Statolith shape and microstructure in studies of systematics, age and growth in planktonic paralarvae of gonatid squids (Cephalopoda, Oegopsida) from the western Bering Sea

Alexander I.Arkhipkin and Vyacheslav A.Bizikov[1]

Atlantic Research Institute of Marine Fisheries and Oceanography (AtlantNIRO), 5 Dm. Donskoy Street, Kaliningrad, 236000 and [1]Russian Research Institute of Marine Fisheries and Oceanography (VNIRO), 17a V.-Krasnoselskaya Street, Moscow, 107140, Russia

Abstract. The microstructure, morphology and ontogenetic development of statoliths and age and growth of 405 planktonic paralarvae and 117 juveniles belonging to 10 species of gonatid squids (Cephalopoda, Oegopsida) were studied in the region of the continental slope in the western part of the Bering Sea (57°00′–61°30′N, 163°00′E–179°20′W). The statolith microstructure of all species was characterized by the presence of a large droplet-shaped nucleus and bipartite postnuclear zone divided into two by the first stress check, except for *Berryteuthis magister* which had only one stress check and an undivided postnuclear zone. In *Gonatus* spp., completion of development of the post-nuclear zone coincided with full development of the central hook on the tentacular club. Daily periodicity of statolith growth increments was validated by maintaining 13 paralarvae of the four most abundant species in captivity. All species might be subdivided into two groups based on statolith microstructure, i.e. species with a central position of the nucleus within the first statolith check (*Gonatopsis* spp., *Egonatus tinro* and *B.magister*) and species with the nucleus shifted to the inner side of the first statolith check (*Gonatus* spp.). Comparative analysis of statolith morphology showed that paralarval statoliths have species-specific characters that allowed the construction of keys to identify species of gonatid paralarvae based on their statoliths. Analysis of paralarval growth using statoliths revealed that these cold-water planktonic gonatid paralarvae have fast growth rates, attaining a mantle length of 7–10 mm at 15–20 days and 20–25 mm at 35–70 days.

Introduction

The Bering Sea is unique, because it is inhabited by squids belonging to only three families; Gonatidae, Onychoteuthidae and Cranchiidae. Except for two species of meso-bathypelagic planktonic cranchiids and a rare giant nektobenthic onychoteuthid *Moroteuthis robusta*, the squid fauna is dominated by 11–12 gonatid species which occur from the continental slope to the open sea (Nesis, 1973, 1987). Gonatids represent two major ecological types: eurybathic forms (*Gonatus* spp., *Gonatopsis borealis*, *Berryteuthis anonychus*) and nektobenthic forms (rest of the *Gonatopsis* species and *Berryteuthis magister*) (Okutani *et al.*, 1988). Because of their high abundance (Laevastu and Fiscus, 1978; Kubodera and Jefferts, 1984), gonatids are the important members of the trophic webs in the Bering Sea, consuming large quantities of planktonic crustaceans, fish and squids. They are also important prey for numerous pelagic and (demersal) slope fishes, sea birds and marine mammals (Kuznetsova and Fedorets, 1987; also a review in Okutani *et al.*, 1988).

Except for *B.magister* (Natsukari *et al.*, 1993; Arkhipkin *et al.*, 1996) and *G.borealis* (Okutani *et al.*, 1988), data on the biology of other North Pacific gonatid species remain scarce. Based on changes both in tentacular club armature

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and body proportions, Kubodera and Okutani (1981) have described growth stages of eight North Pacific gonatids and have produced a key for the species identification of paralarvae and juvenile gonatids. Tentacular and arm hooks develop at specific sizes in each gonatid species, which enables the identification of different species even at the paralarval stage (Okutani *et al.*, 1988). Changes in allometric growth of different parts of the body have revealed some inflection points indicating possible shifts in the ecology of paralarval and juvenile gonatids (Kubodera, 1982; cited in Okutani *et al.*, 1988). Kubodera (1982) has suggested an exponential growth form for the early life stages of *Gonatus madokai*. Direct investigations of paralarval and juvenile age, growth and possible ecological onto-genetic shifts in gonatid squids have not yet been carried out.

