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First Marking of Squid (*Illex illecebrosus*) Statoliths with Tetracycline and Strontium in Captivity

by

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Abstract

Efforts to validate the use of statoliths as a tool for ageing squid have led to the successful development of techniques to put a 'time' mark on the statoliths using tetracycline and strontium. Evidence suggests that cold shocking squid may also result in a recognizible mark being left on the statoliths.

Introduction

Management of squid fisheries throughout the world have been hampered by the lack of a valid ageing technique, without which such population parameters as mortality rate, growth, and recruitment cannot be estimated accurately. Early research efforts were aimed at comparing size-frequency distributions with growth rate information to identify different 'cohorts' of squid and backdating to their time of spawning (Squires 1967, Summers, 1971, Ikeda and Kawahara 1975, Meshil 1977). This often proved to be troublesome 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).

Young(1960) first reported the presence of growth rings in octopod statoliths, and since that observation Clarke(1965,1966) has described concentric growth lines in squid beaks and statoliths in various cephalopod species but could not relate them with time.

Most of the recent research has been directed towards the analysis of growth rings found in statoliths. Spratt(1979) and Brothers et al.(1976) found that for Loligo opalescens these

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growth increments correlate best with daily growth in juveniles and monthly growth in animals over six months of age. Kristensen(1980) showed peridoical growth rings in statoliths of Gonatus fabricii to have daily, fortnightly and monthly interpretations based on known growth rates.Rosenberg et.al.(1981) described daily and fornightly rings observed in Todarodes sagittatus to best correlate with growth rate information. Hurley and Beck(1979) and Lipinski(1981) could not assign a clear periodicity to the rings in Illex illecebrosus although the number of rings suggested a daily interpretation was most likely. Either poor readablity or poor definition of cohorts may have been a factor.

Rearing studies offer the best chance of validating the age.EPO of cephalopods. Choe(1963) showed that the shell stripe line of cuttlefishes formed one per day under adequate nutritive and environmental conditions in known-age animals.R. Hixon(Biomedical Institute, Galveston,Texas pers. comm.) has tentative evidence to support the 'one ring per day' hypothesis for young known age L. opalescens reared in captivity.The application of this approach to I. illecebrosus is hampered by the difficulty of maintaining larval animals for more than a few days(O'Dor et al. 1977).

An alternative might be to to put a 'time' mark on the statoliths as suggested by Hurley and Beck(1979). Results of successful efforts in this direction at marking statoliths of I.illecebrosus for the first time are presented below.

Materials and Methods

Squid were obtained from a trap net located in St. Margaret's Bay on October 20 1982.They were held in a containor of chilled seawater(4 C) for no more than two hours during transport to Dalhousie University in Halifax, Nova Scotia.They were then placed in the pool tank at the Aquatron Laboratory, a description of which is given by O'Dor et al.(1977).They were maintained at a constant increased photoperiod of 16/8 hours: light/dark.Water Temperature were maintained at between 12 and 15 C. Salinity varied from 30.8 to 32.1% during the experiment.

For the purposes of this paper the results of marking trials on two squid specimens are presented. One of these squid was fed a whole, cooked shrimp stuffed with 75 milligrams of tetracycline HCL on November 19, 1982.The other specimen was fed 2 shrimp on November 17, 1982 that had been soaked previously in a solution of 1.2 grams of strontium chloride($SrCl2.6H2O$) per milliliter of distilled water for 24 hours.

Individual identification after marking was made possible by recognition of specific skin abrasion patterns peculiar to each animal.Feeding during maintenance was ad libitum with untreated shrimp.

The tetracycline-marked individual died on December 13,1982 while the strontium-marked individual died on December 4,1982. Both animals were females in a very mature condition when sampled(Table 1). Extraction of the statoliths was performed by making a single oblique transverse cut through the statocyst exposing the paired statoliths in their maculae.The statoliths were transferred to a glass microscope slide using a fine pair of forceps.Only one statolith of the pair was ultimately prepared for analysis.It was mounted with the antero-lateral (concave side) down on the glass slide in a synthetic medium(PROTEXX).The mounting medium was allowed to harden (minimum 24 hours) before grinding was attempted.The grinding was done using a succession of carborundum papers of 600 ,1/0, and 3/0 grit until the grinding surface extended to the edge of the statolith close to the maximum radius. This coincided with the exposure of most of the outer rings on the grinding surface which helped to resolve the rings better.Each statolith underwent a final polishing with 1 micron diamond paste.

Progression of grinding and polishing and general viewing of the statoliths under bright field was accomplished using a Zeiss standard Phot 1 microscope at 200 and 500X.Photographs were taken using a 35 mm camera with Panatomic-X film. Since the rings seem to be visible on different planes a drawing arm was used to map the

rings.Agreement of the counts of two readers to within ± 5% was considered to be acceptable.

