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IMAGE ANALYSIS OF WALLEYE (STIZOSTEDION VITREUM VITREUM) OPERCULA FOR AGE AND GROWTH STUDIES

by

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ABSTRACT

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An image analysis system (IAS) was used to determine effects of within-structure variation in opercula on estimates of age and growth of walleye. Three axes on opercula were considered for ageing and growth measurement. Frequency of successful annulus interpretation in unclear areas near the foci of opercula was increased by as much as 29% with automated and semi-automated detection methods provided by the IAS. ANOVA confirmed thatall three axes provided intersections perpendicular with annuli and were valid for back calculation of length. However, annuli interpretations were incomplete in two of these andwould result in over-estimated back calculated mean lengths with larger variance.

Key words: Image analysis; walleye; opercula; age and growth; back calculation.

RÉSUMÉ

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On a utilisé un système d'analyse d'images (SAI) pour évaluer les effets, sur les estimations de l'âge et la taille des dorés jaunes, de la variation à l'intérieur des structures de l'opercule. Afin de déterminer l'âge et la taille des poissons, on a examiné trois axes sur l'opercule. Le taux de succès relatif à l'interprétation des anneaux dans les zonesfloues situées à proximité des foyers des opercules a augmenté dans une proportion atteignant jusqu'à 29 p. 100 grâce aux méthodes de détection automatisées ou semi- automatisées fournies par le SAI. L'analyse de la variance a confirmé que les trois axes étudiés fournissaient des intersections perpendiculaires aux anneaux et se prêtaient à un rétrocalcul valide de lataille. Cependant, les interprétations des anneaux se sont avérées incomplètes dans deux cas et, par conséquent aboutiraient à une surestimation des longueurs moyennes et à une variance élevée.

Mots-clés: Analyse d'images; erreur de mesurage; doré jaune; opercule; âge et croissance; rétrocalcul.

INTRODUCTION

Opercula have been used for age and growth studies of freshwater fish for more than 45 years. LeCren (1947) and Bardach (1955) used opercula to estimate growth of Eurasian yellow perch (Perca fluviatilis) and North American yellow perch (Perca flavescens) respectively. Campbell and Babaluk (1979) showed that opercula of the related walleye provided age determinations that were comparable to ages determined from otoliths, and were preferable to scales, pelvic, and pectoral fin rays. They recommended the use of opercula because they exhibit the clearest patterns of growth, and are easy to remove and process. Babaluk and Campbell (1987) validated opercula as ageing structures.

A standardized method for collection of data for back calculation of walleye length from opercula is lacking. LeCren (1947), Bardach (1955), and Babaluk et al. (1993) showed from which axis on the operculum they collected data, but they differed in their choices and none of them presented data to support their choice. Presumably, their choice of axis location would have provided intersections perpendicular to annuli, and also where the interpretation of annuli was consistently easiest, especially for early annuli which are not always clear. Because the pattern of growth of walleye opercula is not radial, like it is in spines or vertebra, and also that frequency of annuli interpretation may vary by axis location, the effect that choice of axis has on back calculated fish lengths is unknown. Without this information the selection of an axis to make measurements for back calculation of length is somewhat arbitrary.

Image analysis systems (IAS) have proven to be a valuable asset in fishery investigations of age and growth. Some systems are simple and inexpensive to purchase (Campana 1987), while others can be programmed for detailed and automated analysis of stock composition (Cook and Guthrie 1987), and are ideally suited for scale pattern recognition and discriminant statistical analyses (Cook and Lord 1978). Cooley and Franzin (1995) examined the accuracy and precision of measurement for an IAS, and determined how much error in back calculated fork length was due to the configuration of the system. IAS's are particularly advantageous for interpretation of annuli. Data can be extracted from the image and viewed with, e.g. xy plots. Graphical interfaces simplify the interpretation of growth because the radiometric sensitivity of the system exceeds that of the interpreter, especially in areas where annuli are faint or crowded. Annuli recognition using automated or semi-automated methods of an IAS probably increases the precision of annuli interpretation, and also decreases processing time.

