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SHORT COMMUNICATION

Statolith microstructure and age of early life stages of planktonic squids *Galiteuthis phyllura* and *Belonella borealis* (Oegopsida, Cranchiidae) from the northern North Pacific

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Abstract. Statolith morphology and microstructure were studied in two common species of panktonic cranchiid squids, *Belonella borealis* [four juveniles with mantle length (ML) 375–450 mm] and *Galiteuthis phyllura* (13 paralarvae and juveniles, ML 9–235 mm), caught near the bottom and in pelagic layers over the continental slope of Siberia in the northwest Bering Sea. The total number of growth increments within the statoliths ranged from 277 to 294 in *B.borealis* and from 10 to 209 in *G.phyllura*. Assuming that these increments were produced daily, both species grow rapidly in length (daily growth rate = 1.13 mm day^{-1}) during the first 8–10 months of their juvenile phase in the mesopelagic layers, prior to migration into deeper waters for maturation.

Statolith ageing techniques have provided a promising alternative to length–frequency analysis in the study of age, growth and population structure of squid. This technique was first utilized in the late 1970s (Lipinski, 1978; Spratt, 1978; Hurley and Beck, 1979). The validation of daily growth increments within the squid statolith made ageing techniques even more effective in population analysis (see Jereb *et al.*, 1991). Most investigations using statoliths have been undertaken on commercially important abundant species of the families Ommastrephidae (Arkhipkin, 1990; Rodhouse and Hatfield, 1990; Villanueva, 1992; and others) and Loliginidae (Natsukari *et al.*, 1988; Jackson and Choat, 1992; and others). However, for ecosystem studies it is necessary to know biological parameters for non-commercial or less abundant cephalopods (Arkhipkin and Murzov, 1990; Bigelow, 1992; Jackson and Choat, 1992).

Cranchiids are one of the most unusual groups of pelagic planktonic squids, and have relatively transparent tissues and large coelomic cavities filled with neutrally buoyant liquid due to high concentrations of NH_4Cl (Clarke *et al.*, 1979). Despite their high abundance in the oceans (Voss, 1980), data on cranchiid growth remain scarce (Nixon, 1983). Up to now, statolith microstructure has been studied only in six specimens of the Antarctic *Galiteuthis glacialis* (Jackson and Lu, 1994). The age, growth and life span of cranchiids are still unknown.

The cranchiid fauna in the Bering Sea consists of two species belonging to the subfamily Taoninae: *Galiteuthis phyllura* Berry, 1911 and *Belonella borealis* Nesis, 1972. Squids of both species can grow to 600–700 mm mantle length (ML) or more (Nesis, 1987). Juveniles inhabit epipelagic and mesopelagic layers, whilst adults descend down to deeper bathyal layers where spawning supposedly takes place (Nesis, 1985).

This report is the first attempt to estimate age and growth in juvenile planktonic cranchiid squids by investigation of statolith growth increments.

Four specimens of *B.borealis* (ML ranging from 375 to 450 mm) and 13 specimens of *G.phyllura* (ML ranging from 9 to 235 mm) were caught during two research programmes for the gonatid squid, *Berryteuthis magister* (Gonatidae), in the northwest Bering Sea. Sampling was carried out by the Russian Federal Institute of Marine Fisheries and Oceanography (VNIRO) aboard two Japanese trawlers, 'TENYU-MARU 57' and 'KAIYO-MARU 28', during June–October 1993 and June–November 1994. Sampling localities covered shelf edge and slope waters off Siberia from Karaginsky Island to Navarin Cape (latitudes 58–62°N and longitudes 163°E–179°W). Squids were captured using a bottom trawl with vertical opening of 8 m, horizontal opening of 80 m and a mesh size in sac of 40 mm. Juveniles > 100 mm ML were captured near the bottom and in pelagic layers during trawl lifting over a depth range of 340–450 m.

