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Age Estimation of Four Oceanic Squids, *Ommastrephes bartramii, Dosidicus gigas, Sthenoteuthis oualaniensis,* and *Illex argentinus* (Cephalopoda, Ommastrephidae) Based on Statolith Microstructure

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Abstract

Statolith preparation and observation methods adopted in Japan were reviewed for 4 ommastrephid squids, *Ommastrephes bartramii, Dosidicus gigas, Sthenoteuthis oualaniensis,* and *Illex argentinus.* Relationships between mantle length and age were exponenitial at the early life stages and almost linear at the subadult and adult stages.

Discipline: Fisheries
Additional key words: growth

Introduction

The flying squid family Ommastrephidae is an important component in the marine ecosytem and has been targeted by commercial fisheries in many coastal and pelagic waters of the world. *Todarodes pacificus*, *Illex argentinus*, *Ommastrephes bartramii* and *Dosidicus gigas* are major species accounting for about 50% of the world cephalopod catches which amounted to approximately 2.8 million t year^{-1} during the early 1990s[1]. Since most of these ommastrephid squids have a one-year life cycle, and are usually composed of several seasonal cohorts[11], it is essential for sustainable use of these stocks to determine their age, hatching date, and growth[6]. Until the late 1980s, growth of squids had been studied by using consecutive series of mantle length frequency distributions. Length-based methods, however, are generally inappropriate for fast-growing and highly migratory squids[6,12]. Statolith microstructure is useful for age determination of squids as are otoliths in teleost fishes, and direct or indirect validation studies revealed daily deposition of increments in squids[11,15]. Therefore, recent studies on squid growth are mostly relying on statolith daily increments, with a few exceptions where gladii were used. The purpose of this report is to describe (1) preparation methods of statoliths that have been adopted for age determination in Japanese fisheries research institutes and (2) growth of 4 species targeted by international fisheries.

Structure of statolith

Statoliths are mainly composed of calcium carbonate in the crystal form of aragonite and are located in statocysts in the cartilaginous cranium[7,15]. A squid statolith consists of a dorsal dome, lateral dome, rostrum and wing (Fig. 1). Posterior plane of a statolith is convex, and anterior plane is slightly concave. Anterior surface of the wing is attached to the statocyst wall *via* connective tissue. Growth increments, with a 3-dimensional structure, are deposited from the core (nucleus) of a statolith.

Statolith preparation

In order to extract a pair of statoliths from a squid, cartilaginous cranium was sectioned along a transverse plane in the center of the statocysts from the ventral side of the cranium with a small knife. Then, each statolith was extracted from a statocyst with fine forceps, cleaned with ethanol, and deposited in a pit (ca. 7 mm in diameter) of plastic trays, "microplate", which had been filled with liquid paraffin. After removal of liquid paraffin with xylene and ethanol, statoliths were suitable for preparation. Daily increments were observed in 4 different ways. Details of the preparation methods were described

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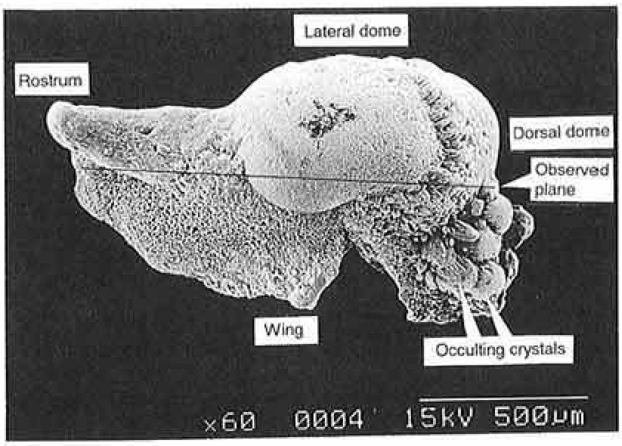

Fig. 1. Scanning electron micrograph (SEM) of the left statolith from *Ommastrephes bartramii* (posterior view) showing major features
Observed plane refers to the sectioning position for SEM and light microscopy observations.