The purpose of this paper is to present a method for collecting data from opercula for estimation of age and growth of walleye. We present data that facilitate choice of axis location, and implement and evaluate the use of the IAS for automated and semi-automated ageing and measurement. Because the populations from which the opercula were collected had extremes in age and rates of growth, these opercula also provided an opportunity to describe the annual patterns of optical density for a group of Precambrian Shield populations from lakes over a range in size.

METHODS

The opercula used in this investigation were collected from walleye populations from six lakes in northwest Ontario (Northwest Ontario Lake Size Series, Fee and Hecky 1992), over a six year period (Fig. 1a). All age determinations and measurements presented in this paper were made by the same experienced reader. Walleye ages were determined from opercula following the criteria of LeCren (1947). Annuli were considered formed according to the validated method of opercular ageing by Campbell and Babaluk (1979).

AGE AND GROWTH DETERMINATION USING IMAGE ANALYSIS

Computer-assisted measurements were obtained according to the methods described by Cooley and Franzin (1995).

To determine if the location of measurement on opercula affected estimates of age and growth, three linear measurements, here termed axes, were made on a static image of the medial surface of each left operculum (Fig. 1b). Axis 1 was closest to the ventral spinous process, axis 2 bisected the structure into approximately equal dorsal and ventral halves, and axis 3 was closest to the dorsal spinous process. Each axis of measurement began at the focus of the structure (LeCren 1947). All measurements at each annulus were determined manually, unless otherwise specified, because the Optical Pattern Recognition System (OPRS software; version 1.10, Biosonics Inc.), in standard configuration, does not recognize annuli according to the criteria of Babaluk et al. (1979) but detects and marks maximum or minimum luminance values only. The system would require further programming to mark annuli on the luminance values that represent time of annulus formation.

Because the interpretation of early annuli can be difficult, the axis which consistently showed the first three annuli most clearly was determined by close inspection of the opercular image. It was noted whether or not one or more annuli were visible on one or both of the other two axes. The OPRS placed marks on annuli along each axis based on maximal luminance; if the system determined an annulus in a position where no annulus was visible by eye, but the distance from the focus to that mark corresponded closely to the distance from focus to the same and clearly visible annulus in a different axis, the computer determined annulus was retained. If the OPRS did not indicate such an annulus, a luminance graph often identified an unclear annulus that then was marked manually. Finally, annuli that were unclear but were not indicated either by the OPRS or observed on the luminance graph were noted.

The operculum length/fork length relationship used for back calculating fish length was determined using linear regressions of transformed (log e) and untransformed data for each of the six populations (n = 1136). The transformed data increased the fit of the model in only two of the populations and were not used.

VARIATION IN ESTIMATES OF AGE AND GROWTH

We could not employ methods that would allow us to validate the position of any annuli on any of the three axes because our samples were from remote populations. Instead, we determined which of the three axes provided estimated lengths that corresponded most closely to lengths of age 1 walleye from similar populations and latitudes, e.g. Carlander (1943), Erickson (1983), and Babaluk et al. (1993) were consulted. Mean lengths at age for a sub-set of each of the triplicate data sets (i.e. one set from each axis; fish captured at time of annulus formation n = 356) were estimated by back calculation after Ricker (1976). The relationships of fork length to operculum length for these populations were linear (r^2 range; 0.96-0.98, unpubl. data). Slopes from linear regressions of fork length and operculum length were used to correct for allometry by the following equation:

$$FL = FL_c + b\,(O_x - O_c);$$

FL = fork length

FL_c = fork length at capture

O_x = operculum length at age x

O_c = operculum length at capture

INTERSECTION OF MEASUREMENT AXIS TO ANNULI

Perpendicular intersection of each axis to annuli was investigated using ANOVA to compare the distance to each annulus as a fraction of the total distance among the axes. In doing this we assume that axis 2 (the axis most likely to be perpendicular) is at right angles to annuli and that those annuli in other axes with statistically similar distances to that of axis 2 are also perpendicular. However, to avoid the influence of missing data on results, it was first necessary to adjust the data from axes which had fewer annuli interpreted to a number equal to the axis with the maximum number of annuli. This was done by offsetting all of the growth data for that axis by a number of columns in the database which equalled the difference in age between that axis and the axis of maximum age.