Three early juveniles of *G.phyllura* (ML 9–33 mm) were also captured by an Isaacs–Kidd midwater trawl (175 cm depressor width) at depths of 140 and 280 m in areas where water depth was >400 m. These squids were caught alive and one of them (ML 33 mm) was placed for 1 day (until death) in a small 3 l aquarium which was kept in a refrigerator, with water temperature close to that of seawater at the depth of sampling (2–4°C).

Squids were identified using the key of Nesis (1987). Mantle length was measured to the nearest 1 mm, sex and maturity stage were assigned after Zuev *et al.* (1985). Statoliths were removed and placed in 96% ethanol for several days, until examination later on board ship. Terminology used is after Clarke (1978) and Lipinski *et al.* (1991). Statoliths were measured and drawn using the 'Olympus' B071 Zoom microscope (× 32 magnification).

For the investigation of statolith microstructure, one statolith from each pair was attached concave side up onto a microscopic slide with Pro-texx mounting medium and ground on wet 1000-grit sandpaper. Statoliths of both species were deeply concave in the region of the spur (Figure 1). Thus, grinding of the convex side was made in two planes (Figure 1). The ground statolith was embedded in Canada balsam and covered with a cover glass to protect it from dust. Growth increments were examined under a Nikon 104 microscope (× 400 magnification) and counted by two observers using the eye-piece micrometer (Arkhipkin, 1991) from the nucleus to the edge of the dorsal dome. The total number of growth increments for each statolith was taken as the mean of two replicate counts if the deviation between them was <5%.

The total statolith length of *B.borealis* varied from 1.72 to 1.85 mm, being 0.40–0.44% ML. They are strongly concave in the midpart. The rostrum is short and curved anteriorly (Figure 1).

Growth increments within the statoliths were discernible from the nucleus to the statolith edge (Figure 2) and can be grouped into three distinct growth zones. The nucleus is small and round, 25–28 μm in diameter. The colour of the nucleus is slightly brownish in transmitted light. There are 22–32 (mean 25) regularly spaced increments (4.1–5 μm in width) in the translucent postnuclear zone (Figure 2). The dark zone has the slightly brownish colour. The width of the growth increments

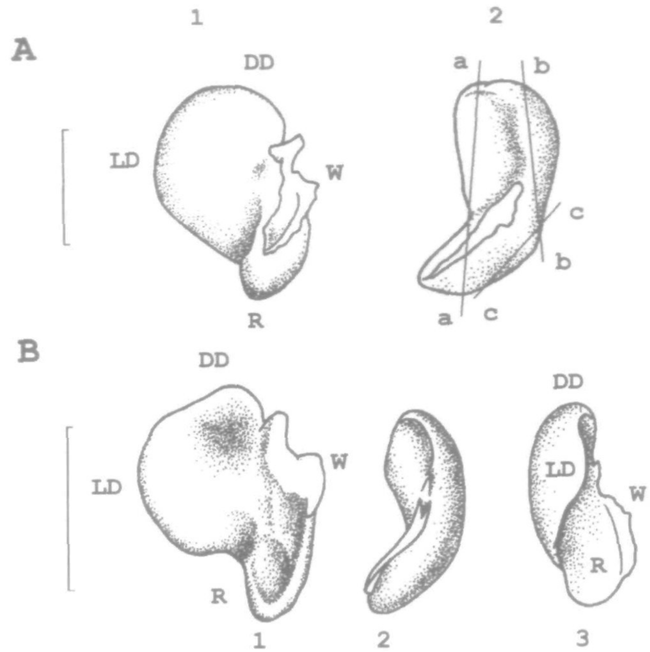


Fig. 1. Statolith morphology of juvenile cranchiids *B.borealis* (ML 400 mm, **A**) and *G.glacialis* (ML 235 mm, **B**). R, rostrum; W, wing; LD, lateral dome; DD, dorsal dome. Scale bars = 1 mm. (A) 1, anterior; 2, medial views. Planes of the first (a–a) and second (b–b and c–c) grinding of the lateral dome and rostrum. (B) 1, anterior; 2, medial; 3, lateral views.

within the dark zone ranges from 4.1 to 6 μm, with the total number ranging from 123 to 140. There are 4–5 irregularly located checks within the dark zone (Figure 2). The peripheral zone is translucent and has the narrowest growth increments (3.1–3.6 μm).

