THE VELIGER

The Veliger 33(4):389–393 (October 1, 1990)

The Use of Tetracycline Staining Techniques to Determine Statolith Growth Ring Periodicity in the Tropical Loliginid Squids *Loliolus noctiluca* and *Loligo chinensis*

by

GEORGE DAVID JACKSON

Department of Marine Biology, James Cook University of North Queensland, Townsville, Queensland 4811, Australia

Abstract. The tropical near-shore loliginid squids *Loliolus noctiluca* and *Loligo chinensis* have statolith growth rings similar to those described for other squid species. These rings also appear to be similar in appearance to increments found in fish otoliths. Daily periodicity of these rings was validated by staining the statoliths with tetracycline and comparing the number of rings produced with the elapsed days. These results were considered in relation to previous validation work on statolith rings that has been carried out with other squid species. Microstructural examination of statoliths promises to be a useful tool to obtain future growth information for these tropical loliginid squids.

INTRODUCTION

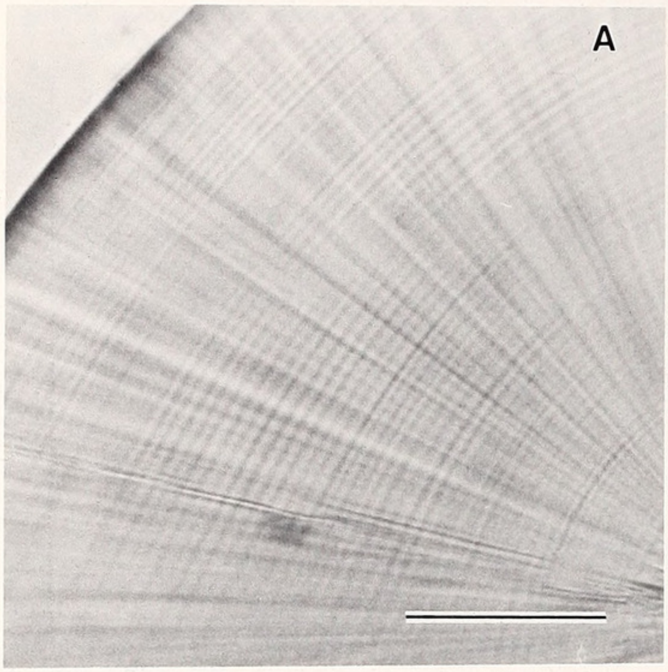
Growth rings within the statolith microstructure of the pelagic squids are gaining increased attention as possible chronological time marks. Currently there is a considerable lack of knowledge on the population dynamics of many squid species; basic biological information is lacking on life span, age at maturity, and growth rates. Research on statolith growth increments promises to yield a useful tool to obtain some of this vital information. However, unequivocal demonstration of periodicity in these growth rings is essential.

Statolith growth rings have been observed and counted in several squid species (HURLEY & BECK, 1979; KRISTENSEN, 1980; ROSENBERG *et al.*, 1981; NATSUKARI *et al.*, 1988) and daily ring periodicity has been documented in some (HURLEY *et al.*, 1985; LIPINSKI, 1986; JACKSON, 1989, 1990). The purpose of this study was to investigate statolith ring periodicity in two other tropical loliginids, *Loligo chinensis* (Gray, 1849) and *Loliolus noctiluca* (Lu *et al.*, 1985), using tetracycline staining techniques. *Loligo chinensis* is found throughout much of the tropical Indo-Pacific (ROPER *et al.*, 1984) while *Loliolus noctiluca* is a smaller near-shore species that is found along the east coast of Australia from New Guinea to Tasmania (LU *et al.*, 1985).

MATERIALS AND METHODS

Loliolus noctiluca

Young individuals of *Loliolus noctiluca* were captured using 1-mm and 8-mm mesh seine nets off the beach at Townsville, North Queensland, Australia. Captured squids were transported back to the laboratory in 20-L plastic buckets and their statoliths were stained by exposing them to an ambient solution of 250 mg of tetracycline per liter of seawater as described in JACKSON (1989). Squids were captured in June and July 1989 and were maintained alive until sacrificed after 30 and 31 days, respectively, although some mortality occurred during maintenance. Squids were kept outside in a 1500-L round fiberglass tank connected to a recirculating seawater system. Three squids captured in June were transferred to a 308-L round tank on day 30 and allowed to grow until they died after 77 and 83 days. Food was supplied *ad libitum* by maintaining live sergestid shrimps *Acetes sibogae australis* (<3