RESULTS AND DISCUSSION

AGE AND GROWTH DETERMINATION USING IMAGE ANALYSIS

Axis 1 was best for consistent identification of annuli on opercula, supporting the choice of Babaluk et al. (1993). Clearly visible annuli were more difficult to determine along axes 2 and 3 because the areas nearest the focus were very thin and translucent whereas the dorsal spinous process often showed dense and irregular calcification associated with structure thickness. The very low and/or high luminance values that resulted obscured the annual patterns of growth, but never beyond annulus 3. The optical density exhibited during annual growth, the approximate location of annulus formation, and the area near the origin where interpretation of annuli was difficult are shown in Fig. 2a-c.

Frequency of annuli interpretation above age 3 was similar along all three axes. However, interpretation of annuli on axes 1 and 3 was easier because these annuli showed greater optical density near the spinous processes where the structure is thicker than the thin area of axis 2. We also learned that most annuli interpreted according to the method of Campbell and Babaluk (1979) were continuous across all three axes and could also be followed through the spinous processes. Axis 2 was shortest and showed the smallest increments of growth making annuli interpretation more difficult in old fish. Because the operculum is thinnest at the outer edge on this axis a slight curvature often developed during drying of the structure which reflected light directly towards the video camera and obscured luminance patterns.

The OPRS assisted recognition of annuli in areas where they were not clear by as much as 29% (Table 1). For our opercula, 24% of the unclear annuli were identified by the IAS where manual interpretation could not. The graphical xy plot provided another valuable method for interpretation by providing the single largest increase in frequency of annuli marked over manual methods. The remaining unclear annuli (53%), not detected manually, by the IAS, or with the graphical interface, but were visible in one or both of the other two axes, were mostly from axis 3. We have not attempted to compare statistically the intervals determined with the 3 different methods of annuli interpretation. The annuli indicated by the OPRS would overestimate annual growth slightly because the IAS was set to recognize and mark pixels with maximal luminance values, which occurs sometime after annulus formation.

Back calculated mean fork lengths from the three axes of our fish at annulus 1, corresponded reasonably closely to published values for mean lengths of age 1 walleye from similar lakes at a similar latitude. However, the unclear annuli (1-3) from axes 2 and 3 increased the means and variances of lengths slightly. Unfortunately, we could not validate the location of annuli in any of the three axes. Because of the consistency of first annulus interpretation in axis 1, and the similarity of computed walleye lengths to those of other studies, ages and growth intervals from axes 2 and 3 were adjusted relative to axis 1 for tests of perpendicularity of the three axes to annuli without the influence of missing data. Walleye lengths estimated from axis 2 and 3, which contained the adjusted data, were graphically identical to lengths from axis 1.

Growth of the opercula is not radial but ANOVA indicated no significant differences between the distances to each annulus along the three measured axes (P>F range 0.12-0.91, n = 1136). This confirmed that all axes used provided intersections with the annuli that were approximately perpendicular, and are valid for use in estimation of back calculated lengths.

PATTERNS OF ANNUAL OPTICAL DENSITY IN PRECAMBRIAN SHIELD POPULATIONS

Although the shape of the luminance curve varied with age and also among opercula, the optical density along the axes showed that three types of annual patterns could be distinguished. Typically, the early and wide and the late and narrow growth rings on the image showed a smooth transition from a highly reflective area near the annulus to a transparent area which preceded the following annulus. The luminance curves which corresponded to such annuli appeared symmetric with a single peak (Fig. 2a; annuli 8-14). A rapid increase in luminance occurred during spring, to a maximum probably during early summer, then decreased to a value similar to the annual minimum of the preceding annulus.

