Experimental validation of periodicity of increment formation in the Statolith of bigfin reef squid *Sepioteuthis lessoniana* (Cephalopoda: Lolignidae) from tropical Indian waters

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Sepioteuthis lessoniana (Lesson, 1830) is a neritic squid that inhabits coral, rocky reefs, seaweed and seagrass beds of the Indo-Pacific region. Increment validation or the periodicity of increment formation in squid statolith is fundamental for age determination, longevity estimation and life history traits, hence it is critical for fishery management and species conservation. Although studies have established one-increment-per-day hypothesis in neritic squids, it has never been validated for tropical Indian Seas. Squids are the dominant component of fauna in the Indian waters, hence an investigation on the statolith increment formation was undertaken for *S. lessoniana*, as a representative of Loliginid squids.

Egg masses of the bigfin reef squid were collected from Vethalai, Mandapam, southeast coast of India at a depth of 3m on 8th February 2019 by SCUBA divers. A single egg cluster contained 182 eggs with a hatching success of 95 % (172) in the laboratory. Hatching occurred at early morning hours from 2 to 5 AM. The planktonic hatchlings ranged between 3.9 and 5.49 mm (average 4.98 mm) in dorsal mantle length. They began to feed after 2-4 hours post hatching. The hatchlings were fed with live crustaceans (*Acetes* sp.) collected from wild, adult *Artemia* and hatchlings of Silver pompano (*Trachinotus blochii*) (1-2 mm). The paralarvae were maintained in captivity for 5 days within the tank. A total of 10 hatchlings were sacrificed each day for 5 days.

The size of the hatchling statolith (total statolith length) ranged from 318 to 418 µm (mean=360 µm). Hatchling statoliths had well-developed dome, wings and rostrum (Fig.1). Increments formed during embryonic development observed were 9-14 numbers (mean=12) in the nuclear region. First solid increment was observed one day after hatching. It was observed that most of the increment counts (82%) were consistent with the actual age of the squids post-hatching, though 18% of statolith counts were 1-2 (increments) days older than the actual age of the squid. The

Table.1 Relationship between age and number of increments in the statolith of *S. lessoniana* from tropical Indian waters under laboratory conditions.

Days	n	Avg. DML(mm)	Increment (range)	Agreement (%)
0	10	4.987	0(0-1)	80
1	10	5.226	1(1-2)	80
2	10	5.469	2(2-3)	90
3	10	5.614	3(3-4)	90
4	10	5.69	4(4-5)	80
5	10	5.834	5(5-7)	70

growth rate of the early stages ranges from 0.07 to 0.24 mm DML/day. The validation data presented here confirms the one day-one ring hypothesis in bigfin reef squid and possibly of similar increment formation in other tropical squids from Indian waters. However, the existing data are still modest and validation of increment formation of full lifecycle for the species continues to be a priority.

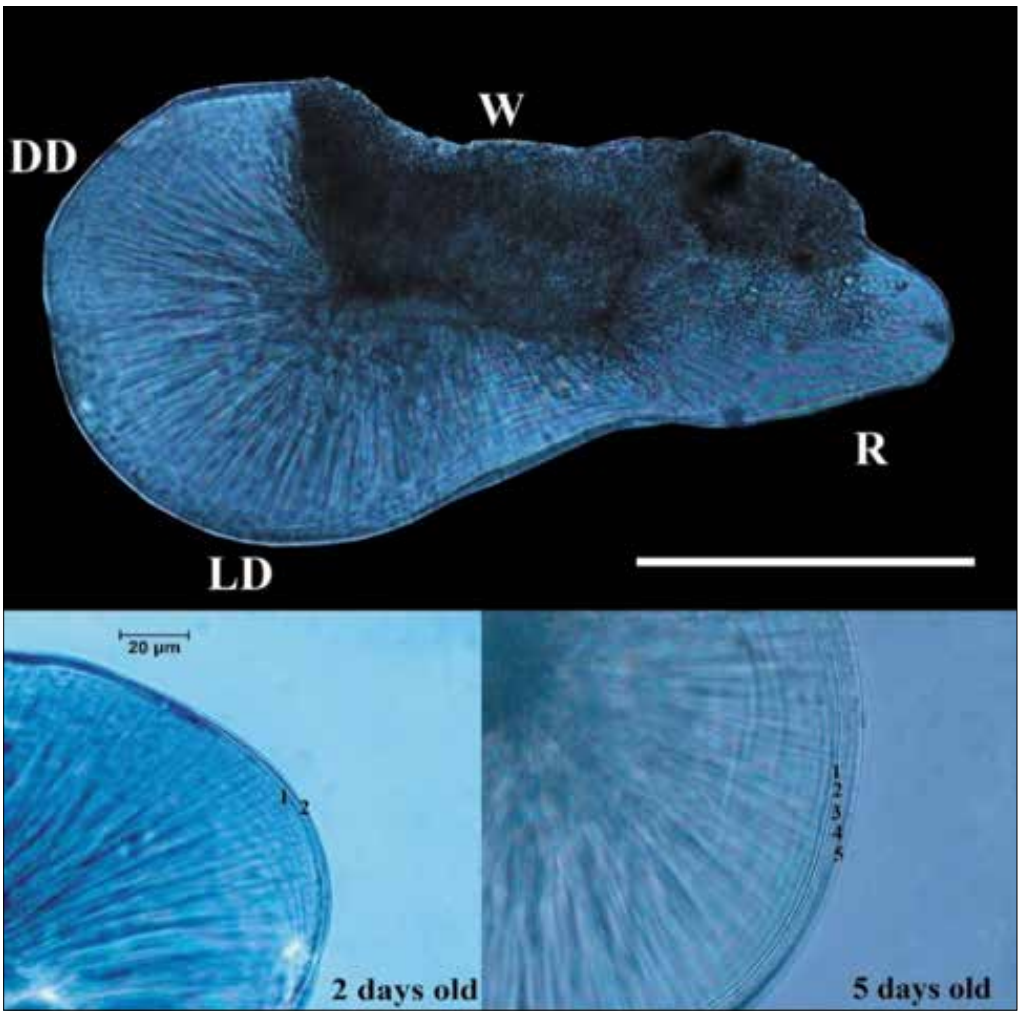

Fig.1. (a) Statolith of newly hatched *Sepioteuthis lessoniana*, DD =dorsal dome, LD=lateral dome, R =Rostrum and W=wing, (b) statolith micrograph of two days old squid with two increments and (c) five days old with five increments.