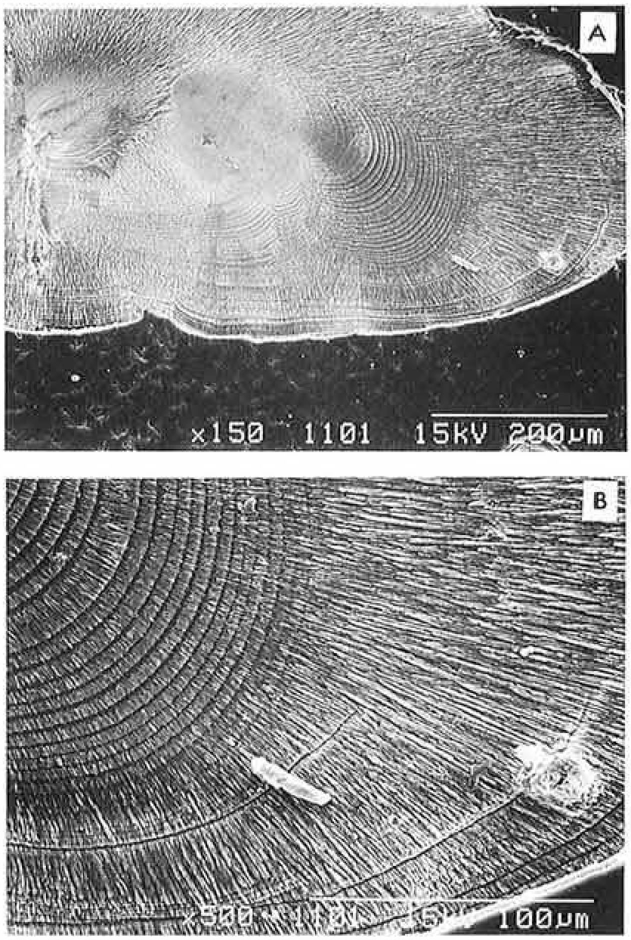

Fig. 2. Scanning electron micrographs of a sectioned and etched statolith from a 390 mm ML *Dosidicus gigas*
A: From core to dorsal dome. B: Dorsal dome area.

in previous reports[5,8,13].

1) Scanning electron microscopy (SEM)

Each statolith was mounted individually in a small mold with epoxy. After hardening, the epoxy block was removed from the mold, attached to a glass slide with the medial (wing) side up and ground on a longitudinal plane until the core was exposed (Figs. 1–3). Fine-grained carbon carbide (1500-grade) waterproof paper was used for grinding and statoliths were polished with 0.3 μm alumina powder. Statoliths were etched with 1% HCl for 150 seconds and coated with gold for SEM observation at 15 kV.

2) Light microscopy (LM)

In order to enhance the contrast of increments, statoliths were heated in small glass tubes filled with silicone oil at about 190°C for 24 h. Then, silicone oil was removed with xylene and ethanol. Heating was not necessary for *I. argentinus* and small individuals (< 300 mm ML) of *O. bartramii*.

(1) Longitudinal sectioning

After the core of a statolith was exposed in a similar way for SEM observation, the epoxy block was detached from the glass slide, attached to a glass slide with the lateral side up, and ground again to the level immediately above the core. This method had been applied for *Ommastrephes bartramii*[18] and *Dosidicus gigas*.

(2) Polishing of posterior plane

Each statolith was mounted on a glass slide with super glue or Eukitt with the posterior (convex) plane up. The posterior plane of the statoliths was slightly ground with fine-grained carbon carbide waterproof paper, and polished with 0.3 μm alumina powder. This method had been used for *Illex argentinus* and *Dosidicus gigas*[14,17].

