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Validation of Daily Growth Increments in Statoliths of Japanese Common Squid *Todarodes pacificus*

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In order to validate daily growth increments in statoliths of Japanese common squid *Todarodes pacificus*, squid were reared for 67 days and fed twice with saury fillets which were immersed in tetracycline HCl (TC) solution. The interval between the first and second oral administrations of TC was 35 days. Two fluorescent marks with TC were observed in the ground and polished statoliths under UV light. The mean number of growth increments between two marks with TC was 34.9 (n=17, SD=1.2). This number corresponded with the number of days elapsed (35 days) between two TC administrations. It was concluded from this result that growth increments in the statoliths of *T. pacificus* were formed daily.

There are probably three groups of the Japanese common squid *Todarodes pacificus*, which is a commercially important species around Japan.[1] The groups have different spawning seasons (winter, summer and autumn) although considerable overlaps of the spawning areas and migration patterns exists.[2] The life span of this species has been assumed to be one year.[2] Because any aging technique for this squid is not established, division of the individuals into the three groups is based on the growth pattern and the season and the timing of sexual maturity.[1] If an aging technique for this squid is established, more reliable information on the main spawning period and growth rate for each group will be provided.

In several squids,[3-13] growth increments similar to daily growth increments in the otoliths of fishes have been observed in the statoliths, and utilization of the growth increments for age determination has been studied. Validation studies of daily growth increments in the statoliths were performed in *Illex illecebrosus*,[3,4] *Sepioteuthis lessoniana*,[5] *Idiosepius pygmaeus*,[6] and *Alloteuthis subulata*[7] by marking the statoliths with tetracyclines or strontium.

In our previous study,[13] it was suggested by marking the statoliths with tetracycline HCl (TC) that growth increments in the statoliths of Japanese common squid were formed daily. In this study, we attempt to confirm the existence of daily growth increments in the statoliths of this squid by using an improved marking method with TC. In addition, alizarin complexone (AC) which is effective in the marking of otoliths of fish[14] was used as a marking chemical in this study.

Materials and Methods

Specimens of Japanese common squid *Todarodes pacificus* were captured by a set net inshore of Usujiri, Hokkaido on the 4th of July 1989. Ninety-eight individuals of the squid (DML 12–18 cm) were transported to the Usujiri Fisheries Laboratory, Faculty of Fisheries, Hokkaido University, at Usujiri and put in a raceway tank (1,200 *l*). Seawater in the tank was filtered and recirculated but a little amount of seawater was continually flowing out and being replaced by fresh seawater. The squid were reared for a maximum of 67 days. Lighting was fluorescent on a 16 h: 8 h day-night cycle, and the temperature of the rearing seawater fluctuated between 13 and 17°C during the rearing period. The squid were fed liberally once a day with saury fillets. Feeding was stopped when several consecutive fillets were ignored.

To mark statoliths, the squid were fed twice with fillets which were immersed overnight in tetracycline HCl (TC) solution (20g/*l* distilled water). In addition, the squid were also fed twice with fillets immersed in alizarin complexone (AC) solution (2g/*l* distilled water). Amounts of TC and AC taken by each squid were varied because the squid were fed liberally with the fillets. The

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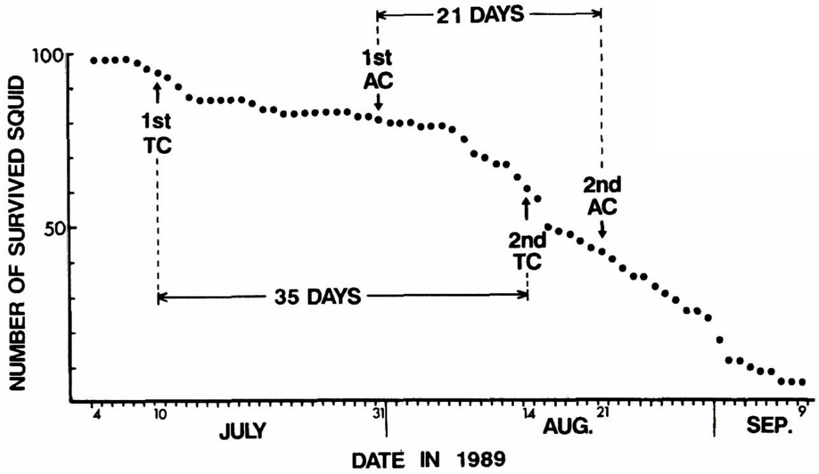