The other two types of annuli appeared to contain bands of different optical density (Fig. 3a, b), and usually showed three peaks on the xy plot (Fig. 3c). Beyond the annual peak, the shape of the rest of an annual curve which contained the 3 peaks was usually descending. This type of annual luminance curve corresponded to areas on the image that appeared to have two translucent bands separated by a very faint and narrow transparent band (3b; annulus 6). Each of these two bands had a relatively homogeneous optical density, but the first was brighter than the second. On the luminance plot, the last peak marked the point where the first band stopped and the second more transparent band started.

The third type of annulus observed was similar to the second. However, this type of two-banded annulus showed a more prominent transparent ring that separated each of the bands (Fig. 3c; annulus 7). This transparent ring showed a decrease in luminance at what would be mid-summer in neighbouring annuli, and also was followed by a greater second peak in luminance before a decline to an annual minimum. Each of these bands was about half the width of nearby normally-shaped annuli in the same individuals and other members of the same cohort in a population. This form of banding usually was discontinuous between the dorsal and ventral spinous processes. Although the transparent ring appeared visually similar to the neighbouring transparent bands which separated clearly recognizable annual growth episodes, the xy plot showed the lowest luminance value at the mid-summer drop was markedly higher than that of the annual minimum of either of the two neighboring annuli. Clay and Clay (1991) also found irregularly spaced and incomplete 'checks' in white hake (Urophycis tenuis) otoliths, and interpreted them, as we have, as false annuli.

CONCLUSIONS

Opercula are ideal for ageing and image analysis of growth episodes because relatively large annual increments are formed which ease interpretation of luminance patterns. Axis 1 consistently provided the clearest images of annuli. Interpretation of annuli along axes 2 and 3 was more difficult, especially for the first annulus. Our data suggest that although all selected axes were approximately perpendicular to annuli, measurements along axes 2 and 3 would lead to over-estimates of back calculated mean fork length at age with greater variance than would measurements along axis 1.

Image analysis systems coupled with graphical interfaces and automated densitometric scanning methods simplify the interpretation of annuli based on patterns of optical density. This can increase the number of annuli detectable, assist identification of false annuli, and once IAS's can export the graphical luminance data, image analysis may provide a means of post-mortem modelling of intra-annual growth.

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Table 1. The number of annuli detected by method of IAS interpretation in unclear areas of Opercula. The columns indicate:1) the number of annuli not clearly visible in one axis, 2) the number of annuli located entirely by the system, 3) located manually with an x-y plot, and 4) the number of annuli which were visible in one of the two other axes, but were not detected by manual, system, or graphical methods of interpretation. Bracketed values are percent of annuli not clearly visible. (n = 1136)

	Number of Annuli not Clearly Visible	Number of Annuli Located by IAS	Number of Annuli Located with XY Plot	Number of Annuli not Marked
Measurement axis 1				
Annulus 1	14	4	3	7
Annulus 2	0	0	0	0
Annulus 3	0	0	0	0
Measurement axis 2				
Annulus 1	139	25	57	57
Annulus 2	24	6	8	10
Annulus 3	0	0	0	0
Measurement axis 3				
Annulus 1	385	102	86	197
Annulus 2	90	19	30	41
Annulus 3	16	1	7	8
Totals	668	157 (24)	191 (29)	320 (48)