(3) Polishing of anterior and posterior planes

Each statolith was mounted on a glass slide with Eukitt with the anterior plane up. Anterior plane of statoliths was slightly ground and polished as described in the previous method. Statoliths were removed by applying xylene, and attached on the glass slide with the posterior plane up. Then, statoliths were similarly ground and polished. This method had been applied for *Sthenoteuthis oualaniensis* and *Dosidicus gigas*[14,16].

Observation of daily increments

A light microscope (LM) equipped with objective lenses (×10, ×20, ×40, and ×60) was connected to a TV camera and a high-resolution monitor, with final magnifications of 900–4500×. For LM observations, cover glasses are not necessary, but a drop of liquid paraffin

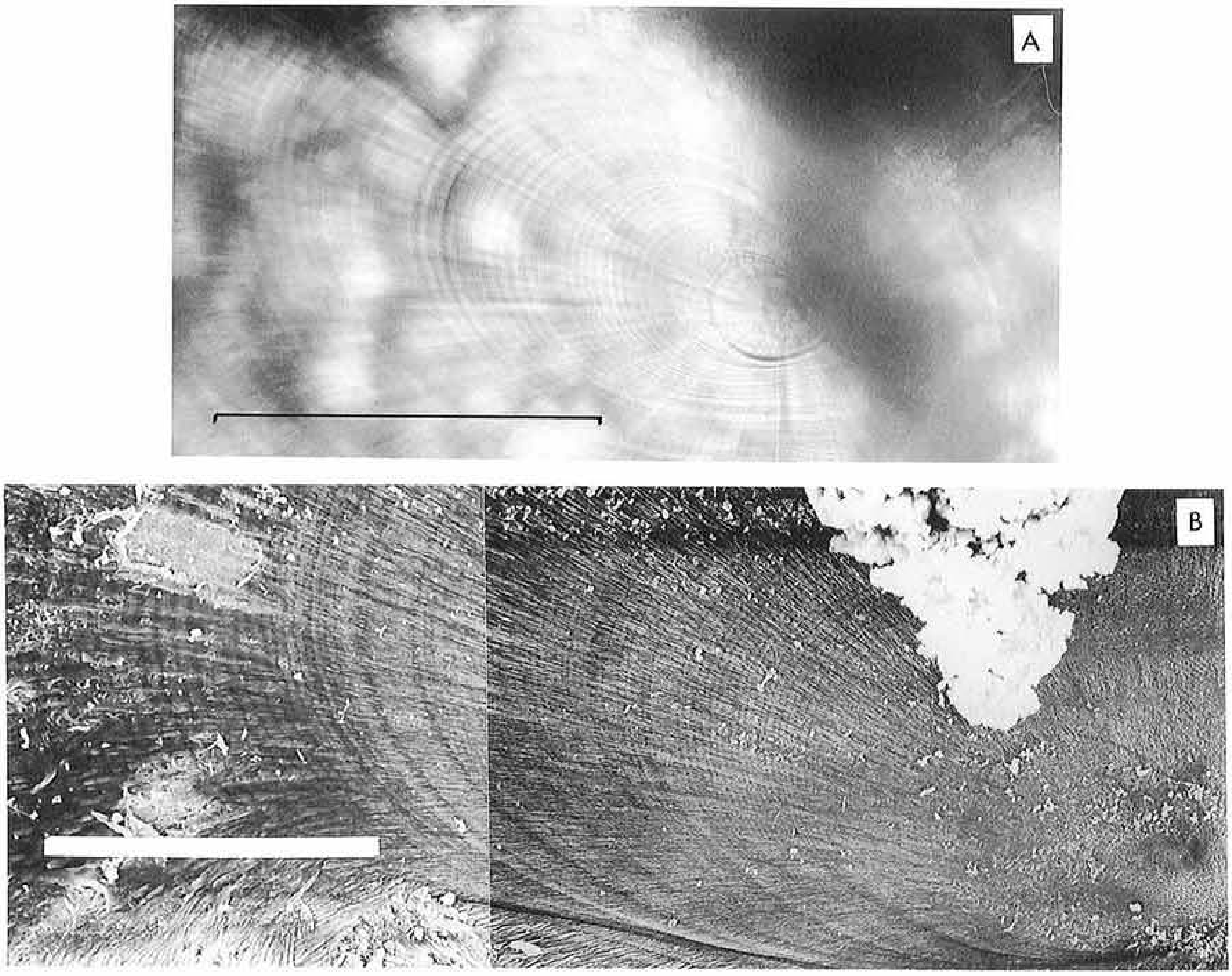

Fig. 3. Light micrograph of the ground and polished left statolith (A) and scanning electron micrograph of the sectioned and etched right statolith (B) from a 244 mm ML *Illex argentinus*

Fine increments around the post-nucleus zone (B) can not be observed by light microscopy at low-magnifications (A). Bar = 50 μm.

markedly enhances the visibility of increments. Visibility may also be improved by controlling transmitted light with manipulation of a condenser and filters. Sketches of tracing increments[8] or graphics from an image-analyzing system are useful for precise counting, i.e. for checking results within multiple counts for a specimen by a reader or among multiple readers.

