Abstract.– Age and growth parameters of the tropical loliginid squid *Sepioteuthis lessoniana* in eastern Australian waters were determined from statolith growth-ring analysis. Juvenile specimens were captured, maintained alive, and their statoliths were chemically marked *in situ* with either tetracycline or calcein. These chemicals produced a fluorescent mark within the statolith microstructure when viewed under UV light. Statoliths were mounted in thermoplastic cement and subsequently ground and polished. This process allowed rings to be visualized without any further preparation. It was thus possible to validate that distinct statolith rings were formed daily and that less-distinct thinner rings were, in fact, subdaily rings.

The results of the age analysis of field-captured individuals revealed that the population of *S. lessoniana* in the study area grows at a very fast rate. Maturity in both sexes was achieved in less than 100 days. All specimens aged were less than 6 months old. The size of large individuals was within the range of *S. lessoniana* captured in other areas, with size ranges being 75–213 mm and 75–184 mm for males and females, respectively.

Growth rates determined for *S. lessoniana* based on statolith ageing are considerably different from previous estimates based on length-frequency data.

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Age and Growth of the Tropical Nearshore Loliginid Squid *Sepioteuthis lessoniana* Determined from Statolith Growth-Ring Analysis

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Estimation of growth rates along with estimates of age-at-maturity and life span are the key elements in understanding the population dynamics of marine organisms. In many instances, such data are difficult to obtain and approximations often have broad confidence intervals. The provision of accurate age estimates is one method by which reliable data on demographic processes can be obtained. For example, research into fish demography has developed rapidly (for relevant reviews see Pannella 1980, Campana and Neilson 1985, Jones 1986) following the discovery and validation of daily microstructure increments within the otolith (Pannella 1971).

Pelagic cephalopods have many fish-like features, yet differ considerably in fundamental ways with respect to metabolism and growth (O'Dor and Webber 1986). Because of their rapid growth and the lack of population statistics in comparison with fish studies, cephalopods are potentially one of the more interesting targets of demographic analysis. Ageing techniques of pelagic cephalopods, employing statolith microstructure analysis, are currently developing along lines similar to those applied to fish biology. Cephalopod statoliths and fish otoliths are physiologically analogous structures (Radtke 1983). Although statolith growth rings were recognized over 20 years ago (Clarke 1966), it has not been until relatively recently that some detailed microstructure analysis has taken place (see Jackson [1989] for relevant literature).

Ongoing research into cephalopod demography is revealing that many species are short-lived and exhibit rapid growth and early maturity (Saville 1987). These features make cephalopods particularly amenable to ageing using daily growth rings. Recent work on growth and ageing on the small tropical sepioid *Idiosepius pygmaeus* has revealed that very small tropical species can have relatively fast growth and maturity rates (Jackson 1989) compared with previous estimates.

The majority of research on squid demography has taken place in temperate waters. Life spans for large temperate squids are generally believed to be annual (Voss 1983, Natsukari et al. 1988, Amaratunga 1987). It was thus of interest to examine age, growth and maturity parameters of a large tropical cephalopod to see if these parameters paralleled similar work on their temperate counterparts.

The tropical loliginid squid *Sepioteuthis lessoniana* was selected for age and growth analysis. This species is one of the larger tropical nearshore squids, reaching lengths of over 30 cm and occurs throughout much of the Indo-Pacific (Roper et al. 1984).

The environmental distribution of *S. lessoniana* in North Queensland waters includes offshore reef environments as well as nearshore/estuarine habitats. Its distribution thus overlaps that of *I. pygmaeus*.

Materials and methods

The two primary components of this study involved (1) the collection of juvenile *Sepioteuthis lessoniana* specimens, which were maintained alive and stained with tetracycline or calcein to determine statolith ring periodicity, and (2) the collection of field specimens, which were fixed shortly after capture and subsequently used for age and gonad analysis. Thus all age estimates were taken from field specimens that were not exposed to artificial maintenance.