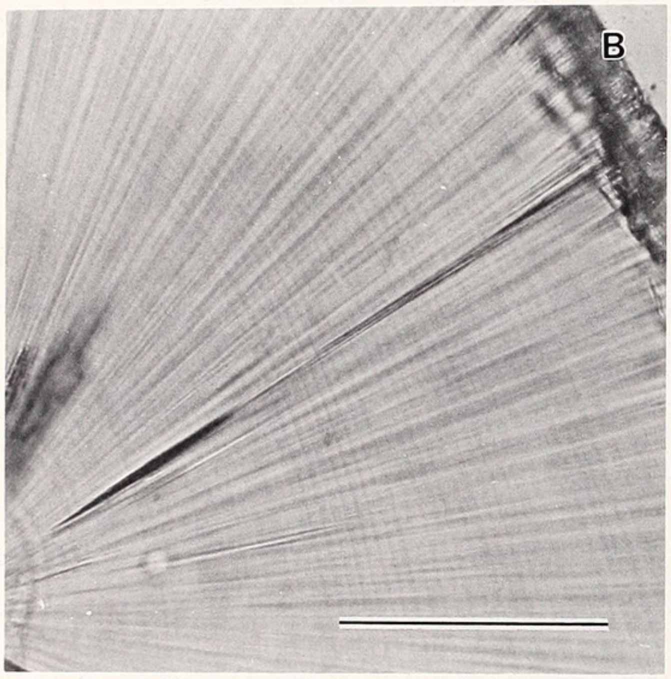


Figure 1

A. Daily growth rings in a whole statolith of a field-captured *Loliolus noctiluca* (female, 59 days, 52 mm dorsal mantle length) mounted in thermoplastic cement. Scale bar = 50 μm. B. Daily growth rings in a whole statolith of a field-captured *Loligo chinensis,* which has been ground and polished on both sides to produce a thin section (male, 78 days, 110 mm dorsal mantle length). Scale bar = 100 μm.

cm in length) with the squids. Large schools of this easily obtainable food source were maintained with *Loliolus noctiluca* and used as a constant and abundant food supply.

Loligo chinensis

Individuals of *Loligo chinensis* were trawled using 40-mm mesh, paired otter trawls in Cleveland Bay off Townsville on 13 July 1989. Although individuals were often killed during trawling, any squids that were in good condition were placed immediately in a 98-L tub with flow-through seawater. Although mortality was high, there was some survival during the course of the day. Because *Loligo chinensis* is large and sensitive to handling, exposing it to an ambient solution of tetracycline-seawater was not suitable; therefore, an injection technique was used. Squids brought back from trawling were injected with a tetracycline-seawater solution (6 mg/mL) at the base of arm I. Previous injection trials indicated that tetracycline was incorporated into the statolith within at least 15 hr of injection, *e.g.*, an individual that was injected in the evening of the day of trawling and found dead the following morning had already taken up the tetracycline into its statolith.

Two individuals of *Loligo chinensis* survived capture and injection and were maintained for 21 and 25 days, respectively, in a 2500-L circular tank that was maintained outside and equipped with a closed recirculating seawater system. Live food organisms kept with the squids were fishes of the families Ambassidae, Mugilidae, and Sillaginidae and juvenile penaeid and *Acetes* crustaceans. Feeding was *ad libitum*.

Statolith Observation

Details regarding grinding techniques, delineation of the tetracycline mark, and counting of subsequent growth rings are the same as described for *Sepioteuthis lessoniana* (Jackson, 1990). Growth rings in the statoliths of *Loliolus noctiluca* were counted directly without any grinding or polishing, as growth ring definition is excellent in statoliths that are not ground (Figure 1a). Rings were most visible in the dorsal dome region. Similarly the outermost rings could be visualized on the dorsal dome of the statolith of the larger *Loligo chinensis* without any polishing or grinding. However, the statolith of the second individual required grinding and polishing on both sides to enhance the visibility of the growth rings. This resulted in the growth rings being most easily delineated on the rostrum. To delineate all the growth rings clearly within the statolith microstructure of *Loligo chinensis* generally requires grinding and polishing of the statolith on both surfaces to produce a thin section (Figure 1b).

RESULTS AND DISCUSSION

The mean value obtained from replicate growth ring counts from the tetracycline mark to the statolith edge for both *Loliolus noctiluca* (Table 1) and *Loligo chinensis* (Table 2)

Table 1

Tetracycline staining and statolith ring counts for *Loliolus noctiluca* (SD = standard deviation).