Statoliths (and in some cases gladii) appear to be the only structures in squid containing information about age (number of growth increments), growth (width of growth increments) and possible ontogenetic events ('checks') and shifts (bands or zones) from hatching until the death of the animal (Arkhipkin and Bizikov, 1991). This has also been found to be true for fish otoliths (Campana and Neilson, 1985). Among gonatids, statolith microstructure was first studied in an Atlantic *Gonatus fabricii* (Kristensen, 1980). In the Northern Pacific, statolith microstructure has been investigated only in *B.magister* (Natsukari *et al.*, 1993; Arkhipkin *et al.*, 1996).

The abundance and ecological diversity of the gonatid squids in the Bering Sea provided a good opportunity to undertake a comparative analysis of the statolith morphology and growth of closely related species possessing different life styles. The main aim of this report is to investigate statolith morphology, microstructure, age and growth of gonatid squids at paralarval and juvenile ontogenetic stages, and to apply these data to their systematics.

Method

Sampling

Gonatid paralarvae and small juveniles [mantle length (ML) < 30 mm] were collected during six plankton surveys carried out above and off the continental slope of the western part of the Bering Sea (57°00′–61°30′N, 163°00′E–179°20′W) by the Japanese fishing vessel 'Tenyu Maru N 57' between June and October 1994 and 1995. The surveys were executed under the 5 year scientific program on resources of the gonatid squid *B.magister* within the Exclusive Economic Zone of Russia, established by the Russian Federal Committee of Fisheries in 1993. Hauls were made in areas where the bottom depth exceeded 400 m using an Isaacs–Kidd midwater trawl (175 cm depressor width, 1 mm mesh). During each plankton station, three horizontal hauls were taken at three depths: 70, 140 and 280 m. The duration of each haul was 30 min with an average vessel speed of 3.3–4.5 km h^{-1}.

Additionally, large juveniles and subadults (ML > 30 mm) were taken both from the mesh and sac of the Japanese commercial bottom trawl (42 m horizontal opening, 6 m vertical opening and 70 mm double mesh at the distal end of the sac). Trawl depth was between 370 and 700 m between the plankton stations in

the daytime. The duration of each trawl was 1 h with an average speed of 7.4–8.3 km h^{-1}.

Catches were analyzed immediately on board ship. A total of ~3000 paralarvae and small juveniles belonging to nine gonatid species were caught during the six surveys. Paralarvae and small juveniles were sorted and first placed in 2 l jars containing seawater. Then, their ML was measured to the nearest 0.1 mm under an Olympus SZH10 zoom microscope in the ship laboratory. They were identified using the key by Kubodera and Okutani (1981). A total of 117 large juveniles and subadults (ML >30 mm; Table I) were caught and identified using two different keys (Kubodera and Okutani, 1981; Nesis, 1987). Their ML was measured to the nearest 1 mm.

Statolith sampling and processing

A total of 405 pairs of statoliths were sampled from nine species of gonatid paralarvae (Table I). Statoliths were removed immediately after measurements and identification of paralarvae aboard ship. Statoliths were dissected under the zoom microscope with a fine needle and first placed into a drop of fresh water to wash off organic debris. After thorough removal of water with a piece of filter paper, each pair of statoliths was placed on a microscope slide, embedded either into Pro-texx or Canada balsam and covered with a cover slip. Pro-texx was found to be unsuitable for small paralarval statoliths. During drying it substantially decreased its volume, causing the statoliths to be crushed by the cover slip, and large air bubbles formed preventing further analysis. The use of Canada balsam overcame these problems and it was chosen as the only embedding medium for paralarval statoliths. Statoliths of paralarvae and small juveniles were transparent which allowed their inner microstructure to be analyzed without grinding.