The total number of growth increments in the four *B.borealis* specimens studied ranged from 277 to 294 (Figure 4).

Statoliths of *G.phyllura* resemble those of *B.borealis*, but are more dorso-ventrally elongated (Figure 1). Therefore, their relative lengths are greater, ranging from 1–1.11% ML in small specimens (9–33 mm ML) to 0.67–0.72% ML in medium-size squid (135–235 mm ML).

In general, the statolith microstructure of *G.phyllura* is similar to that of *B.borealis* (Figure 3) with a clear growth increment sequence. *Galiteuthis phyllura* statoliths have a slightly oval nucleus with a maximum diameter of 30–35 μm, a somewhat larger number of growth increments from the nucleus to the first check (34–38 increments) and an almost uniform maximum width of all growth increments (3.8–4.8 μm).

The statoliths of the *G.phyllura* juvenile which was kept in the aquarium for 1 day from capture had one well-defined check near the statolith edge and one translucent increment outside it.

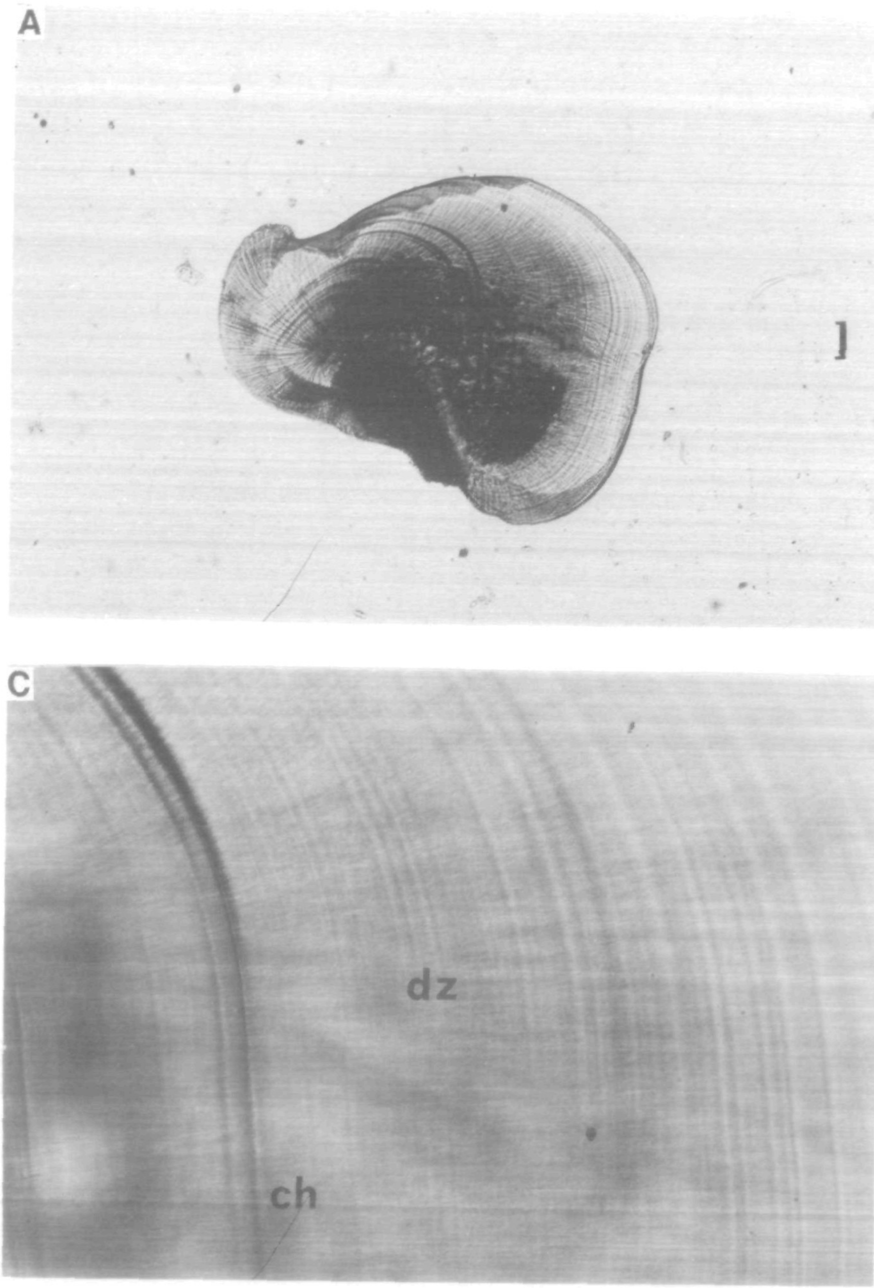
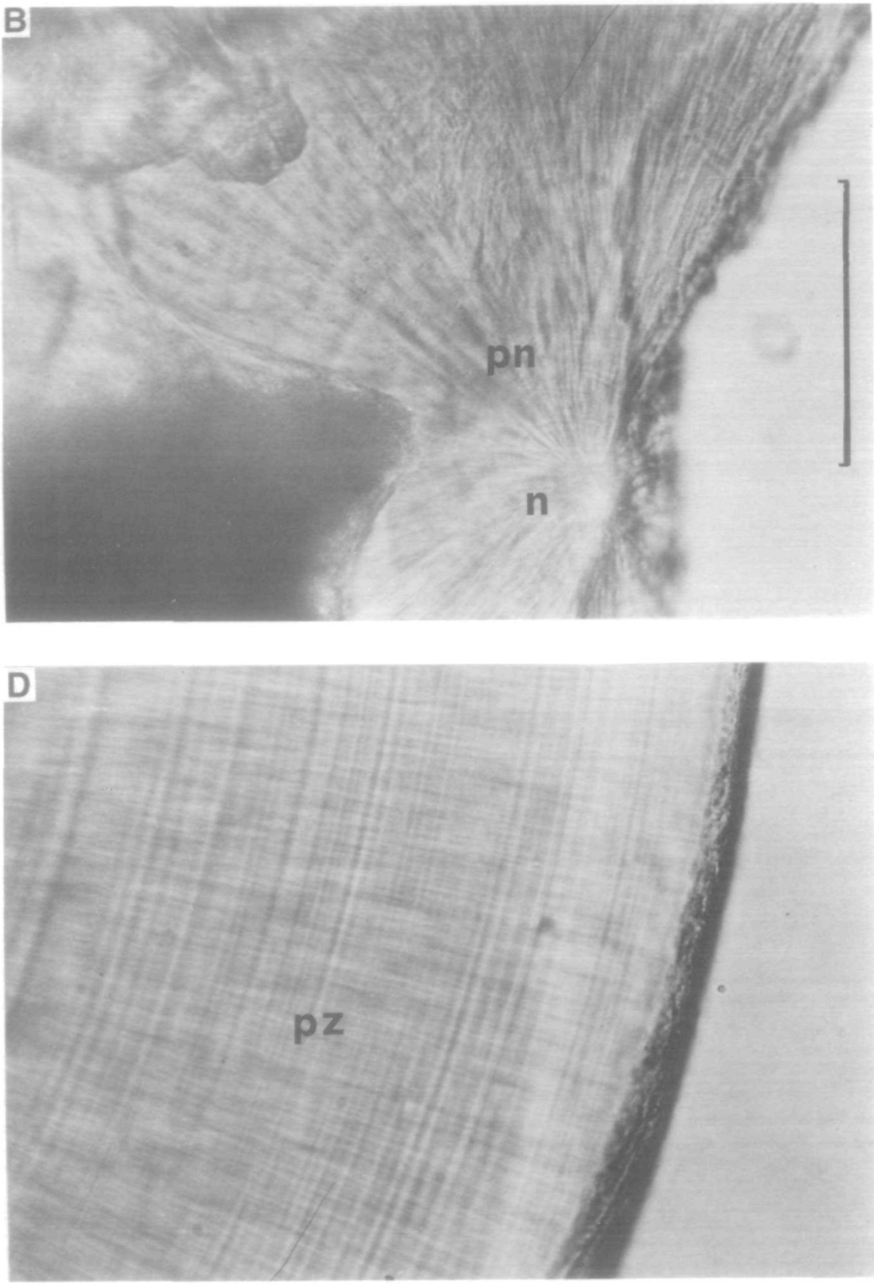