A single increment is composed of a pair of dark and light rings when viewed with LM (Fig. 3A)[11]. The dark rings are more deeply etched with HCl because of the larger abundance of amino acids than in the light rings (Figs. 2 and 3B). In the ommastrephids, increments are usually prominent on the anterior plane of statoliths, and counts were made from the core to the dorsal dome (Fig. 2).

The core (nucleus) is usually dark and oval, with a size of approximately 20–30 μm×15–20 μm for *O. bartramii*, *I. illecebrosus* and *I. argentinus* (Figs. 3 and 4)[2,4]. The size of the core is considered to correspond to that at the time of hatching in *Illex illecebrosus*[3,9]. This assumption has been extended to other ommastrephid squids. In artificially reared *D. gigas*, the maximum diameters of 2 statoliths examined were 37 and 44 μm on the day of hatching, and the size of the statolith linearly increased until the 6th day after hatching, but without distinct increments (Fig. 5)[20]. Bigelow and Landgraph[4] also observed an inconsistent increment structure around the 10–to15–μm area immediately distal to the core. In some squid species, increments may not be produced daily during the embryonic development[11]. Therefore, caution must be paid to uncertainties in age estimation during the earliest life stage.

In *O. bartramii*, increment width increased from 3–4 μm near the core to up to 7μm at a distance of 200 μm,