Statolith measurements were after Clarke (1978). Total statolith length (TSL), statolith width (SW), rostrum length (LRO) and rostrum width (WRO) (Figure 1) were measured to the nearest 0.01 mm using either a Nikon (Type 104)

Table I. Mantle length ranges (in mm), numbers of specimens studied (*N*) and their statoliths analyzed (Nst) of paralarvae and juvenile squids of the family Gonatidae from the western Bering Sea

No.	Species	Paralarvae			Juveniles + subadults	
		ML range	*N*	Nst	ML range	Nst
1	*Gonatus berryi*	8.5–14.0	15	14	51–215	35
2	*Gonatus madokai*	6.0–28.2	37	19		
3	*Gonatus middendorffi*	6.9–18.2	67	67	415	1
4	*Gonatus onyx*	5.7–26.0	215	121	91–120	5
5	*Gonatus pyros*	7.2–16.0	13	13	93–123	9
6	*Eogonatus tinro*	9.5–28.0	26	19	48	1
7	*Gonatopsis borealis*	6.0–27.1	18	18	42–155	38
8	*Gonatopsis makko*				380	1
9	*Gonatopsis okutanii*	9.2–11.5	4	4	200–208	2
10	*Berryteuthis magister*	6.5–20.0	9	9	32–120	25
Total			404	284		117

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Dorsal side
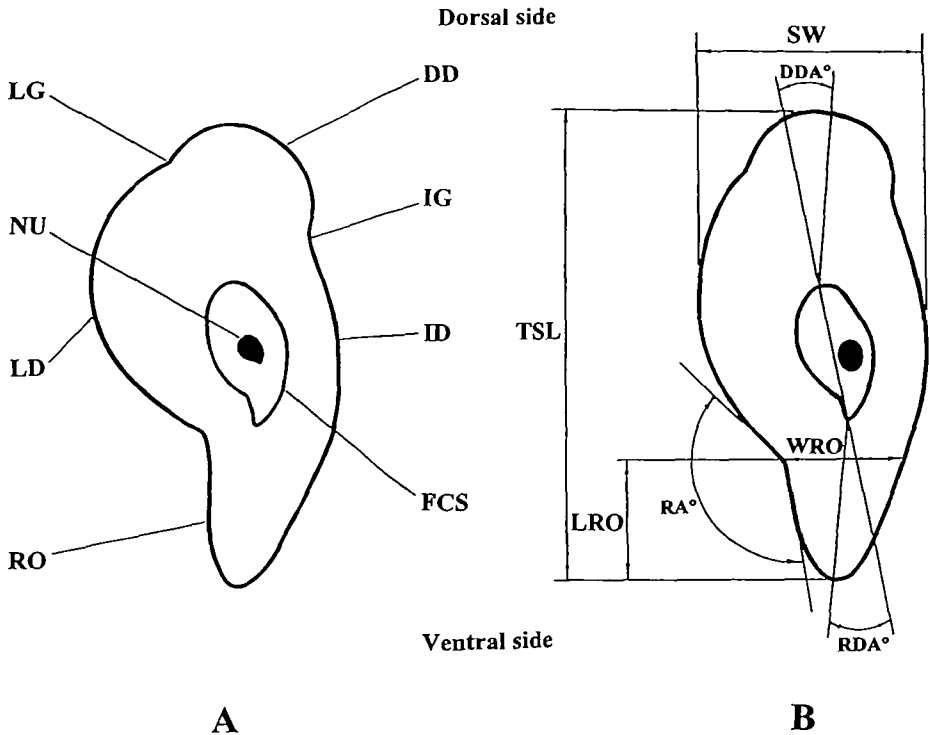

Ventral side

A   B

Fig. 1. Terminology, orientation (A) and measurements ((B) of a paralarval statolith (lateral view, lateral side left, inner side right). (A) Statolith with the nucleus in the center of the first check stato-lith; (B) statolith with the nucleus shifted toward the inner side of the first check statolith. NU, nucleus; LD, lateral dome; RO, rostrum; FCS, first check statolith; ID, inner dome; DD, dorsal dome; LG, lateral groove; IG, inner groove; TSL, total statolith length; SW, statolith width; LRO, rostrum length; WRO, rostrum width; RA°, rostrum angle; RDA°, rostrum deviation angle; DDA°, dorsal dome devi-ation angle.

light microscope (statoliths of paralarvae) or zoom microscope (statoliths of juven-iles). Additionally, the rostrum angle between the rostrum and lateral dome (RA°), the rostrum deviation angle (RDA°) and the dorsal dome deviation angle (DDA°) were measured on micrographs or drawings of some statoliths (Figure 1).