Sex	Mantle length (mm)	Date stained	Date experiment terminated	Number of days	Replicate statolith ring counts	Mean	SD
F	35.0	6 June 1989	20 June 1989	14	14, 14, 13	14	0.58
J	19.8	6 June 1989	21 June 1989	15	15, 14, 16	15	1.00
F	38.0	7 June 1989	7 July 1989	30	30, 29, 28	29	1.00
M	32.0	7 June 1989	7 July 1989	30	28, 27, 28	28	0.58
F	34.0	7 June 1989	7 July 1989	30	29, 29, 31	30	1.15
M	30.0	7 July 1989	7 August 1989	31	30, 30, 31	30	0.58
M	38.0	7 June 1989	23 August 1989	77	79, 75, 75	76	2.31
F	45.0	7 June 1989	30 August 1989	83	86, 82, 89	86	3.51
F	54.0	7 June 1989	30 August 1989	83	77, 82, 84	81	3.61

corresponded to, or was very close to, the number of days the squids were maintained. Individuals of *Loliolus noctiluca* were maintained for periods of 13 to 83 days. The degree of correspondence between the days maintained and ring number decreased and the among-count variance increased with the length of time maintained. This reflects the problems associated with counting large numbers of relatively narrow rings.

Statolith growth ring analysis promises to be the most useful method for establishing squid age. However, the technique is of value only when ring counts are highly accurate, which requires experience and familiarity with the ring structure of the species studied.For example, validation of daily rings in *Sepioteuthis lessoniana* has highlighted the presence of sub-daily rings (JACKSON, 1990), which if counted would lead to an over estimation of squid age. Because of the specificity involved in ring counting, it is often difficult to obtain independent counts from multiple observers. Avoiding observer bias is therefore important. This is most easily achieved by using a hand counter during counts so the observer is not biased by previous trials. In addition, replicate counts should be made of each statolith to provide estimates of variance in ring numbers. It would be of use in future work to establish a core of cephalopod workers with expertise and experience in statolith ring counting. In this way both the intra- and inter-observer counting biases could be addressed systematically.

The growth rings present in the squid statolith are similar to those found in fish otoliths. Having the ability to obtain accurate age estimates from fishes has proven to be valuable to the understanding of their biology (*e.g.*, CAMPANA & NEILSON, 1985). It is becoming increasingly apparent that squid statoliths can be used in a similar way to ascertain important biological parameters that would be difficult to obtain by other means (*e.g.*, NATSUKARI *et al.*, 1988). However, the results obtained are only tentative until the periodicity of statolith growth rings can be calibrated.

Techniques used in the analysis of growth ring data from fish otoliths can be applied to the study of statolith microanatomy. These include delineation of growth rings using scanning electron microscopy on polished and etched statolith surfaces (RADTKE, 1983; HURLEY & BECK, 1979; LIPINSKI, 1986). Alternatively, light microscopy has been used to observe growth rings in whole untreated statoliths (BALCH *et al.*, 1988; JACKSON, 1989) or in statoliths that have been ground and polished using various techniques (KRISTENSEN, 1980; ROSENBERG *et al.*, 1981; NATSUKARI *et al.*, 1988; JACKSON, 1990).

Artificially inducing a chemical time mark on the statolith is perhaps one of the most convenient methods for the validation of daily statolith ring periodicity. Culturing squids from hatching (thereby knowing the age of individuals) is the only other means to calibrate ring periodicity (*e.g.*, YANG *et al.*, 1986), and this method is often quite difficult and time consuming. Exposing squids to an ambient solution of tetracycline or calcein-seawater is most easily used with small individuals that can easily be maintained in relatively small confines during the staining process, with minimal damage, *e.g.*, *Idiosipius pygmaeus*

Table 2

Tetracycline injection and statolith ring counts for *Loligo chinensis* (SD = Standard Deviation).

Sex	Date injected	Date experiment terminated	Number of days	Replicate statolith ring counts	Mean	SD	Area of statolith observed
M	13 July 1989	7 August 1989	25	24, 26, 26, 26, 23, 24	25	1.33	Rostrum
M	13 July 1989	3 August 1989	21	20, 20, 20, 22, 21, 22	21	0.98	Dorsal dome

Table 3

Summary of species and number of individuals used in chemical staining of statoliths for the determination of ring periodicity.

Species	Number of individuals	Chemical used	Technique employed	Reference
Illex illecebrosus	4	strontium	given with food	HURLEY *et al.*, 1985
Illex illecebrosus	8	strontium-tetracycline	with food, force feeding	DAWE *et al.*, 1985
Alloteuthus subulata	11	tetracycline	injection	LIPINSKI, 1986
Idiosepius pygmaeus	6	tetracycline	ambient exposure	JACKSON, 1989
Sepioteuthis lessoniana	7	tetracycline-calcein	ambient exposure	JACKSON, 1990
Loliolus noctiluca	9	tetracycline	ambient exposure	Jackson, this report
Loligo chinensis	2	tetracycline	injection	Jackson, this report

(JACKSON, 1989) and juvenile *Sepioteuthis lessoniana* (JACKSON, 1990). However, larger more active species are too sensitive for this type of method, and such other techniques as injection (LIPINSKI, 1986) or inclusion of a statolith-staining drug in the food have been used (DAWE *et al.*, 1985; HURLEY et al., 1985).