Fig. 2. Light photomicrographs of the statoliths of *B.borealis* (juvenile, ML 400 mm, **A**, **C**; juvenile, ML 440 mm, **B**, **D**). (A) General view; (B) nucleus (n) and postnuclear zone (pn);)C) dark zone (dz) with check (ch); (D) peripheral zone (pz). Scale bars = 100 μm. Scale bar of (B) also applies to (C) and (D).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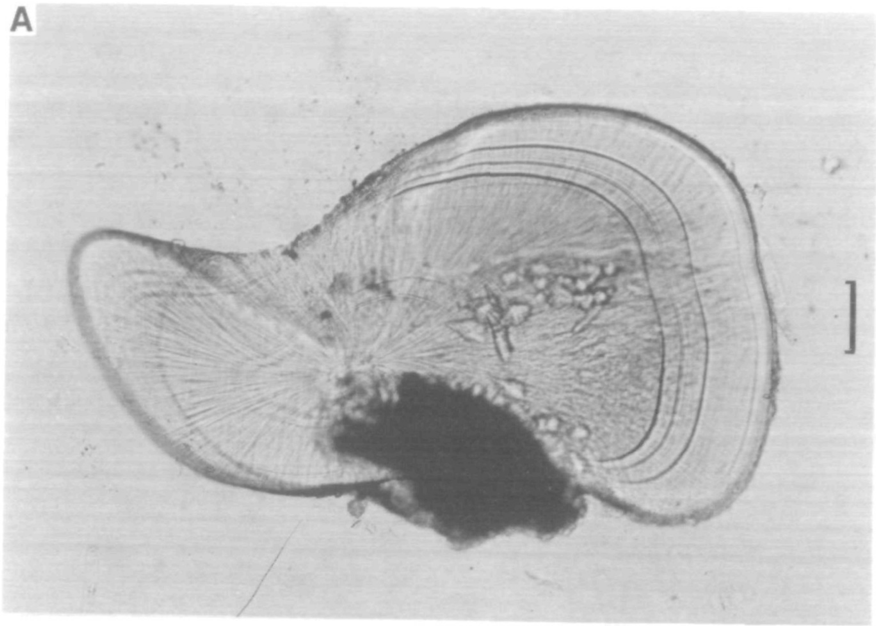