Tetracycline fluoresence was observed using a Zeiss photomicroscope with a No.2 Exciter filter(350nm) and No. 50 barrier filter which cut out all ultraviolet light below 500nm.Photographs were taken at 100X (Figure 2a) using Tri-X film.

The strontium statolith was examined in the JEOL odel 35 electron microprobe.The X-ray peak for strontium using the wavelength spectrometer was determined using a strontium standard.A strontium X-ray map of the statolith was obtained(Figure 1a) together with a photo of the back-scattered electron image(Figure 1b). The X-ray map shows a well-defined strontium line near the edge of the statolith.A bright line, corresponding to the strontium X-ray line, is seen in the back-scattered electron image in the compositional or atomic contrast mode.

Images of the tetracycline and strontium statoliths were projected on their respective drawing- arm maps in order to count to the outer edge for age validation .

Results

Strontium and Tetracycline

Both strontium chloride and tetracycline HCl were successfully used in this experiment to put a recognizable mark on statoliths of I.illecebrosus .The strontium mark was much narrower in diameter than the tetracycline band which seemed to spread over 6 or 7 growth rings.A similar phenomonen occurs in fish otoliths marked with tetracycline,probably due to the slow release of the chemical into bony tissues(Campana and Neilsen,1982).Based on the procedure of overlaying the projected images of the chemical-induced marks on the drawing- arm maps, the number of rings that could be counted from the interior side of the mark to the outer edge of the ground statolith were 26 and 17 rings for the tetracycline and strontium statoliths respectively.These counts conform closely to the difference in number of days between the time of marking and death of the two animals (Table 1. Tetracycline-24 days; strontium-17 days).

Transmitted light

Growth rings were counted on each statolith with some difficulty because the density of rings seemed to diminish gradually towards the outer edge (Figures 1c and 2b). This made the mapping of the rings more difficult than has been found for'wild' animals.In all pool tank animals examined to date there appears to be a definite line which shows up in transmitted-light photos as a white ring towards the interior of the ground surface(Figures 1c and 2b).The appearance of the same type of line is only occasionly seen in 'wild' animals.Counting back from the date of marking both strontium and tetracycline animals, the calculated number of days based on the 'one ring per day' hypothesis were 26 and 32 respectively, which conforms approximately with the date of entry (October 20) of the animals into the pool tank. The fact that they were in a very mature condition with nidamental glands of lengths 90 and 95 mm suggests that they had spent between 40 and 55 days in the pool(O'Dor et al. 1977).This also conforms with the ring count intervals of 56 and 43 between the ' white ' ring and the outer edge of the statolith for the two animals.

Discussion

The results summarized above are the first reported evidence of successful chemical marking of cephalopod statoliths in the laboratory.The possibility of physically marking statoliths by cold shocking is also suggested. The use of strontium as a 'time mark' in a marine animal in this study hopefully will motivate furthur age validation work using this technique.

Tetracycline-derivatives have been used in several studies to mark the bony parts of fish for ageing studies(Kobayashi et al. 1964,Jensen and Cummings 1967,Weber and Ridgway 1967, Holden and Vince 1973, Odense 1974 ,Wild and Foreman 1980).This technique is based on the fact that antibiotics are absorbed by the fish and deposited in newly formed otolith crystals.These antibiotics become chelated in developing bone and fluoresce under ultraviolet light of the appropriate wavelength.Although not precisely known, the dosage administered in this experiment seems to be within the range if not slightly lower than that recommended as a suitable level for fish(Yamada 1971,Weber and Ridgway ibid). From the intensity and width of the tetracycine ring presented in Figure 2a ,we can surmize if anything that the dosage could be reduced in order to narrow its appearance.

Strontium marking of fish otoliths to date has been limited to use in distinguishing between hatchery-reared and wild stocks of salmon (Yamada et al. 1978,1981).Analysis of strontium in these studies was by chemical means rather than by the electron microprobe employed here.The principle of inducing a mark in bony tissue by replacing the Ca++ ion with another non-toxic divalent cation such as strontium is the same.The definite mark seen in both the X-ray (Figure 1a) and the back-scatter images(Figure 1b) lent itself readily to projection on the ring map .The fact that the presence of strontium can be verified by comparing to a standard and its relative non-toxicity is an improvement in some respects over the tetracycline method.

Perhaps the best technique for ageing squid will prove to be by cold-shocking them.Figures 1c and 2b , seem to show similarily identifiable rings which closely approximate the date of transport of these animals to the Aquatron in iced seawater.Mugiya and Muramatsu(1982) observed that of several treatments, including tetracycline, cold shocking left the most distinct markings in goldfish otoliths viewed in the scanning electron microscope.Taubert and Coble (1977) also reported that below a certain temperature, rings are not formed in sunfish otoliths and that low-temperature induced annuli formed, as evidenced by deeply etched rings.It would seem plausible then that any disruption in statolith growth would be visible also in transmitted light.