Evidence for daily periodicity in statolith growth rings, derived from chemical marking experiments, now exists from 47 individuals out of five species of squids and one sepioid (Table 3). There is considerable scope to extend ring validation work to other cephalopod species, as the species that have been worked on represent only a minute portion of the total cephalopod fauna. Ring validation work on the larger oceanic squids is particularly needed as statolith growth ring analysis promises to be one technique that can be used to establish important biological parameters for many of these species. Furthermore, it is necessary to increase the sample size of validated individuals to extend age validation work beyond the scope of preliminary findings.

It can only be assumed that the statolith ring deposition process that has been observed for *Loliolus noctiluca* and *Loligo chinensis* under these artificial conditions is similar to what occurs in the natural environment. The maintenance conditions were especially suitable for providing a good environment for statolith incremental growth, since the tanks were located outside and thereby exposed to the natural diel light regime. Furthermore, the tanks were relatively large, which promoted a more natural behavior of the squids, especially *Loliolus noctiluca* that appeared to live and grow relatively undisturbed in the tanks. The demonstration of daily statolith rings in *Loliolus noctiluca* and *Loligo chinensis* indicates that these structures will be useful in determining important age and growth parameters for these tropical squids.

ACKNOWLEDGMENTS

I thank J. H. Choat for assistance during the project and in preparation of the manuscript and F. Hoedt for assistance with field collections. I further thank C. C. Lu, who assisted with taxonomic identification of the species studied. This research was supported by grants through James Cook University of North Queensland.

LITERATURE CITED

BALCH, N., A. SIROIS & G. V. HURLEY. 1988. Growth increments in statoliths from paralarvae of the ommastrephid squid *Illex* (Cephalopoda: Teuthoidea). Malacologia 29:103–112.

CAMPANA, S. E. & J. D. NEILSON. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42:1014–1032.

DAWE, E. G., R. K. O'DOR, P. H. ODENSE & G. V. HURLEY. 1985. Validation and application of an ageing technique for short-finned squid (*Illex illecebrosus*). Journal of Northwest Atlantic Fishery Science 6:107–116.

HURLEY, G. V. & P. BECK. 1979. The observation of growth rings in statoliths from the ommastrephid squid *Illex illecebrosus*. Bulletin of the American Malacological Union, Inc., for 1979:23–25.

HURLEY, G. V., P. H. ODENSE, P. K. O'DOR & E. G. DAWE. 1985. Strontium labelling for verifying daily growth increments in the statolith of the short finned squid (*Illex illecebrosus*). Canadian Journal of Fisheries and Aquatic Sciences 42:380–383.

JACKSON, G. D. 1989. The use of statolith microstructures to analyze life-history events in the small tropical cephalopod *Idiosepius pygmaeus*. Fishery Bulletin, U.S. 87:265–272.

JACKSON, G. D. 1990. Age and growth of the tropical nearshore loliginid squid *Sepioteuthis lessoniana* determined from statolith growth ring analysis. Fishery Bulletin, U.S. 87. In press.

KRISTENSEN, T. K. 1980. Periodical growth rings in cephalopod statoliths. Dana 1:39–51.

LIPINSKI, M. 1986. Methods for the validation of squid age from statoliths. Journal of the Marine Biological Association of the U.K. 66:505–524.

LU, C. C., C. F. E. ROPER & R. W. TAIT. 1985. A revision of *Loliolus* (Cephalopoda; Loliginidae), including *L. noctiluca*, a new species of squid from Australian waters. Proceedings of the Royal Society of Victoria 97:59–85.

NATSUKARI, Y., T. NAKANOSE & K. ODA. 1988. Age and growth of loliginid squid *Photololigo edulis* (Hoyle, 1885). Journal of Experimental Marine Biology and Ecology 116:177–190.

RADTKE, R. L. 1983. Chemical and structural characteristics of statoliths from the short-finned squid *Illex illecebrosus.* Marine Biology 76:47–54.

ROPER, C. F. E., M. J. SWEENEY & C. E. NAUEN. 1984. Cephalopods of the world. FAO fisheries synopsis. No. 125, vol. 3. Rome: FAO. 277 pp.

ROSENBERG, A. A., K. F. WIBORG & I. M. BECH. 1981. Growth of *Todarodes sagittatus* (Lamarck) (Cephalopoda, Ommastrephidae) from the Northeast Atlantic, based on counts of statolith growth rings. Sarsia 66:53–57.

YANG, W. T., R. F. HIXON, P. E. TURK, M. E. KREJCI, W. H. HULET & R. T. HANLON. 1986. Growth, behavior, and sexual maturation of the market squid, *Loligo opalescens,* cultured through the life cycle. Fishery Bulletin, U.S. 84: 771–798.