Juvenile *S. lessoniana* were often seen along breakwaters in the Townsville region and occasionally in a local estuary. Specimens commonly sheltered under flotsam or floating pieces of ropes, singly or in schools of up to 20. Juveniles were dipnetted and transported back to the lab for subsequent tetracycline staining and maintenance. Collections were made between May 1988 and February 1989.

Larger individuals for ageing were captured using squid jigs at night off the Picnic Bay jetty (lat. 19°11′S, long. 146°50′E) on Magnetic Island (a continental island ~7 km off Townsville) and the Australian Institute of Marine Science jetty south of Townsville (lat. 19°17′S, long. 147°03′E). Night jigging took place between November 1988 and February 1989.

Squids were aged (i.e., statolith rings counted) and examination of reproductive structures was carried out to ascertain the level of maturity. Maturity was determined by the presence of spermatophores in the spermatophore sac in males and the presence of mature oocytes in females.

Maintenance and tetracycline staining

Captured *S. lessoniana* were transported back to the lab and were maintained in 1500-L and 2500-L round tanks located outside so that squids would maintain normal diel periodicity. Specimens were kept alive as long as possible to provide maximum statolith growth before being sacrificed for examination, although in many instances the maintenance was terminated by death of the squid or sometimes by individuals jumping out of the tank. Squids which survived the first few days of captivity were maintained for between 9 and 39 days. Seawater from a closed recirculating system continually flowed through the tanks. Feeding was *ad libitum*. Common food organisms maintained in the tanks were the crustacean *Acetes sibogae australis* and Ambassid, Mugilid, and Clupeid fish species. On several occasions squids were induced to eat previously frozen prawns. Up to seven squids were maintained concurrently in one tank. Their typical behavior was to hover motionless in a school, aligned in the same direction.

The methods for tetracycline staining were the same as used for *Idiosepius pygmaeus* (Jackson 1989). Specimens were normally stained on the day of capture, and if they survived long enough they were exposed to a second staining at least 9 days later, with an interval of 9–20 days between stainings. In one experimental treatment, five squid were initially exposed for 1.5 hours to 100 mg of dissolved calcein per liter of seawater, and then stained a second time 11 days later with tetracycline (250 mg/L, 2 hours). The calcein staining produced a very faint green fluorescence compared with the very strong more yellow-green tetracycline hydrochloride fluorescence. Exposure to calcein at a higher concentration or for a longer time period is likely to produce more prominent fluorescence under UV irradiation.

Preparation and observation of statolith microstructure

Sepioteuthis lessoniana specimens were preserved in 10% borax-buffered seawater formalin to fix tissues and gonads. All length measurements refer to dorsal mantle length (DML). Statoliths were removed the next day within 12 hours to avoid damage from prolonged exposure to formalin. Statoliths were exposed to a 1% bleach solution for several minutes to remove excess tissue, rinsed in distilled water, dehydrated with 100% ethanol and mounted in the thermoplastic cement Crystal Bond. Crystal Bond was found to be an excellent mountant, as it is completely translucent, does not fluoresce under UV irradiation, and statoliths can be easily manipulated or turned-over because it quickly melts at a low temperature and hardens relatively rapidly after removal from heat.

Statoliths were then ground by hand on wet 1200-grade carborundum paper. The scratches from the grinding were removed by polishing the specimens either by hand on wet suede with 0.05 μ alumina powder, or by using a modified gem polishing machine equipped with a microscope slide-holding arm, in which the statolith was lowered onto a 6-inch rotating disc to which was attached a wet Leco Lecloth impregnated with alumina powder.