Statoliths of large juveniles (ML > 30 mm) had an opaque surface and had to be ground for their microstructure analysis (Dawe and Natsukari, 1991). Usually, one statolith from each pair was ground on both sides using wet waterproof sand-paper (600 grit) and polished with fine sandpaper (1000 grit). Ground statoliths were embedded in Canada balsam and placed in an oven (80–90°C) for 1 h to dry the balsam. If the statolith was overground, another statolith of the same speci-men was ground. Statoliths of large juveniles were used for a comparative analy-sis of their microstructure (mainly postnuclear and dark zones) with that of paralarvae and small juveniles.

Patterns of the statolith microstructure were examined under the light micro-scope (400×). The maximum diameters of the nucleus (ND) and paralarval

Table II. Minimum (min), maximum (max) and average (x) values of different features in the statolith microstructure of paralarvae and juvenile gonatid squids from the western Bering Sea

		Paralarvae					Juveniles + subadults			
		ND	Ch1	Ch2	NGI1	NGI2	Ch1	Ch2	NGI1	NGI2
Gonatus	min	30	90	270	6	21	100	350	7	15
berryi	max	45	180	270	16	23	205	525	16	43
	x	35.3	112.3	270	9.2	22	141.96	414.57	11.8	27.9
	SD	3.85	24.68	0	2.78	1	25.82	36.68	2.35	6.69
	n	15	15	2	15	2	28	23	34	31
Gonatus	min	20	80		4					
madokaı	max	50	135		11					
	x	30.8	104.4		7.47		No specimens			
	SD	7.52	11.23		1.6					
	n	34	34		36					
Gonatus	min	25	75	205	5	7				
middendorffi	max	45	240	355	13	14				
	x	33.3	118.6	268.17	6.56	9.19	130	310	8	12
	SD	4.98	24.64	32.474	1.25	1.85	0	0	0	0
	n	65	65	41	66	41	1	1	1	1
Gonatus	min	20	75	175	4	6	150	400	9	27
onyx	max	50	165	580	14	44	160	420	11	44
	x	36.3	119.7	328.2	8.26	18.7	155	410	10	33.6
	SD	5.9	13.18	95.399	2.42	12.1	5	10	0.81	7.4
	n	213	206	64	208	62	2	2	3	3
Gonatus	min	30	90		5		80	265	5	18
pyros	max	45	200		20		110	500	12	42
	x	34.6	115		9.38		92.78	334	8.88	28.6
	SD	4.98	32.64		3.58		7.86	85.4	1.96	7.88
	n	13	13		13		9	5	9	5
Eogonatus	min	35	135	240	6	8				
tinro	max	55	260	365	19	10				
	x	46.1	182	332.85	11.8	8.71	195	350	13	10
	SD	9.46	32.78	40.076	3.61	0.88	0	0	0	0
	n	27	27	7	27	7	1	1	1	1
Gonatopsis	min	35	75	185	5	4	105	175	8	6
borealıs	max	50	210	260	18	16	190	260	36	19
	x	42.2	162.7	225.83	13	8.2	133.87	202	15	10.4
	SD	4.77	31.72	23.525	3.28	4.11	25.68	23.21	7.6	3.06
	n	18	18	6	17	5	31	20	38	21
Gonatopsis	min									
makko	max									
	x	No specimens					280	460	15	15
	SD						0	0	0	0
	n						1	1	1	1
Gonatopsis	min	25					160	275	14	12
okutanii	max	35					260	275	22	12
	x	30					210	275	18	12
	SD	3.53					50	0	4	0
	n	4					2	1	2	1
Berryteuthis	min	35	160		7		170		9	
magister	max	55	250		16		240		15	
	x	43.3	198.3		11		189.5		11.7	
	SD	6.66	24.03		2.78		19.7		2.52	
	n	9	9		9		25		25	

SD, standard deviation; *N*, number of observations; ND, nucleus diameter (in µm); Ch1, Ch2, maximum diameters of the first and second checks, and numbers of growth increments between: nucleus and Ch1 (NGI1), Ch1 and Ch2 (NGI2).

statoliths outlined by the first check (Ch1) and second check (Ch2) were measured to the nearest 2 μm using an eyepiece micrometer. Growth increments were counted by two observers separately in the following parts of the statolith microstructure: from the nucleus to the first check (NGI1), between the first and second checks (NGI2), and between the second check and statolith edge (Table II). The sum of the growth increments within all growth zones of a statolith was taken as the total number of growth increments. Total increment number was taken as the mean of the counts made by each observer if the deviation between the two counts was <5%.