Fig. 1. Daily change in the number of surviving squid *Todarodes pacificus* during the rearing period and dates and intervals of the first and second oral administrations of tetracycline HCl(TC) or alizarin complexone (AC).

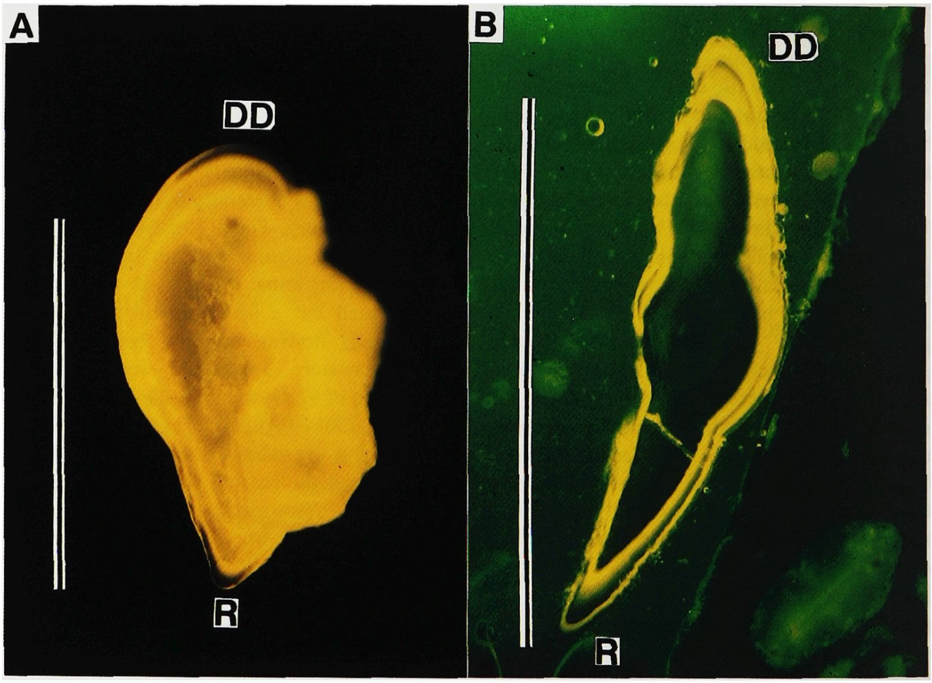

Fig. 2. Micrographs under UV light of the statolith of the Japanese common squid *T. pacificus* marked with tetracycline HCl twice with a 35-day interval. (A) Whole view of intact statolith from a 170 mm DML squid. (B) Longitudinal section of the ground and polished statolith from a 168 mm DML squid. DD: dorsal dome; R: rostrum. Scale bar = 1 mm.

dates of two oral administrations of TC were July 10, corresponding to the 7th day from the beginning of rearing, and August 14 (35-day interval) (Fig. 1). The dates of AC administrations were July 31 and August 21 (21-day interval) Squid were sacrificed on the 19th day after th

