

Estimated life span of the Japanese pygmy squid, *Idiosepius paradoxus* from statolith growth increments

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Growth increments in statoliths of Idiosepius paradoxus were observed for aging analysis. The rate of increment formation was examined by staining the statoliths with tetracycline at 5–14 d intervals. In 6 of 16 specimens, increments formed daily, but in the other specimens, increments formed less than one per day. The maximum number of increments observed in males and females were more than 150 and 140, respectively, suggesting a life span of ≥ 150 and ≥ 140 d. During the cool season (from November to April), the dorsal mantle length (DML) and the number of increments gradually increased. Specimens collected in June showed bi-modal patterns in DML and the number of increments. Only small specimens were collected from August to October. These results suggest that two generations alternately occurred in the cool season and in the warm season.

Keywords: *Idiosepius paradoxus*, life span, statolith, age validation

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INTRODUCTION

The life histories of cephalopods are highly variable (e.g. Challier *et al.*, 2002; Jackson *et al.*, 2003). This variability is considered to be derived from specific growth plasticity (e.g. Natsukari *et al.*, 1988; Jackson *et al.*, 1997). Growth patterns are affected by various biotic and abiotic factors. Water temperature is the most important factor on the growth rate and longevity (Forsythe, 1993), and squids live longer and grow slower at lower temperatures (Arkhipkin, 2004).

The pygmy squids (*Idiosepius* spp.) comprise seven species that occur from South Africa to Japan and southern Australia, and are the smallest squids in the world (Lu & Dunning, 1998). Most species live for less than 100 d (Jackson, 1989; Jackson & Choat, 1992), and the longest-lived individual reported for this genus is 115 d old in *Idiosepius notoides* (Tracey *et al.*, 2003). This life span is the shortest known for squids and sepoids (Jackson, 1994).

The Japanese pygmy squid (*I. paradoxus*) is the northernmost distributed species in the genus, occurring in Japan, South Korea, southern Russia and central China (Lu & Dunning, 1998; Nesis *et al.*, 2002). Kasugai & Segawa (2005) showed that in central Honshu, Japan, there are two generations with a different body size each year. The study did not validate that increments form daily. In the present study, we examined the periodicity of

ring formation and analysed the daily growth in *I. paradoxus*.

MATERIALS AND METHODS

Statolith validation

Sixteen individuals were collected with a hand-net at 3–8 m depth using SCUBA on 13 September and 2 October 2005, and 13 September 2006 in nearshore waters of Usujiri, southern Hokkaido, Japan (41° 56' N 140° 56' E). Water temperature and the light cycle in a transparent aquarium (45 × 30 × 30 cm) were the same as those in the collection area: 20 ± 1°C, 12 h light/12 h dark. Tetracycline staining was conducted using the method described by Jackson (1989). One day after collection, the specimens were placed for two hours in a bucket containing 250 ppm of tetracycline dissolved in 2 l of seawater and then returned to the aquarium tank. The specimens were fed live amphipods (*Ampithoe* sp.) once a day after the tetracycline staining. Each specimen was placed in the tetracycline solution again 5, 8, 11 or 14 d after the first staining. The maximum time was set by referring to Jackson (1989). Statoliths were removed from statocysts under a stereomicroscope, fibrous tissues around the statoliths were removed with sharpened needles, and then the statoliths were embedded in enamel resin on a clear microscope slide. After the enamel resin hardened, the surface of the statolith was polished with 0.3 µm sandpaper until all increments between the core and the edge in the lateral area were visible (Figure 1). The number of increments between the two fluorescent tetracycline marks was counted in the left and right

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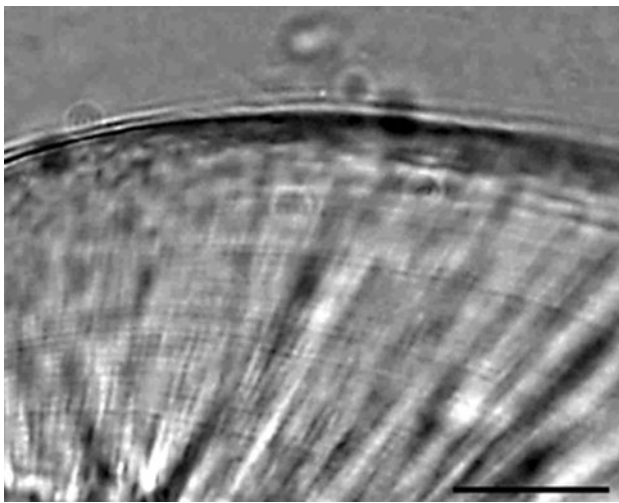


Fig. 1. Light micrographs of a statolith of *Idiosepius paradoxus* (scale bar = 15 μm). Increments were distinct in the centre of the statoliths, but not at their edges.

statoliths with an ultraviolet lighting microscope, and the mean of the two numbers was calculated.