Statoliths rings were usually best visualized by grinding and polishing the statolith on the anterior (concave) surface only; however, in some statoliths that were particularly opaque or thick, the ring structure was

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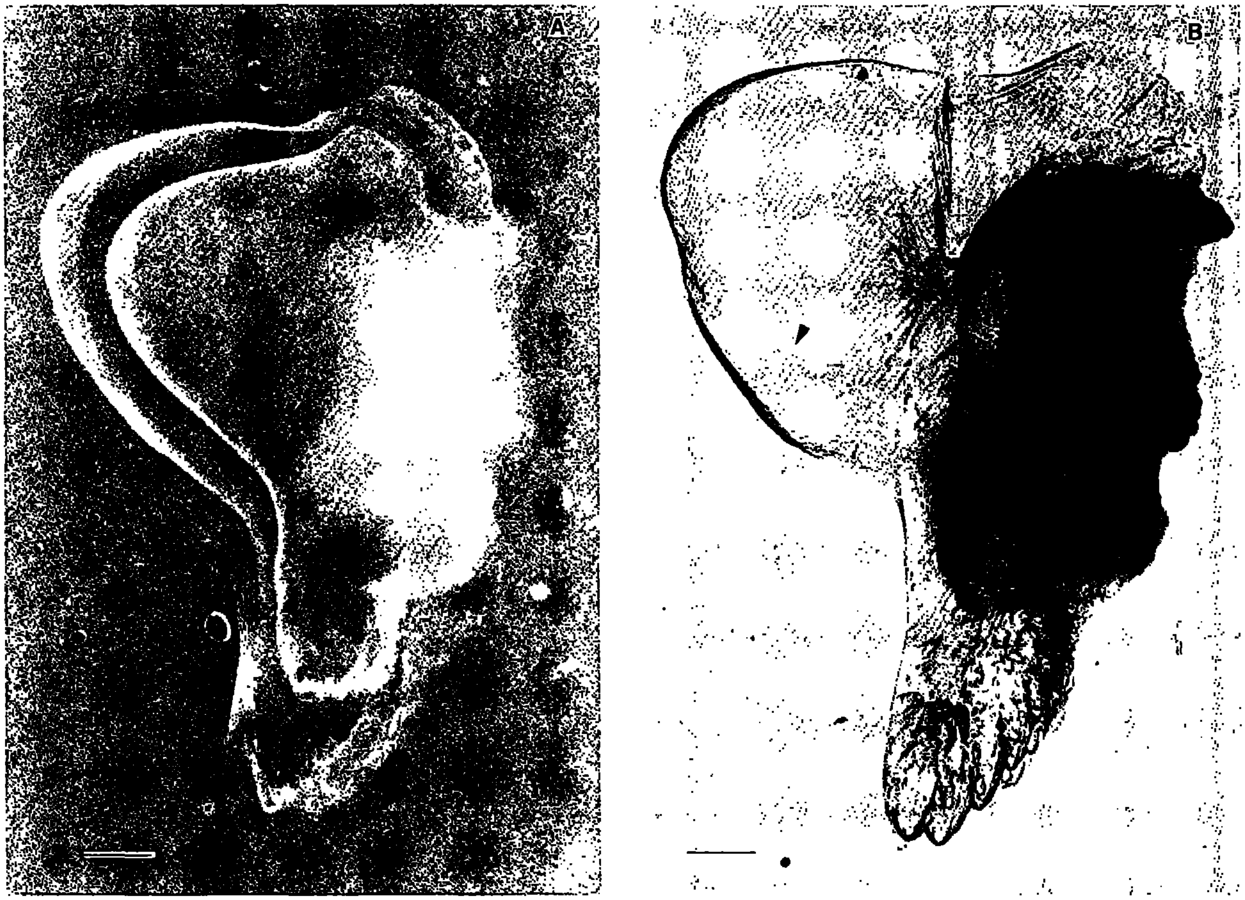