The mechanism controlling the formation of rings in Cephalopod statoliths is unknown.Studies are currently underway to investigate factors responsible for ring formation in fish otoliths such as variability in feeding and temperature regimes. The application of induced 'time marks' described above makes possible the opportunity to validate the use of statoliths as an ageing tool for squid, even for those species which are difficult to rear in captivity.

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Table 1.

Marking method	Date marked	Date Dyed	Mantle length (mm)	Nidamental gland length (mm)	Maturity stage
Strontium	17/11/82	04/12/82	247	90	5
Tetracycline	19/11/82	13/12/82	248	95	4++

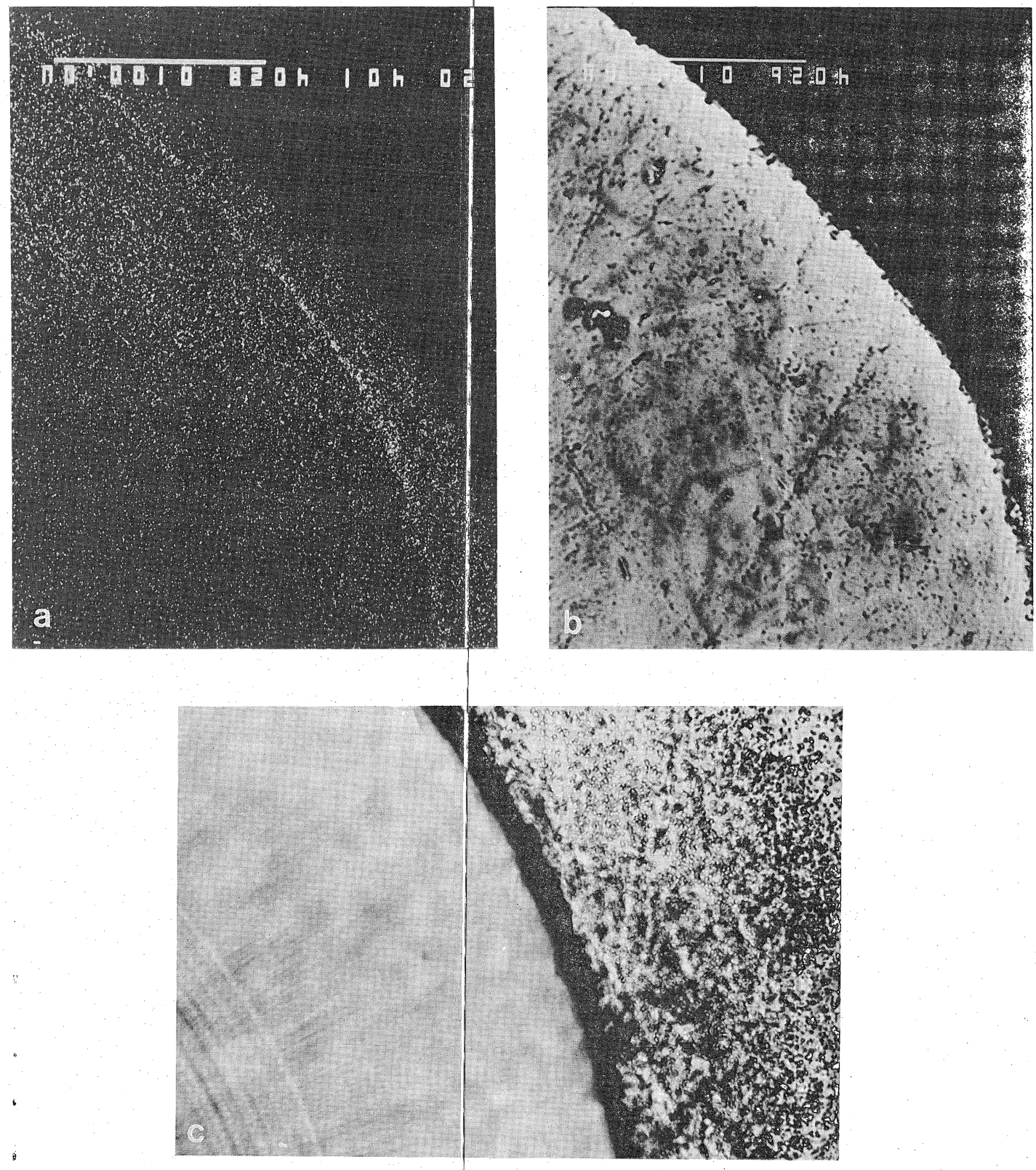
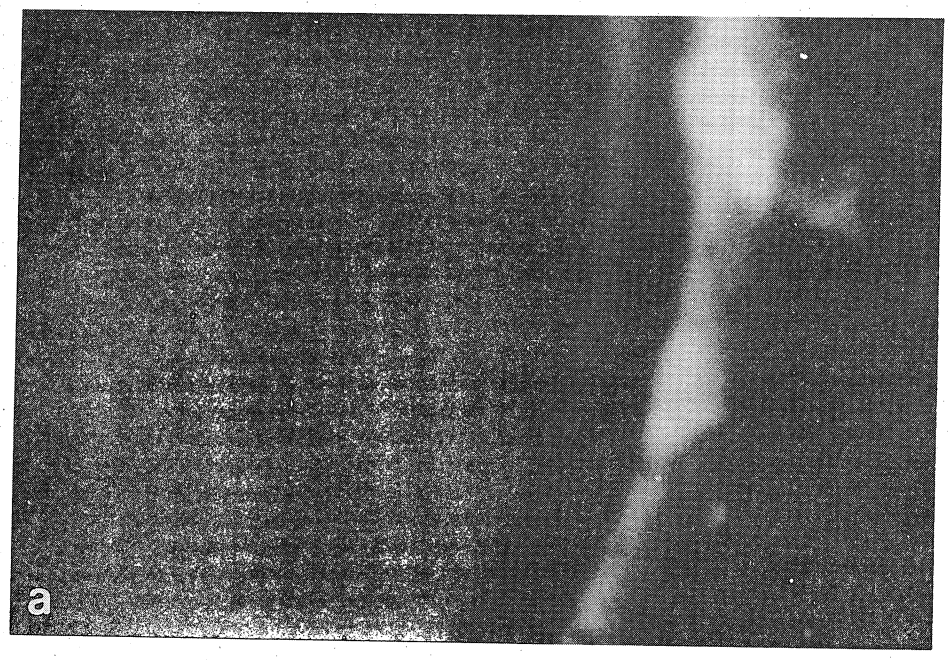