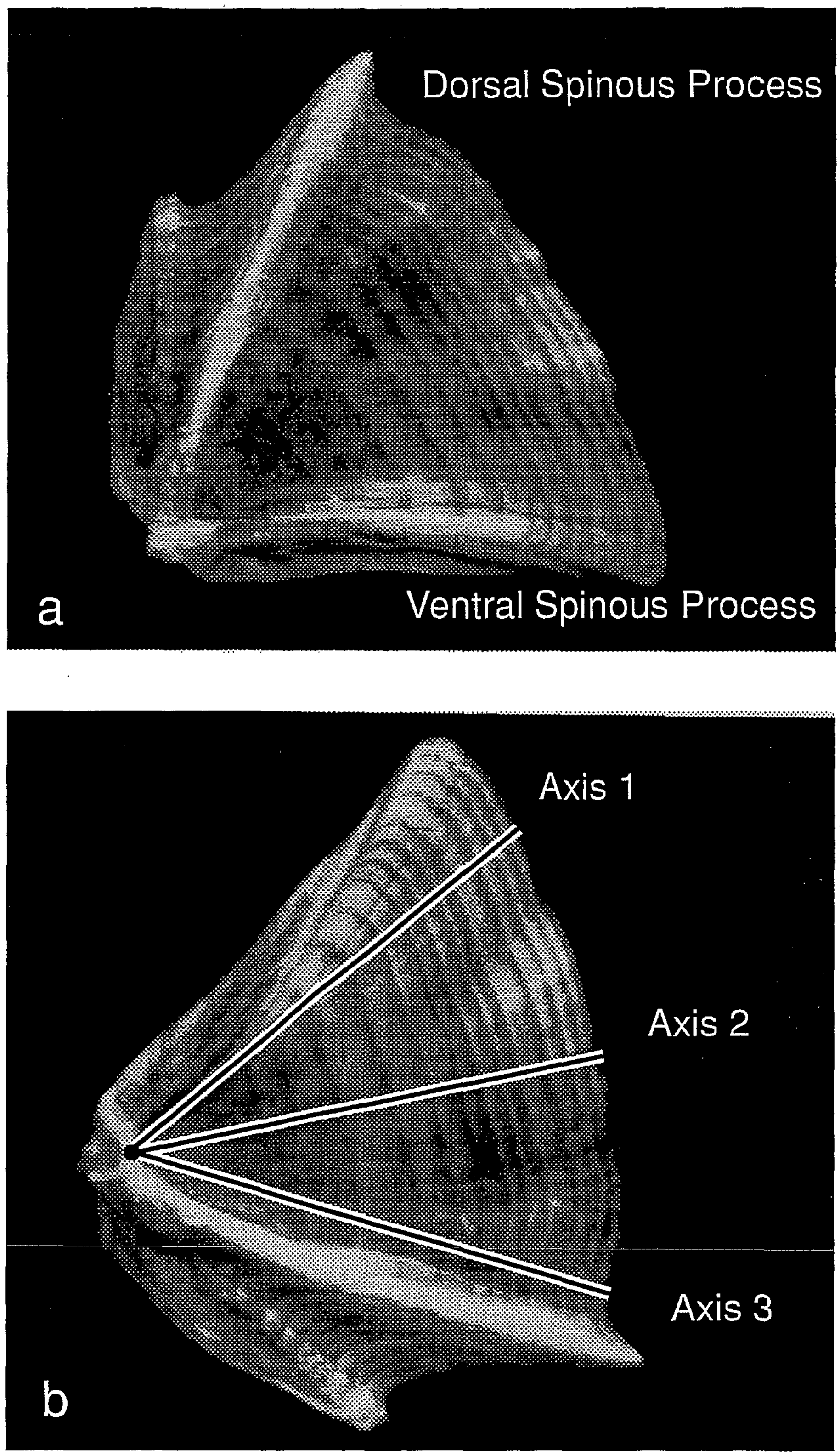

Figure 1. a) lateral surface of left walleye operculum, b) medial surface of left walleye operculum showing the origin and orientation of the three axes used for ageing and measurement.

