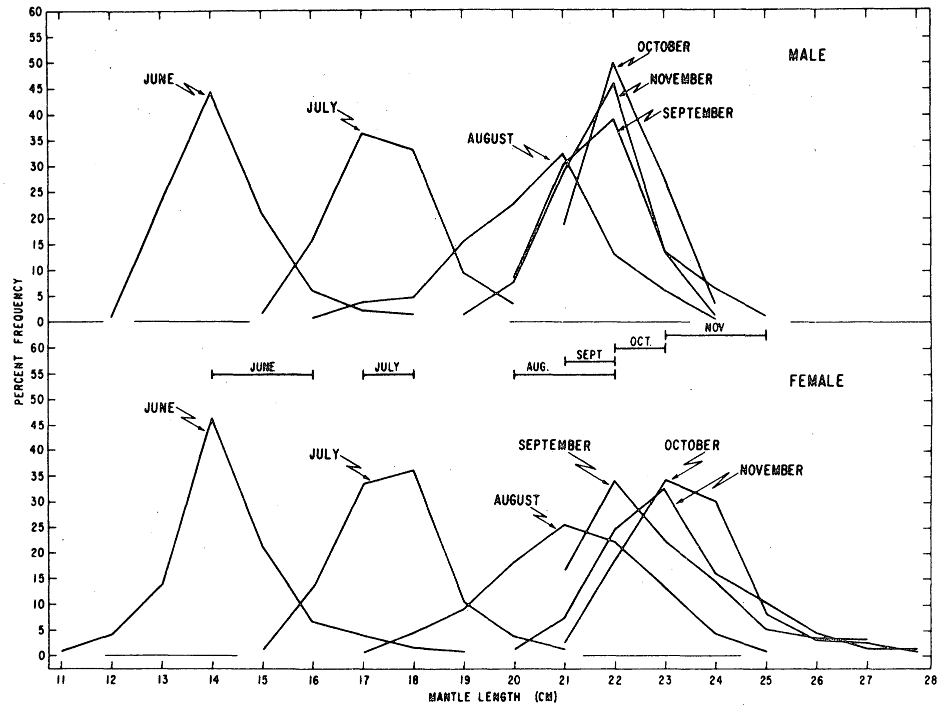

Fig. 7. Size distributions from inshore samples taken in 1978 from June to November. Horizontal solid lines show length intervals for each sample from which statoliths were read.

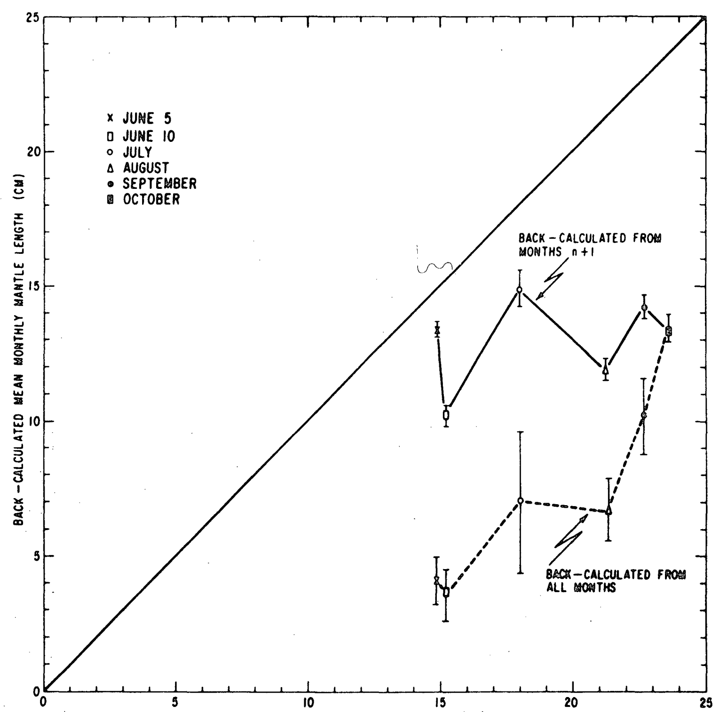

Fig. 8. Monthly mantle length mode from size distributions — monthly back calculated mean mantle length relationship from 1978 samples. Solid line represents mean mantle length back calculated from the immediately successive sample. Dashed line represents mean mantle length back calculated from all successive samples. Equisector represents expected modal mantle length values.