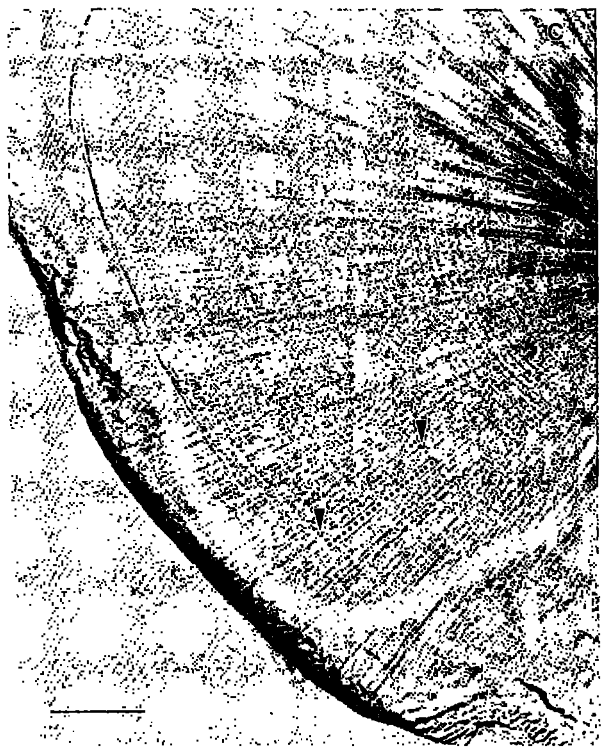

Figure 1
(A) UV micrograph of statolith from *Sepioteuthis lessoniana* from eastern Australian waters (age 68 days, DML 67 mm) stained with tetracycline twice at a 20-day interval and then allowed to grow for a further 18 days. Scale bar = 100 μm. (B) Light micrograph of same specimen in Fig. 1A, ground and polished on both anterior and posterior surfaces. Scale bar = 100 μm. (C) Light micrograph of lateral region of dorsal dome (indicated by arrow in Fig. 1B). Arrows indicate checks that correspond to tetracycline stainings. Scale bar = 50 μm.

Table 1
Statolith ring validation information for *Sepioteuthis lessoniana*.

Mantle length (mm)	Stain 1	Stain 2	Date experiment terminated	Number of days	Number of increments	Comments
53	11 May 88	—	20 May 88	9	9	
50*	18 May 88	—	27 May 88	9	9	
Not taken	14 Oct. 88 (calcein)	25 Oct. 88	8 Nov. 88	11	11	Between stains
52	14 Oct. 88 (calcein)	25 Oc.t 88	9 Nov. 88	11	11	Between stains
73	14 Oct. 88 (calcein)	25 Oct. 99	20 Nov. 88	26	26	From 2nd stain to edge
67	20 Oct. 88	9 Nov. 88	28 Nov. 88	20	20	
50	8 Feb. 89	20 Feb. 89	20 Feb. 89	12	11	Died during 2nd staining

*Development of hectocotylus observed on this specimen.

clarified by grinding and polishing on both surfaces. Growth rings were counted on the polished surface with no further preparation. Rings were accentuated by observing the specimen under polarized light and were enumerated with a hand counter by following the ring sequence with a pencil via a camera lucida. Specimen age in days (i.e., statolith ring number) was established by taking the mean of at least three counts that deviated less than 10%. Specimen age was rounded to the nearest whole number.

The statolith size and shape of a newly hatched *S. lessoniana* were determined from a specimen which was hatched from an egg trawled-up in Cleveland Bay off Townsville. This specimen further provided information on DML at hatching (5.3 mm). Growth rate of aged specimens was thus taken as the increase in mantle length in mm/day minus 5.3 (i.e., DML − 5.3/age).

Results

The notable characteristics of *Sepioteuthis lessoniana* statoliths are a rounded dorsal dome and a relatively long, thin rostrum (Fig. 1B). Unlike the sepioid *Idiosepius pygmaeus* in which rings were most obvious in the lateral region of the statolith (Jackson 1989) or *Loligo* (*Photololigo*) *edulis* (Natsukari et al. 1988) which has a clearly countable ring sequence in the rostrum, *S. lessoniana* statolith rings were most discernable in the dorsal dome. Rings were very difficult to discern in the rostrum.