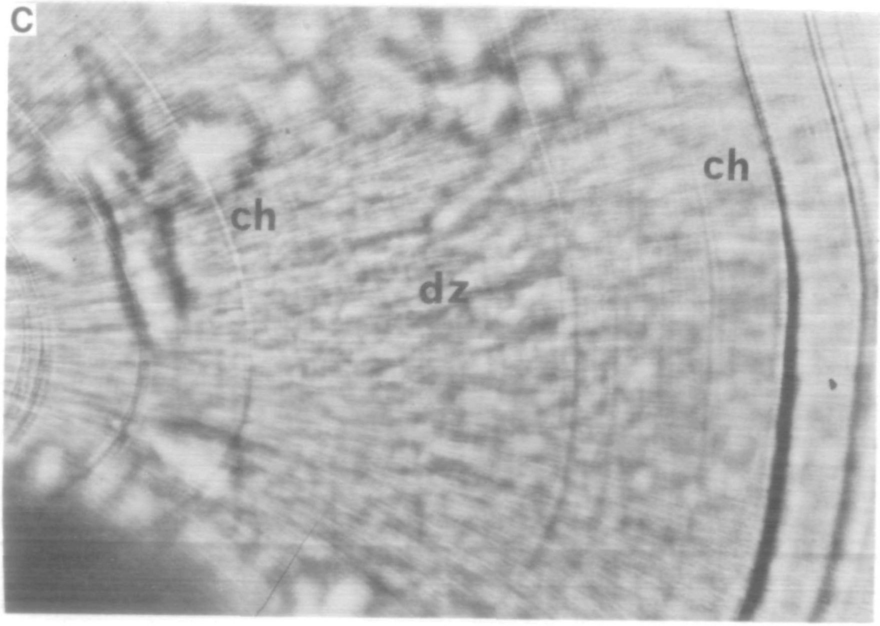
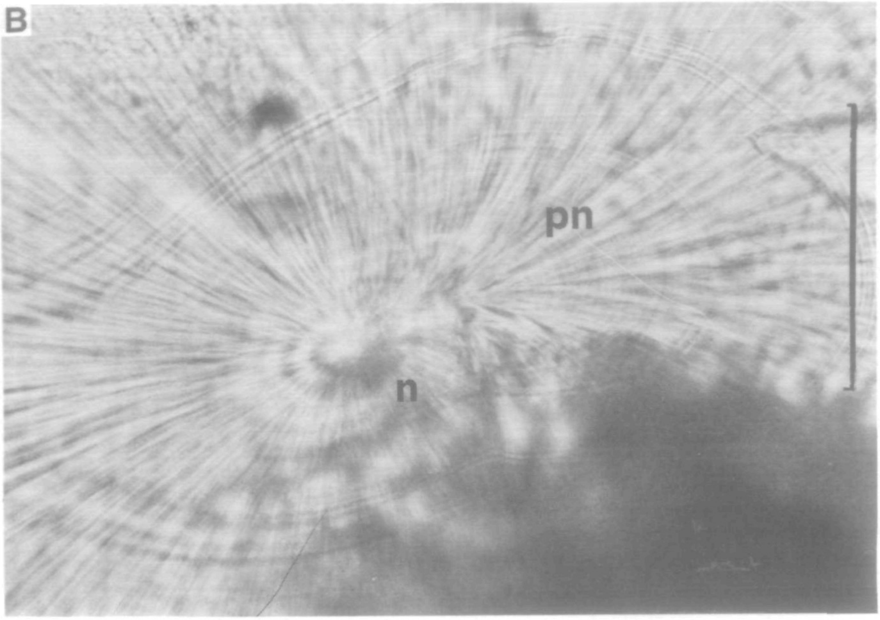