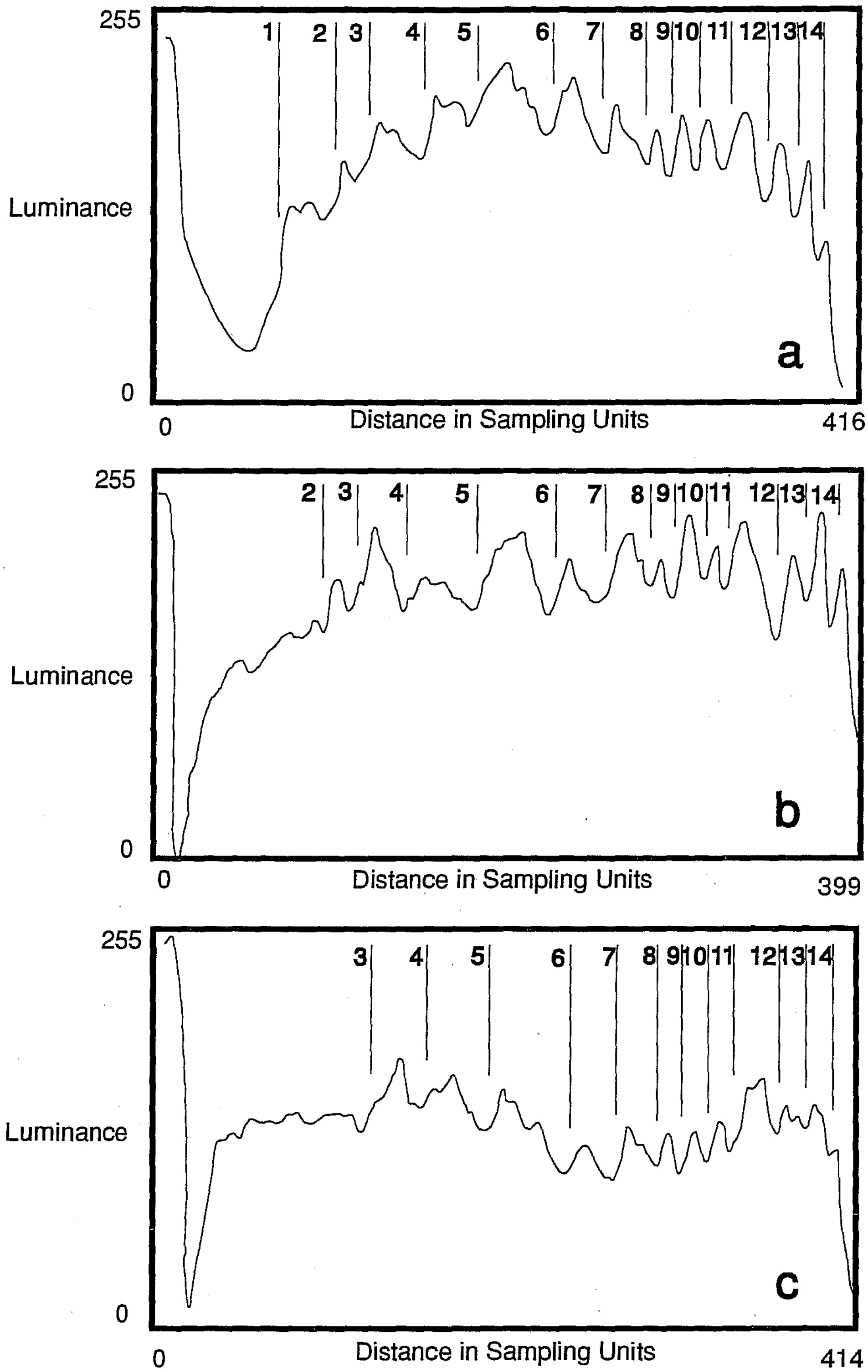

Figure 2. Luminance plots from the axes of figure 1b showing complete interpretation of annuli near the focus in axis 1 (a), whereas axes 2 and 3 (b,c respectively) produced incomplete interpretations in the area near the focus and dorsal spinous process. Vertical bars indicate location and number of annuli.