Validation of the periodicity of increment formation within statoliths

To validate the periodicity of increment deposition, it was assumed that stress during capture by trawl could induce the development of a well-defined stress mark ('check') within the statolith. The same phenomenon had been observed in fish otoliths (Campana and Neilson, 1985). For increment validation, live gonatid paralarvae (total 13 specimens of four species; Table III) were kept for 1–4 days (until death) in small aquaria (3 l) placed in a refrigerator, with water temperature close to that at the sampling depth (2–4°C). After death, individuals were measured and identified. Their statoliths were examined for the presence of any check near the statolith margin. If such a check existed, the number of growth increments between it and the statolith edge was counted and compared with the number of elapsed days between capture and death of the paralarva (Table III).

Growth parameters

Relationships between ML and TSL, and between the total number of growth increments (age?) and ML, were analyzed separately for each gonatid species. Growth curves were constructed using the method of iterative non-linear least

Table III. Results of validation of the periodicity of increment formation within statoliths of paralarval gonatid squids in the western Bering Sea in 1994

No.	Species	ML	Date	ED	PC	NI
1	G.onyx	14.0	October 6	1	No	
2	G.onyx	16.3	September 18	3	Yes	3
3	G.onyx	17.0	October 6	1	Yes	1
4	G.onyx	18.5	October 10	4	Yes	3
5	G.onyx	19.0	October 10	4	Yes	4
6	E.tinro	9.0	June 21	1	Yes	1
7	E.tinro	9.5	June 19	1	Yes	1
8	E.tinro	12.0	June 15	3	Yes	2
9	E.tinro	13.2	June 18	2	Yes	2
10	G.borealis	9.2	June 19	1	Yes	1
11	G.borealis	9.8	June 19	1	Yes	1
12	B.magister	9.5	June 19	1	No	
13	B.magister	20.0	September 18	3	Yes	3

ML, mantle length (mm); Date, date of capture; ED, elapsed days in captivity until death; PC, presence of check near the statolith edge; NI, number of increments between the check and statolith edge.

squares. The following curves were used to fit the data: power, exponential, logarithmic and logistic curves. The formula of the best fitted curve was determined by both the least coefficients of variance of estimated parameters and the highest R^2. The growth curves were constructed predominantly for growth rate estimation, therefore no biological meaning was applied to their parameters. In the case of an exponential relationship between age and ML, the instantaneous growth rate (G) was considered to be equal to the exponent index. Growth curves were not constructed for the relationship between the total number of growth increments and TSL because the rostrum and dorsal dome of the statolith change the direction of their growth during ontogenesis (Figure 1).

Terminology of statolith microstructure and shape

Statolith microstructure is described following the terminology of Lipinski *et al.* (1991). The nucleus is situated under the statolith spur, and outlined by the first growth increment (Figure 1). Usually, it is possible to see a dark concretion in the center of the nucleus, called the 'focus', i.e. the central point of statolith formation. Outside the nucleus, there are alternating dark and translucent concentric rings. One dark and one translucent growth ring comprise each growth increment. Growth increments can also be grouped into growth zones (i.e. postnuclear, dark and peripheral zones). Sometimes there were more pronounced growth increments or 'checks' differing in color intensity from the nearby growth increments. They were considered as stress checks resulting from hatching, sudden disturbance during capture or even a storm (Campana and Neilson, 1985). The paralarval statolith at the time of the first check formation is referred to here as the 'first-check statolith'.