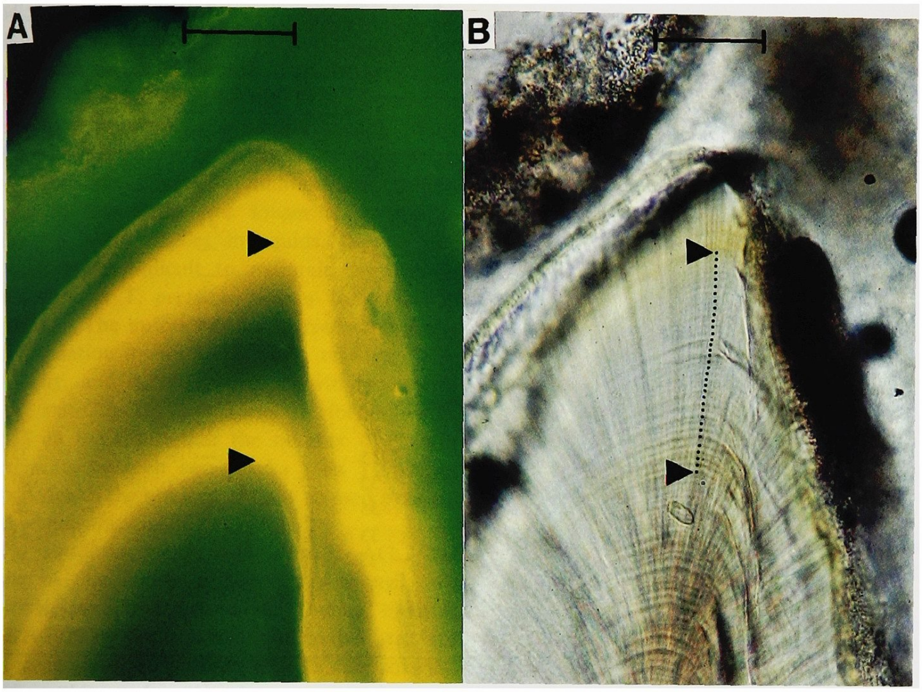

Fig. 3. Micrographs of the dorsal dome of a statolith marked with tetracycline HCl(TC) twice with a 35-day interval from a 176 mm DML squid *T. pacificus*. (A) Micrograph under UV light. (B) Micrograph of the same preparation under normal transmitted light. Arrows indicate innermost margins of the first and second marks with TC, respectively. Growth increments between two marks with TC are dotted. Scale bar = 50 μm.

second administration of AC.

Squid died or sacrificed during the rearing period were frozen. About two months later, statoliths of the squid were removed after measuring the dorsal mantle length and body weight. The statoliths were rinsed with distilled water, blotted and kept in liquid paraffin. All of the intact statoliths were observed under UV light by a light microscope equipped with an apparatus for UV irradiation to ascertain whether fluorescent marks were visible or not. The statoliths had the fluorescent marks were embedded each in a block of five minute epoxy on a glass slide with the anterior (concave) or posterior (convex) side up. After hardening, each block was trimmed parallel to the longitudinal axis of the statolith. The block was detached with a razor blade from the glass slide and glued again with cyanoacrylate adhesive to the glass slide with the lateral dome side downward. Then, the statolith was ground and polished with waterproof papers of silicon carbide (#1,000) and a graded series of aluminium oxide lapping films (9, 3, 1 μm grit size) to a plane just above the nucleus. After detaching, turning over and gluing the block, the other side of statolith was ground and polished to a thichness of about 0.1 mm. The polished surface was covered with a drop of Canada balsam and a cover glass.

Growth increments and fluorescent marks in the ground and polished statoliths were photographed under normal transmitted or UV light, sometimes under both lights, by the light microscope. Growth increments between two fluorescent marks were counted on the micrograph under normal transmitted light, enlarged to a final magnification of 430x, as comparing with the micrograph of the same preparation under UV light. When the numbers of growth increments counted were different in left and right statoliths, the mean of the two numbers was rounded to the nearest whole number.