The ring sequence within the statolith commenced from a prominent check, although some faint ring structure was visible inside this check. This check ring corresponded closely in size to the outer margin of the statolith of the newly hatched *S. lessoniana* (which also had some ring structure at hatching), indicating that this ring represents a hatching check. This feature has also been documented in the loliginid squids *Alloteuthis subulata* (Lipinski 1986) and *Loligo* (*Photololigo*) *edulis* (Natsukari et al. 1988).

Tetracycline staining

The growth ring sequence could be clearly visualized and counted in the vicinity of the tetracycline or calcein marks in seven specimens maintained in captivity. The counts of these specimens corresponded to a daily periodicity in ring formation (Table 1). Rings were generally more easily counted between two stain marks (Fig. 1C) than from the stain mark to the edge, as the edge rings are the most difficult to discern. A statolith check was often induced within the statolith, which corresponded to the date of capture and staining (Figs 1A, C).

When using high magnification or very sharp focus, numerous subdaily rings could be discerned which often made counting of daily rings difficult. This was especially true in areas of the statolith where rings were quite thick (wide). Using a lower magnification or changing the plane of focus helped to delineate the true daily rings that were superimposed over the numerous subdaily rings. A similar phenomena has been shown to exist in the otoliths of the freshwater fish *Coregonus* spp. (Eckmann and Rey 1987).

Age and growth

A total of 23 individual squids were aged (Table 2) to produce a growth curve (Fig. 2) and to ascertain maturity. Growth in *S. lessoniana* is rapid, with a large size reached in less than 6 months. Growth rates determined for larger specimens were considerably greater

Table 2
Variability in replicate statolith ring counts for field-captured *Sepioteuthis lessoniana* specimens. SD = standard deviation.

Specimen no.	Sex	Replicate	Mean	SD
1	J	37, 36, 35	36.0	1.00
2	J	10, 10, 10	10.0	0
3	J	34, 37, 37	36.0	1.73
4	J	37, 37, 39	37.7	1.15
5	J	34, 34, 35	34.3	0.58
6	J	38, 40, 41	39.7	1.53
7	F	191, 190, 182	187.7	4.93
8	M	89, 85, 85	86.3	2.31
9	F	66, 65, 75	68.7	5.51
10	M	67, 67, 67	67.0	0
11	M	62, 55, 62	59.7	4.04
12	M	98, 95, 101	98.0	3.00
13	M	85, 96, 89	90.0	5.57
14	M	85, 77, 81	81.0	4.00
15	M	87, 89, 90	88.7	1.53
16	M	117, 119, 119	117.7	1.15
17	F	53, 57, 54	54.7	2.08
18	F	68, 64, 68	66.7	2.31
19	F	90, 90, 87	89.0	1.73
20	F	121, 122, 119	120.6	1.53
21	M	151, 159, 150	153.3	4.93
22	M	132, 142, 132	135.3	5.78
23	M	55, 57, 41	57.7	3.05