Aging

Specimens were collected with a small drag net (1 \times 2 m, mesh size: 1.5 mm) near small stocks of *Zostera marina* in nearshore waters of the Chita Peninsula, central Honshu Island, Japan (34° 43' N 136° 58' E), monthly from November 2004 to October 2005, except in May and July 2005. Specimens were preserved in 99% ethanol immediately after collection. The dorsal mantle length (DML) of about 10 individuals was measured to the nearest 0.01 mm at each sampling, and a total of 96 males and 94 females were investigated.

The statolith increments were counted and total statolith length (TSL) was measured using a light microscope at 400 \times magnification with a video camera attached to a personal computer. Images on the computer screen were analysed

with a software program (RATOC system engineering ARR version 5.00). The number of counts was estimated by averaging three counts of the increments in the right and left statoliths along the axis from the natal ring to the lateral dome. If one of the six counts deviated more than 10% from the mean, a seventh count was taken and then all seven counts were averaged.

RESULTS

Most statoliths were opaque, and increments in the edge zone indistinct (Figure 1). Increments formed daily in 6 of the 16 statoliths, including all 4 individuals exposed to the 8-day treatment interval and half of those exposed to the 5-day treatment interval. Samples in the longer interval treatments had fewer increments than the number of days (Table 1).

The relationship between TSL and DML was expressed by the following allometric equations:

$$y = 100.4 \ln(x) + 113.17 \quad (r^2 = 0.5774, P < 0.0001, N = 96, \text{ Figure 2}) \text{ for males}$$

$$y = 99.74 \ln(x) + 103.32 \quad (r^2 = 0.6108, P < 0.0001, N = 94) \text{ for females.}$$

Where y is TSL; x is DML. These results indicate that TSL increased logarithmically with an increase in DML.

Both sexes showed a similar seasonal change in DML and the number of increments (Figure 3). DML and the number of increments gradually increased during the cool season (from November to April), and recruitment of squids in a small size-class began in June. DML and the number of increments showed little change during the warm season (from August to October). The maximum ages of males and females sampled in June were 150 d and 140 d, respectively.

Table 1. Age validation information for *Idiosepius paradoxus*. Increments were counted from the natal ring to the end of the lateral. Number of increments is the mean of right and left statoliths.

| Dorsal mantle length (mm) | Total statolith length (μm) | First staining date | Second staining date | Interval (days) | Number of increments |
|---------------------------|--|---------------------|----------------------|-----------------|----------------------|
| 5.37 | 263.62 | 4 September 2005 | 9 September 2005 | 5 | 5 |
| 6.42 | 321.15 | 4 September 2005 | 9 September 2005 | 5 | 2.5 |
| 6.72 | 267.91 | 4 September 2005 | 9 September 2005 | 5 | 5 |
| 7.76 | 319.29 | 4 September 2005 | 9 September 2005 | 5 | 2.5 |
| 4.53 | 248.56 | 14 September 2005 | 22 September 2005 | 8 | 8 |
| 5.73 | 303.41 | 14 September 2005 | 22 September 2005 | 8 | 7.5 |
| 7.50 | 283.48 | 14 September 2005 | 22 September 2005 | 8 | 8 |
| 10.37 | 291.98 | 14 September 2005 | 22 September 2005 | 8 | 8 |
| 6.24 | 294.91 | 15 September 2006 | 26 September 2006 | 11 | 8.5 |
| 5.68 | 289.26 | 15 September 2006 | 26 September 2006 | 11 | 5 |
| 5.95 | 286.09 | 15 September 2006 | 26 September 2006 | 11 | 8 |
| 5.66 | 277.64 | 15 September 2006 | 26 September 2006 | 11 | 7 |
| 6.95 | 287.95 | 15 September 2006 | 29 September 2006 | 14 | 8 |
| 6.58 | 318.18 | 15 September 2006 | 29 September 2006 | 14 | 7 |
| 6.23 | 254.63 | 15 September 2006 | 29 September 2006 | 14 | 7.5 |
| 7.18 | 286.94 | 15 September 2006 | 29 September 2006 | 14 | 8 |

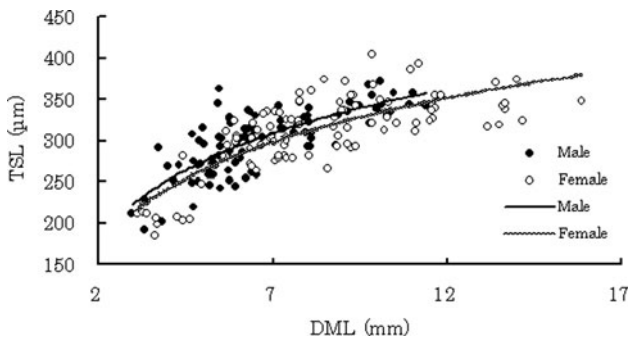


Fig. 2. The relationship between dorsal mantle length (DML) and total statolith length (TSL).