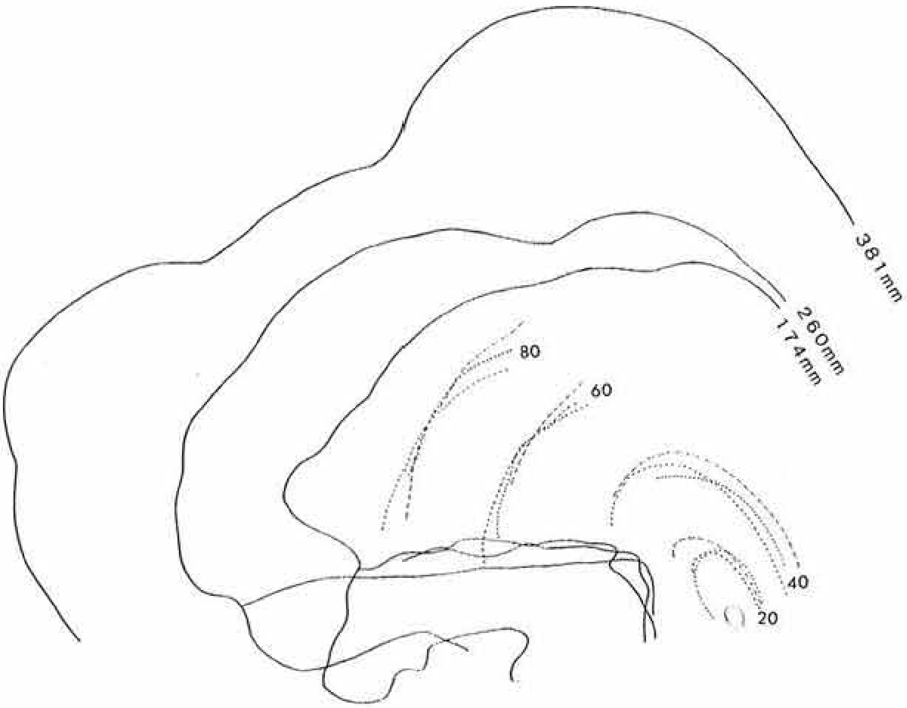

Fig. 4. Posterior view of ground and polished statoliths from three *Ommastrephes bartramii* (174, 260 and 381 mm ML), overlaying locations of 20th, 40th, 60th and 80th increments counted from the core

then gradually decreased to 2 μm or less after a distance of approximately 400 μm[19]. A similar pattern was also observed in *S. oualaniensis* and *D. gigas* (Fig. 2). In *Illex argentinus*, increment width increased from 1.5–3 μm in the post-nucleus zone, to 2–5 μm in the dark zone and decreased to 1.5–3 μm in the peripheral zone of the dorsal dome[2]. Increment width is also subject to variation with environmental conditions, mainly temperature, which in turn results from the difference in the hatching season[2]. In a stock of *I. argentinus* which spawns in summer, increment width may be as narrow as 0.9 μm in the post-nucleus zone (Fig. 3). Such fine increments can be observed with SEM or LM equipped with a high magnification lens (×60 objective lens).

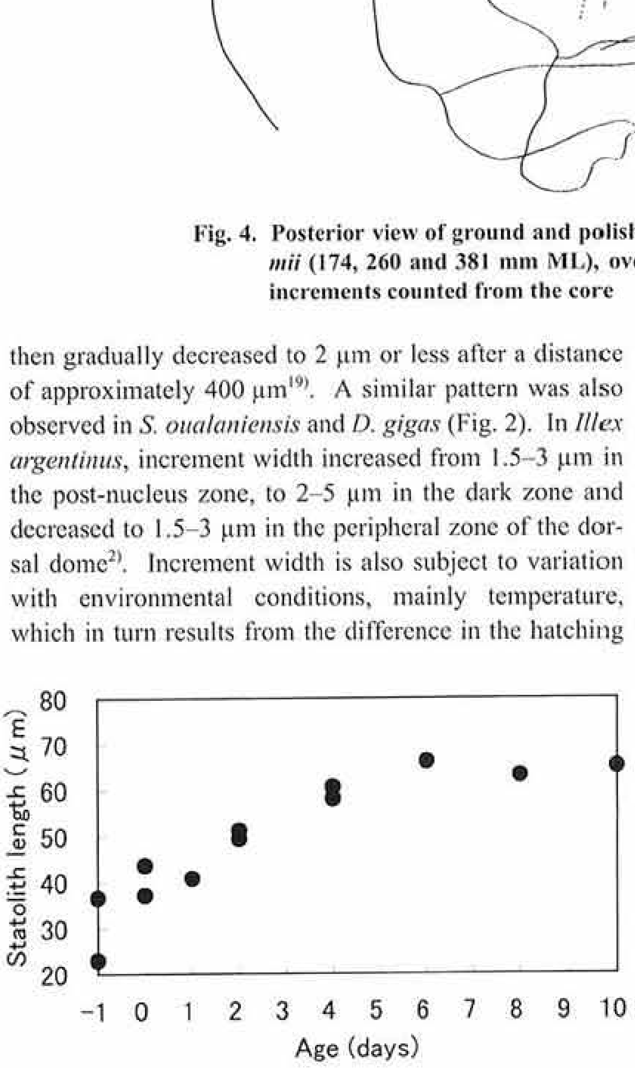

Fig. 5. Relationship between age and statolith length (maximum diameter) of artificially fertilized and reared *Dosidicus gigas* (Yatsu et al., in press[20])

Growth in older paralarvae may be underestimated because they were starved after exhaustion of yolk, about 8 days after hatching.