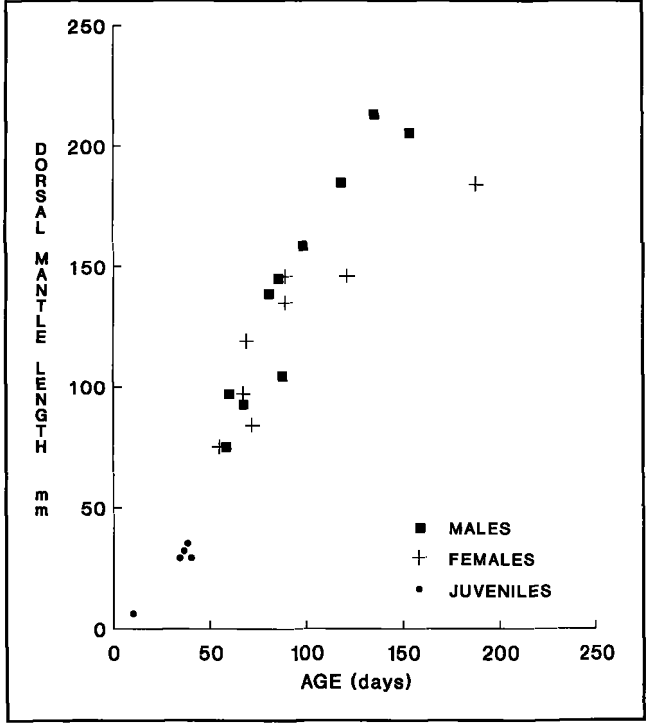

Figure 2
Age-length relationship for field-captured male, female, and juvenile *Sepioteuthis lessoniana*.

Table 3
Age, length, and growth-rate parameters for field-captured *Sepioteuthis lessoniana*. DML = dorsal mantle length; SD = standard deviation.

		Age	DML	Growth rate		
	N	range (days)	range (mm)	Range (mm/day)	Mean	SD
Paralarva	1	10	6.1	0.08	—	—
Juveniles	5	34–40	29–35	0.59–0.78	0.71	0.073
Males	9	58–153	75–213	1.11–1.65	1.42	0.197
Females	9	60–188	75–184	0.95–1.65	1.34	0.239

than in the paralarvae or juveniles (Table 3). Maturity was found to take place as young as 67 days and 69 days in males and females, respectively.

Discussion

Ageing research with *Sepioteuthis lessoniana* provides further evidence that statolith growth rings in tropical loliginids are a valuable tool that can be applied to tropical squid demography. Daily statolith ring periodicity has also been demonstrated to occur in *Illex illecebrosus* (Hurley et al. 1985, Dawe et al. 1985), *Alloteuthis subulata* (Lipinski 1986), *Loligo opalescens* (Yang et al. 1986), and *Idiosepius pygmaeus* (Jackson 1989). Furthermore, statolith ring sequences have been observed and counted in other temperate-water squid species (Kristensen 1980, Rosenberg et al. 1981, Natsukari et al. 1988).

The presence of subdaily rings within the statolith microstructure of *S. lessoniana* illustrates the need for validation when attempting to enumerate growth rings. Validation is the only way to conclusively delineate whether less-prominent rings are in fact subdaily rings.

Statolith growth-ring analysis suggests that *S. lessoniana* in Australia has a short life cycle and rapid growth. Estimates of age and growth of this species from size-frequency analysis at other localities suggest a very different pattern of growth. Earlier work on growth analysis in India (Rao 1954, Silas et al. 1982) has estimated that *S. lessoniana* takes up to three years to reach maximum size. This has also been further supported by ELEFAN 1 computer program analysis of Rao's (1954) length-frequency data (Longhurst and Pauly 1987).

Data from these different tropical populations of *S. lessoniana* suggest different patterns in the life cycle and growth of this species. The question is raised as to whether this is a locality difference or a reflection of different methodologies in analysis of growth rate.

This study emphasizes the need to critically compare different methods of estimating growth in tropical squids. It is essential to review size-frequency analysis in the light of statolith age findings at a variety of different geographic localities in the range of a species. Growth comparisons incorporating both size frequency and statolith ageing methods of other tropical Australian squid species are currently being investigated.

Artificial culture conditions provide a third method to estimate squid growth. Based on laboratory culture and field observations, Segawa (1987) has reached conclusions similar to the present study of a short life span and rapid growth for *S. lessoniana* in temperate waters around Japan. Segawa further provides growth data from a captive female *S. lessoniana* spawning at 113 days and 143 mm in tropical waters in the Philippines. This value fits well in the size-age correlation for female squid in tropical Australia.

Forsythe and Van Heukelem (1987) have suggested that development and refinement of ageing techniques are the most important tool needed in the study of cephalopod growth in natural populations. While statolith rings are a valuable tool, it is important to use a variety of methods to assess growth on the same population to distinguish between locality and method-specific differences.