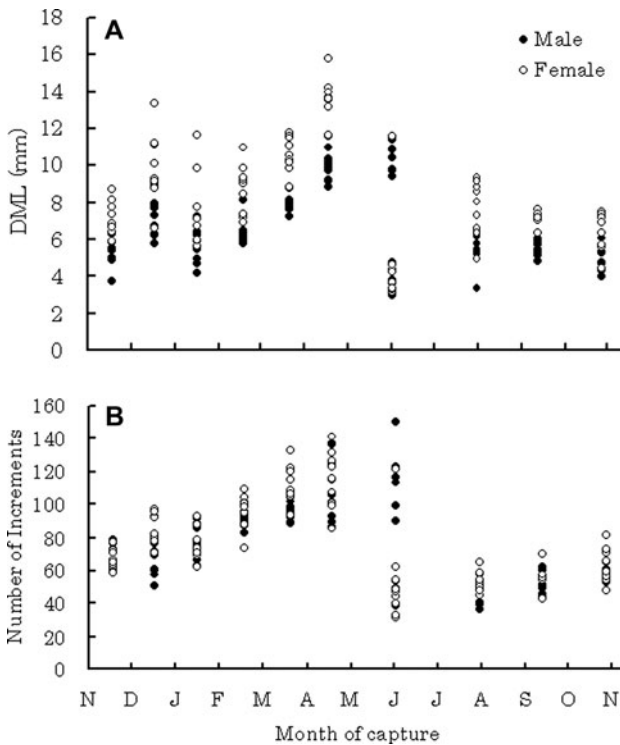


Fig. 3. The seasonal change in: (A) size dorsal mantle length (DML) structure; and (B) number of increments counted from November 2004 to October 2005.

DISCUSSION

Counting statolith increments is the standard method for aging squids, but daily increment formation has been validated in only one *Idiosepius* species (*I. pygmaeus*; Jackson, 1989). In the present study, in 6 of 16 samples, increments formed daily, but in the other specimens, increments formed less than once per day. One possible reason for the less than daily deposition is that the stress of captivity might disturb periodical increment deposition on the statolith. In this result, statolith increment counts may underestimate age.

Specimens sampled during the cool season (October to June) are considered to form a single cohort (Kasugai & Segawa, 2005). We would expect to see an increase in age over time within this cohort, however, we found no increase in ring number from October to June within the cohort. This suggests that ring number might underestimate the age

of specimens collected in the field. The growth rate of statolith logarithmically decreased with growth of body size. Therefore it may be difficult to accurately count increments because of the narrow ring distance and opaque background at the edge of statoliths in large individuals.

Daily increments in *I. pygmaeus* seem to be clearer (Jackson, 1989) than those in *I. paradoxus* because the increments are longer in *I. pygmaeus*. This supposition is supported by the fact that tropical species grow faster than temperate species (Jackson & Choat, 1992). The age analysis of *I. notoides* was conducted by Tracey *et al.* (2003) without the statolith age validation. *Idiosepius paradoxus* is a temperate species as is *I. notoides*, so the increment number in *I. notoides* might also underestimate the true age.

The longevity of *I. paradoxus* was about 1.5 times longer than that of *I. notoides* (91 d in males and 115 d in females; Tracey *et al.*, 2003) and about twice that of *I. pygmaeus* (67 d in males and 79 d in females; Jackson, 1989). In the present study the increment count was less than the age, suggesting that *Idiosepius paradoxus* may live longer than 150 d, which would make it the longest lived *Idiosepius* species reported to date. Many studies have reported that the growth rates of cephalopods decrease at low water temperature (Forsythe & Hanlon, 1988; Forsythe, 1993), therefore the longer longevity of *I. paradoxus* may be caused by slower growth rate in lower temperature.

Based on seasonal changes in body size and maturation, Kasugai & Segawa (2005) reported that *I. paradoxus* may have at least two generations (a cool season generation with large body size that spawns from March to May, and a warm season generation with small body size that spawns from June to September). Our age analysis showed that warm season individuals were born from March to May and cool season individuals were born from June to September. In addition warm season individuals were not single generation, but multiple generations. Our results confirm the occurrence of two generations per year as proposed by Kasugai & Segawa (2005).

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