Fig. 1. (a) Strontium X-ray map of statolith SM 06/12-2-7. Magnification 401X.

(b) Strontium back-scattered electron image of statolith SM 06/12-2-7. Magnification 401X.

(c) Transmitted light photo of growth rings of statolith SM 06/12-2-7. Magnification 500X.



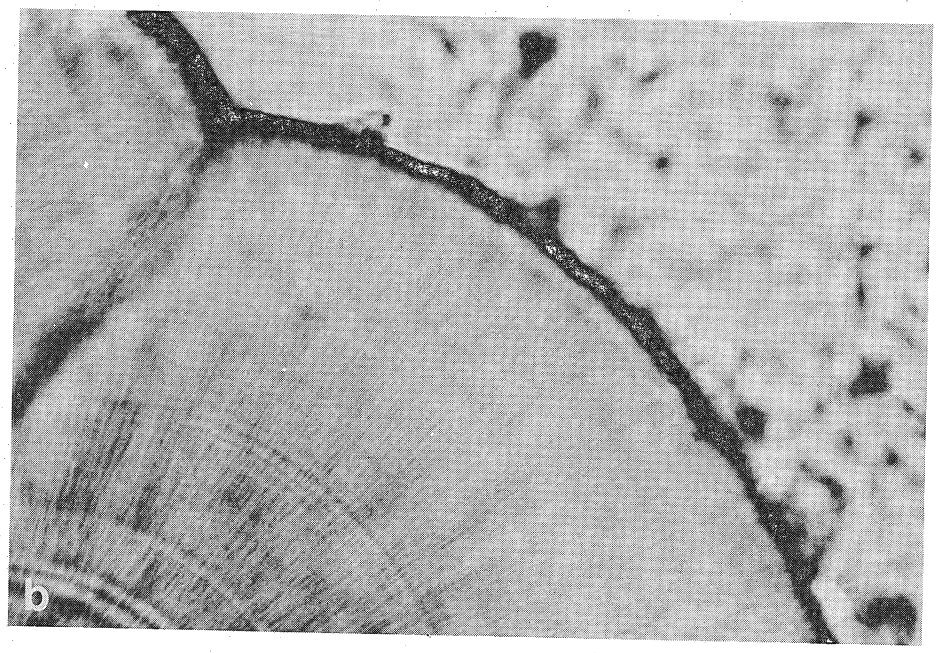


Fig. 2. (a) Tetracycline band on statolith SM 13/12-1-5. Exciter filter 350 nm. Barrier filters # 50. Magnification 100X.

(b) Transmitted light photo of growth rings of statolith SM 13/12-1-5. Magnification 500X.