Comparison of preparation methods

In SEM observations, it was difficult to count the total number of increments from the core to the dorsal dome because some areas were not sufficiently etched for counting (Fig. 2). Increment width can be precisely measured with SEM photographs for adequately etched areas. In LM observations, increments were diffuse or duplicated depending on the thickness of the samples, magnification levels, and depth of focus. For example, subdaily rings (faint increments formed through non-endogenous rhythm, see Fig. 4 of Bigelow and Landgraph[4]) may become more prominent in over-ground statoliths for LM observation[11]. For accurate (unbiased) age determination, therefore, SEM observation is recommended for a limited number of samples before routine counting with LM, where several hundreds of readings are required in fisheries research[13].

The sectioning method for LM observation was effective for all the 4 species, and led to more reliable counting than the 2 other methods for LM, because it was

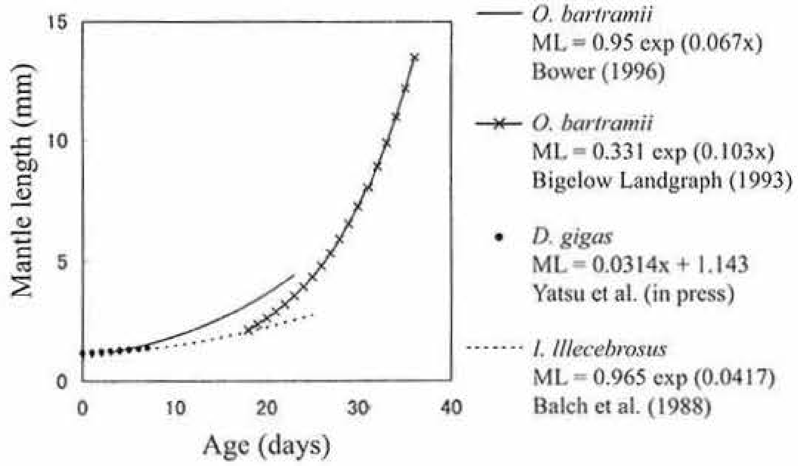

Fig. 6. Growth curves of paralarvae of *Ommastrephes bartramii*, *Dosidicus gigas* and *Illex illecebrosus* estimated from statolith microstructure
ML: Mantle length (mm). x: Age (days).

possible to avoid occulting crystals and dark zones, where increments are sometimes not sufficiently clear to obtain consistent counts. Sectioning method is, however, more labor-intensive and it is difficult to estimate earlier growth (back-calculation) owing to the "curved axis" of the statoliths (Fig. 4)[8]. The polishing method of the posterior plane is suitable for *Illex argentinus* whose statoliths have few occulting crystals. Polishing of both anterior and posterior planes is indispensable for *Sthenoteuthis oualaniensis* because of the existence of abundant occulting crystals.