Results

The number of squid survived during the rearing period changed as shown in Fig. 1, consequently 84 squid died during the rearing period and 5 squid survived the full 67 day period. Those

Table 1. Numbers of growth increments between the two marks with tetracycline HCl in left and right statoliths of Japanese common squid *T. pacificus*

No. of squid	Sex	Dorsal mantle length (mm)	Number of growth increments		
			Left	Right	Mean
1	Male	165	35	35	35
2	Female	202	33	35	34
3	Male	168	35	33	34
4	Male	172	34	—	34
5	Female	180	35	—	35
6	Male	185	35	36	36
7	Female	176	—	34	34
8	Male	180	—	37	37
9	Male	176	—	35	35
10	Female	180	35	—	35
11	Male	206	—	37	37
12	Male	178	35	—	35
13	Male	200	33	—	33
14	Female	167	35	36	36
15	Female	174	33	33	33
16	Female	202	35	—	35
17	Male	189	34	36	35
					Mean 34.9
					SD 1.2

deaths were mainly caused from skin damages, mostly injured the top of fin by dashing themselves against the walls of the tank.

When intact statoliths were observed under UV light, two fluorescent yellow-green marks with TC were visible in 24 individuals out of 89 (Fig. 2A). The distance between two marks with TC was wide in parts of dorsal dome and rostrum (Fig. 2B). Fluorescent marks with AC were not observed in any statoliths from the 89 sguid.

Growth increments between two growth increments which respectively correspond to the innermost margins of the first and second marks with TC were discernible (Fig. 3).

Growth increments between two marks with TC were countable in left and/or right statoliths from 17 squid (Table 1). In 10 of 17 squid, growth increments between two marks with TC were countalbe in only one statolith of a pair because of failure in polishing or faint growth increments in the other statolith.

Numbers of the growth increments between the marks with TC ranged from 33 to 37 (Table 1). The mean and standard devation of the counts in 17 squid were 34.9 and 1.2, respectively. Thus, the number of growth increments between two marks with TC corresponded with the number of days elapsed (35 days) between the first and second administrations of TC.

Discussion

In our previous study,[13] the statoliths of *Todarodes pacificus* were marked with two TC administrations but the two separate marks were not discernible due to overlapping. Therefore, counting of growth increments for validation was made from the innermost margin of the mark to the edge of statiolith. In that case, two problems remained. First, if there is a time lag in the deposition of TC to the statoliths, the numbers of growth increments counted will be understimates. Second, growth increments near the edge of statolith are often difficult to discern. In this study, the concentration of TC solution was decreased to 40% of the previous one, and marking interval with TC was extended from 14 days in the previous study to 35 days. In addition, the rearing period after the second administration of TC was prolonged from 4 days in the previous study to 26 days. Owing to those improvements, two marks with TC were observed in this study, and growth increments between two marks were more discernible than increments near the edge of statolith.

In validation studies of daily growth increments in the statoliths of other squids,[3-7] the statoliths of reared squids were marked once or twice with tetracyclines or strontium to validate. In those studies, the numbers of increments counted for validation and the differences between those counts and the days elapsed after marking were respectively 3–42 and mainly 0–3. Compared with those numbers, the counts in this study (33–37) were rather large and the differences (0–2) were not large. In addition, the rearing period in this study was longer than that in other validation studies in squids, despite that it is generally difficult to maintain an oceanic squid such as *T. pecificus* over a long term in captivity. The authors consider from the above that validation in this study has a relatively high reliability, and conclude that growth increments in the statoliths of *T. pacificus* are formed daily, based on the result that the number of growth increments between two marks with TC corresponded with the number of days elapsed between two administrations of TC.

No statoliths were marked with AC. The cause of this failure is not clear, though two possible causes are suspected. One of them is that AC which has a low solubility to water had not been completely dissolved in the solution used in this study. Another possible cause is that the squid did not sufficiently eat the fillets containing AC. However, these possibilities could not be ascertained since amounts of AC taken by each squid were not estimated.

To establish a method of daily age determination for *T. pacificus*, it is necessary to determine an increment formed at hatching. However, there is no available data on statolith size or the number of growth increments in statoliths of hatching larvae of *T. pacificus*. Therefore, we cannot help making the assumption that the increment formed at hatching is the innermost increment with a generally constant diameter. However, errors resulting from this assumption are probably small, one or two in number of increments, because the period from formation of statocysts to hatching in *T. pacificus* is short (about one day at 14–21°C).[15]

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