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Citations

Amaratunga, T.
1987 Population biology. *In* Boyle, P.T. (ed.), Cephalopod life cycles, vol. II: Comparative reviews, p. 239–252. Acad. Press, London.

Campana, S.E., and J.D. Neilson
1985 Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014–1032.

Clarke, M.R.
1966 A review of the systematics and ecology of oceanic squids. Adv. Mar. Biol. 4:91–300.

Dawe, E.C., R.K. O'Dor, P.H. Odense, and G.V. Hurley
1985 Validation and application of an ageing technique for short-finned squid (*Illex illecebrosus*). J. Northwest Atl. Fish Sci. 6:107–116.

Eckmann, R., and P. Rey
1987 Daily increments on the otoliths of larval and juvenile *Coregonus* spp., and their modification by environmental factors. Hydrobiologia 148:137–143.

Forsythe, J.W., and W.F. Van Heukelem
1987 Growth. *In* Boyle, P.R. (ed.), Cephalopod life cycles, vol. II: Comparative reviews, p. 135–156. Acad. Press, London.

Hurley, G.V., P.H. Odense, R.K. O'Dor, and E.G. Dawe
1985 Strontium labelling for verifying daily growth increments in the statolith of the short finned squid (*Illex illecebrosus*). Can. J. Fish. Aquat. Sci. 42:380–383.

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

Jones, C.
1986 Determining age of larval fish with the otolith increment technique. Fish. Bull., U.S. 84:91–103.

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. J. Mar. Biol. Assoc. U.K. 66:505–524.

Longhurst, A.R., and D. Pauly
1987 Ecology of tropical oceans. Acad. Press, San Diego, 407 p.

Natsukari, Y., T. Nakanose, and K. Oda
1988 Age and growth of loliginid squid *Photololigo edulis* (Hoyle, 1885). J. Exp. Mar. Biol. Ecol. 116:177–190.

O'Dor, R.K., and D.M. Webber
1986 The constraints on cephalopods: why squid aren't fish. Can. J. Zool. 64:1591–1605.

Pannella, G.
1971 Fish otoliths: daily growth layers and periodical patterns. Science (Wash., DC) 173:1124–1127.
1980 Growth patterns in fish sagittae. *In* Rhoads, D.C., and R.A. Lutz (eds.), Skeletal growth of aquatic organisms. Biological records of environmental change, p. 519–560. Plenum Press, NY.

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

Rao, K.V.
1954 Biology and fishery of the Palk-Bay squid, *Sepioteuthis arctipinnis* Gould. Indian J. Fish. 1:37–66.

Roper, C.F.E., M.J. Sweeney, and C.E. Nauen
1984 Cephalopods of the world. An annotated and illustrated catalogue of species of interest to fisheries. FAO Fish. Synop. 125(3), 277 p.

Rosenberg, A.A., K.F. Wiborg, and 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.

Saville, A.
1987 Comparisons between cephalopods and fish of those aspects of the biology related to stock management. *In* Boyle, P.R. (ed.), Cephalopod life cycles, vol. II: Comparative reviews, p. 277–290. Acad. Press, London.

Segawa, S.
1987 Life history of the oval squid *Sepioteuthis lessoniana* in Kominato and adjacent waters central Honshu, Japan. J. Tokyo Univ. Fish. 74(2):67–105.

Silas, E.G., K.S. Rao, R. Sarvesan, K.P. Nair, and M.M. Meiyappan
1982 The exploited squid and cuttlefish resources in India: A review. Tech. Ext. Ser. Mar. Fish. Inf. Serv., Cochin 34, 17 p.

Voss, G.L.
1983 A review of cephalopod fisheries biology. Mem. Nat. Mus. Vic. 44:229–241.

Yang, W.T., R.F. Hixon, P.E. Turk, M.E. Krejci, W.H. Hulet, and R.T. Hanlon
1986 Growth behavior, and sexual maturation of the market squid, *Loligo opalescens*, cultured through the life cycle. Fish. Bull., U.S. 84:771–798.