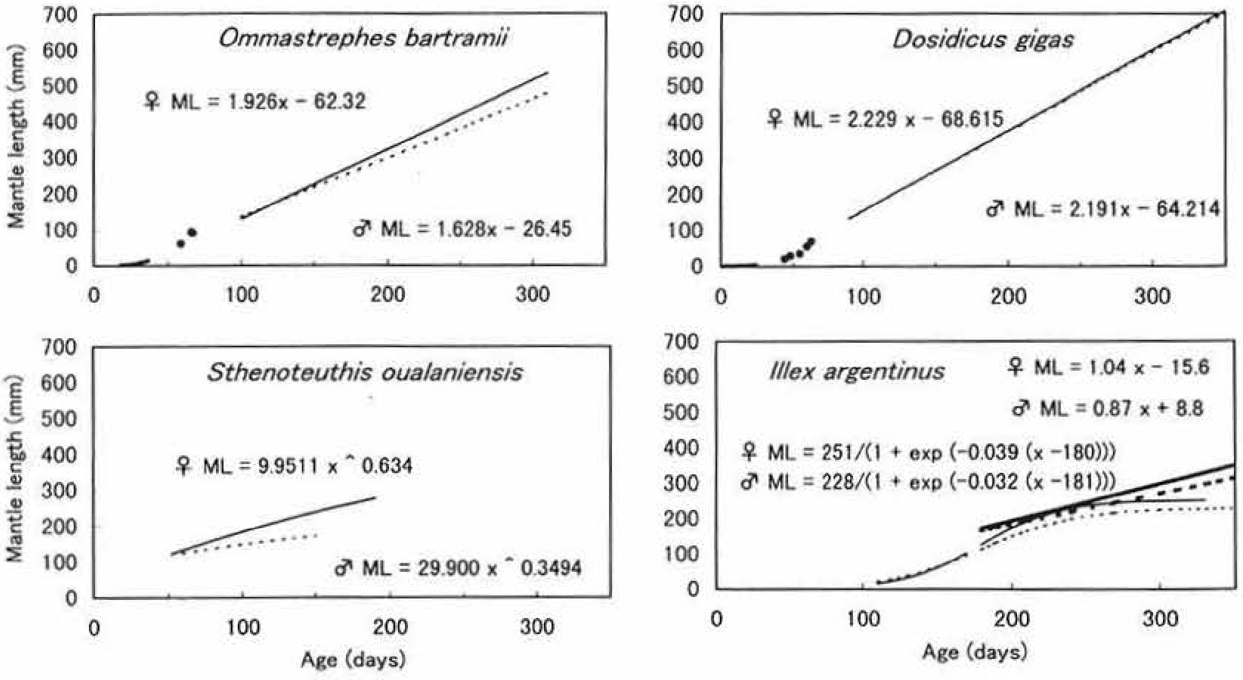

Fig. 7. Composites of ML-age relationships in selected seasonal cohorts of *Ommastrephes bartramii*, *Dosidicus gigas*, *Sthenoteuthis oualaniensis*, and *Illex argentinus*, showing early exponential growth and later linear or linear-like growth
Paralarval growth data were derived from Fig. 6. Adult and subadult growth data were cited from Yatsu et al. (1997) and unpublished data for *O. bartramii*, Masuda et al. (1998) for *D. gigas*, Takagi and Yatsu (1998) for *S. oualaniensis*, and Uozumi and Shiba (1993) for *I. argentinus* (logistic curves for autumn brood and lines for winter brood).
ML: Mantle length (mm). x: Age (days).

Growth

Growth at the early life stages has been estimated to be exponential based on statolith daily increment counts for either wild or artificially reared paralarvae (Fig. 6). ML-age relationships at the subadult and adult stages are more or less linear (Fig. 7)[11]. Altough Uozumi and Shiba[17] applied a logistic curve for the autumn brood of *Illex argentinus* (Fig. 7), a linear model can be also valid for their data[10]. Growth rates vary with sex, seasonal cohorts, geographic area, and year[2,14,18]. Females grew faster than males in all the 4 species examined, although the difference was small for *D. gigas*[14]. Since ambient temperature, food availability and maturation affect squid growth, these environmental and physiological factors should be considered for future analyses[11]. Such growth histories may be reconstructed from increment width and trace element analyses[11,19].

Conclusion

Squid growth is affected by geographic, seasonal, and year-to-year changes in environmental conditions. The growth variability, together with extended spawning season (seasonal cohorts) and short life span (usually 1 year), is an important aspect of squid ecology. For age and growth studies of squids, it is preferable not to use length-based methods and not to fit *a priori* asymptotic growth curves (or any single model), which are usually applied in fin-fish studies[6,11]. Therefore, routine age estimation studies are essential for cephalopod fisheries management, although such a task is labor-intensive.

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