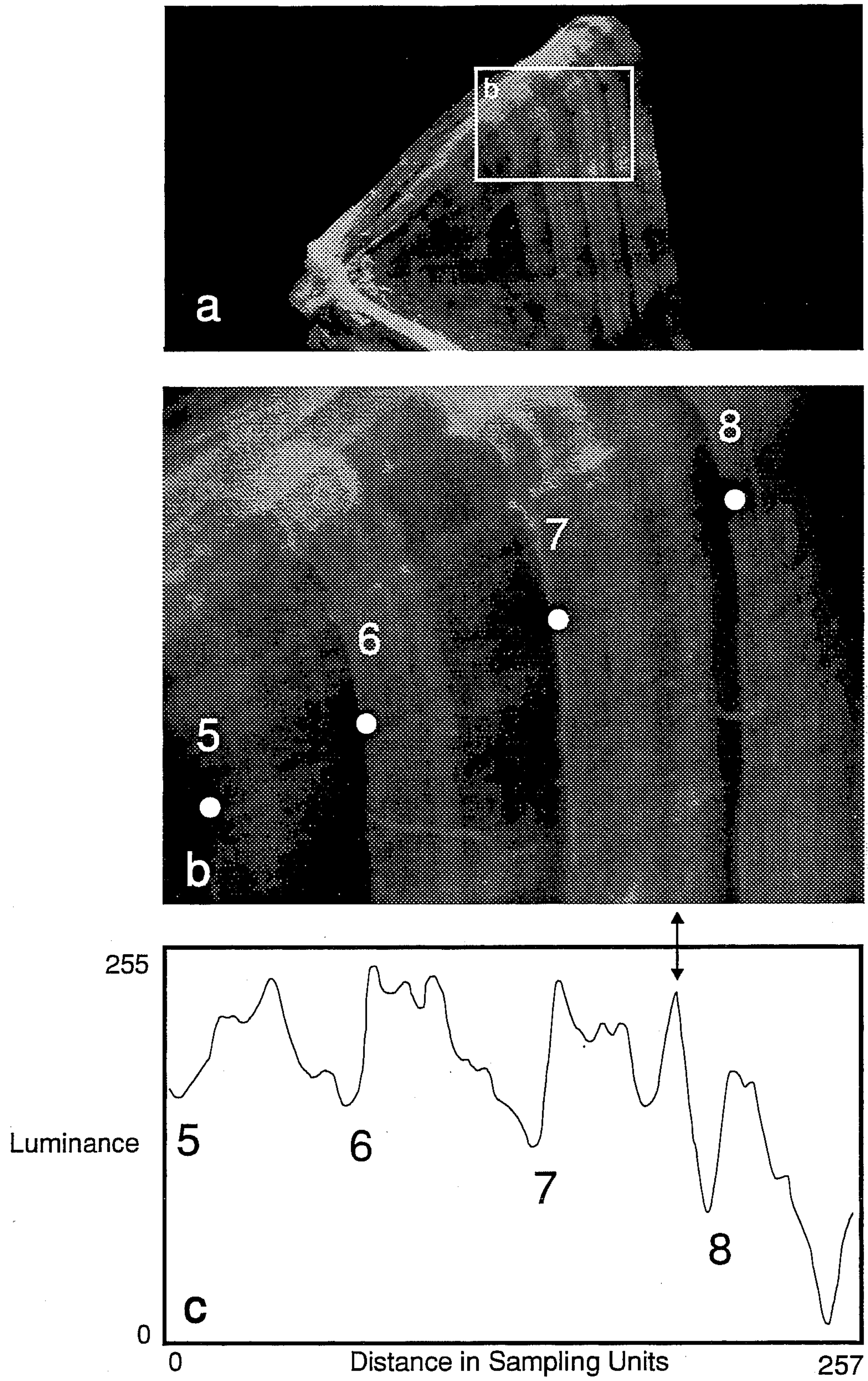

Figure 3. a) operculum showing common patterns of annual optical density (in framed area). b) higher magnification of patterns of optical density from framed area of (a). Annuli are numbered and are indicated by dots. c) a luminance plot from axis 1 (b above)) showing typical multi-peaked form of annuli (e.g 5,6,8) and a suspected false annulus (indicated with vertical arrow). False annuli are preceeded by minimum luminance values that are notably higher than neighboring clear annuli. b and c are registered vertically.