Fig. 3. Light photomicrographs of the statolith of *G.phyllura* (juvenile, ML 150 mm). (A) general view; (B) nucleus (n) and postnuclear zone (pn): (C) dark zone (dz) with checks (ch); (D) peripheral zone (pz) with checks (ch) near the statolith edge. Scale bars = 100 μm. Scale bar of (B) also applies to (C) and (D).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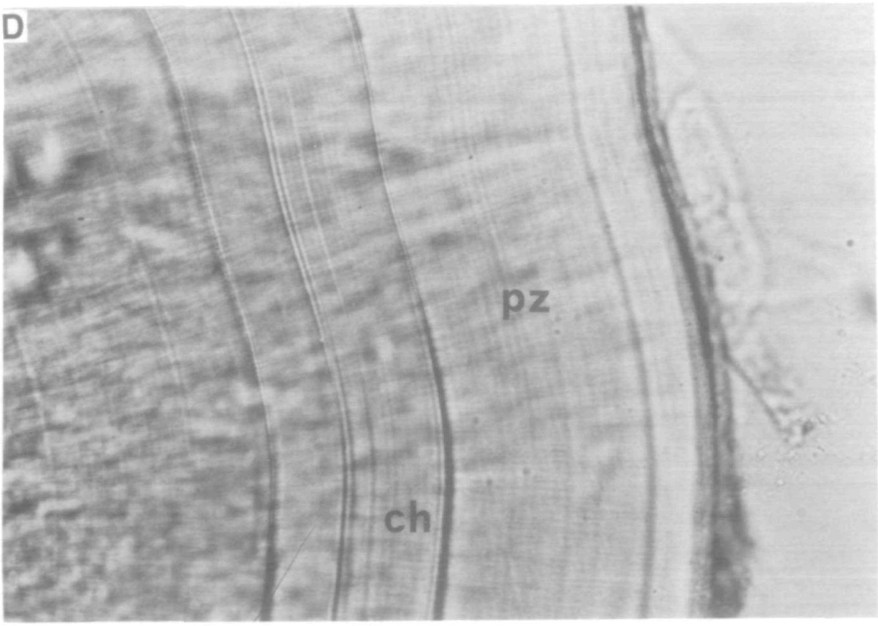
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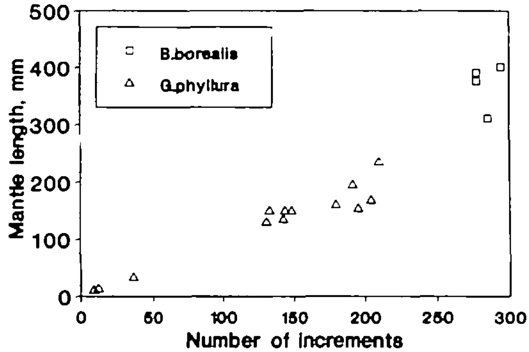

Fig. 4. Relationship between the number of growth increments within statoliths and mantle length of *B.borealis* and *G.glacialis*.

The total number of growth increments ranged between 10 in the paralarva of 9 mm ML and 209 in a juvenile of 235 mm ML (Figure 4). The average daily growth rate was 1.13 mm day^{-1}, and the relative daily growth rate in ML was 1.63% ML day^{-1}.

Adult statoliths of both studied species had a typical teuthid structure: smooth and well-developed dorsal and lateral dromes, rostrum and wing (Clarke, 1978). Specific features of *B.borealis* and *G.phyllura* statoliths are the dorsal dome bent in the midpart like a scoop and a short rostrum strongly curved anteriorly. They are similar in shape to statoliths of the cranchiid *Egea inermis* (Clarke, 1978).

The ground statoliths of both species had well-defined countable growth increments.

The width and appearance of statolith growth increments of *B.borealis* and *G.phyllura* resemble validated (daily) increments in *Illex illecebrosus* (Hurley *et al.*, 1985), *Alloteuthis subulata* (Lipinski, 1986) and others (for a review, see Jackson and Choat, 1992). It is interesting that the statoliths of the *G.phyllura* specimen which was kept alive for 1 day had a prominent check and then one translucent ring. Thus, it is possible to consider the growth increments within *G.phyllura* statoliths as daily.

It is notable that all the specimens studied (including squids of maximum sizes 235 mm ML *G.phyllura* and 400 mm ML *B.borealis*) were juveniles aged 209 and 294 days respectively. The age of *G.phyllura* (235 mm ML—209 days) was somewhat less than that of comparatively sized individuals of the Antarctic *G.glacialis* (198 and 218 mm ML—214 and 254 days, respectively) (Jackson and Lu, 1994). However, larger sample sizes are needed before further comparisons can be made between these two polar species. Based on maximum sizes of *B.borealis* and *G.phyllura* captured in epi- and mesopelagic waters (400–500 mm; Nesis, 1985), both of these species appear to grow quickly (only in length, not in weight) during the first 8–10 months of their juvenile phase in the upper mesopelagic layers, and then migrate into deep bathyal waters for maturation.

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