   Statoliths of juvenile gonatids consist of four main parts: lateral dome, dorsal dome, rostrum and wing (Clarke, 1978). Statoliths of paralarvae have some specific characters in their morphology. The wing was either absent (in small paralarvae) or handle shaped, being attached with its upper part to the dorsal dome and with its lower part to the rostrum. The spur is absent. In the present report, some new terminology is suggested to describe the morphology and development of paralarval statoliths: 'inner dome', the inner convex part of a paralarval statolith; 'inner groove', the groove between the dorsal and inner domes; 'lateral groove', the groove between the dorsal and lateral domes; 'rostrum deviation angle' (RDA°), the angle between the longitudinal axis of the first-check statolith and the rostrum axis; 'dorsal dome deviation angle' (DDA°), the angle between the longitudinal axis of the first-check statolith and the dorsal dome axis (Figure 1).

Results

Validation of statolith growth increments

The results of validation experiments are shown in Table III. Among 13 specimens studied, two paralarvae (one *G.onyx* and one *B.magister*) had no distinct check within the statolith microstructure near the statolith edge. Both squids
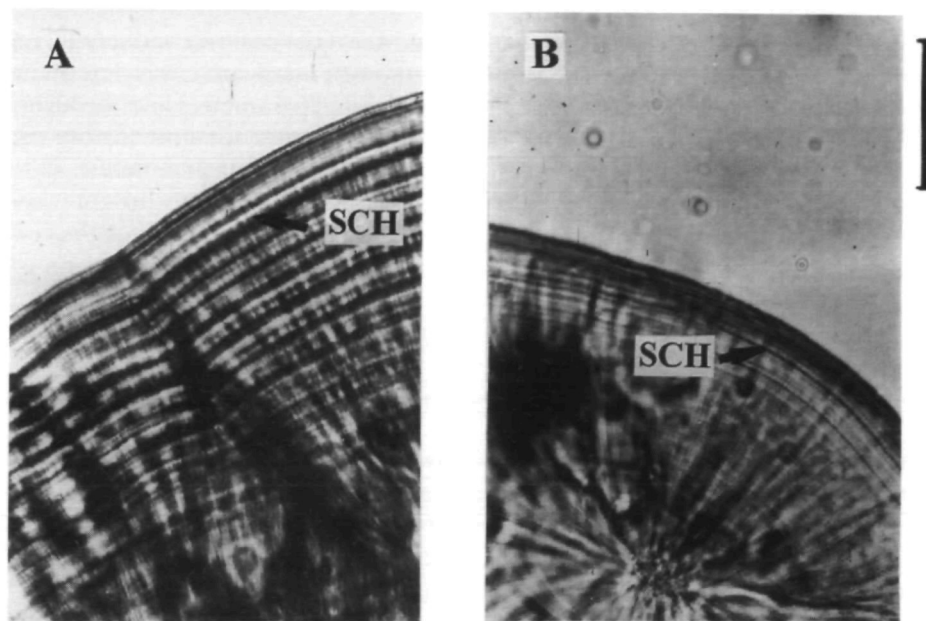
Fig. 2. Light photomicrograph of the statolith of paralarvae of *Berryteuthis magister* (20 mm ML) (**A**, **B**) and *Gonatus onyx* (16.3 mm ML) kept for 3 days in an aquarium. SCH, stress check (three growth increments outside the check). Scale bar = 50 μm.

lived for 1 day in aquaria and were possibly not in good condition, constantly remaining near the bottom. The rest of the squid were quite active in aquaria, especially during the first day of maintenance, moving either in the midwater or near the bottom. A distinct stress check was observed near the statolith edge in all of these specimens, belonging to four gonatid squids (Table III, Figure 2). The number of growth increments outside this stress check was similar to the number of days elapsed between capture and death of the paralarvae in all but two para-larvae which had one less ring (Table III). In all specimens analyzed, the stress checks were situated outside the first check, being either between the first and second check (as in small *E.tinro*) or outside the second check (as in *G.onyx* of 18.5–19.0 mm ML). Thus, it was possible to consider growth increments within statoliths of gonatid paralarvae as daily, at least in the portion outside the first check, in all four species examined.

Statolith microstructure and development; age and growth

Patterns of statolith microstructure and development, as well as age and growth, were described separately for paralarvae and small juveniles of each gonatid species studied. Patterns of statolith microstructure within the same growth zones were practically similar in both paralarval and juvenile statoliths of each species (Table II). Owing to the absence of large paralarvae in our samples (as in *Gonatus berryi* and *Gonatus pyros*), microstructural peculiarities of the outer part of the
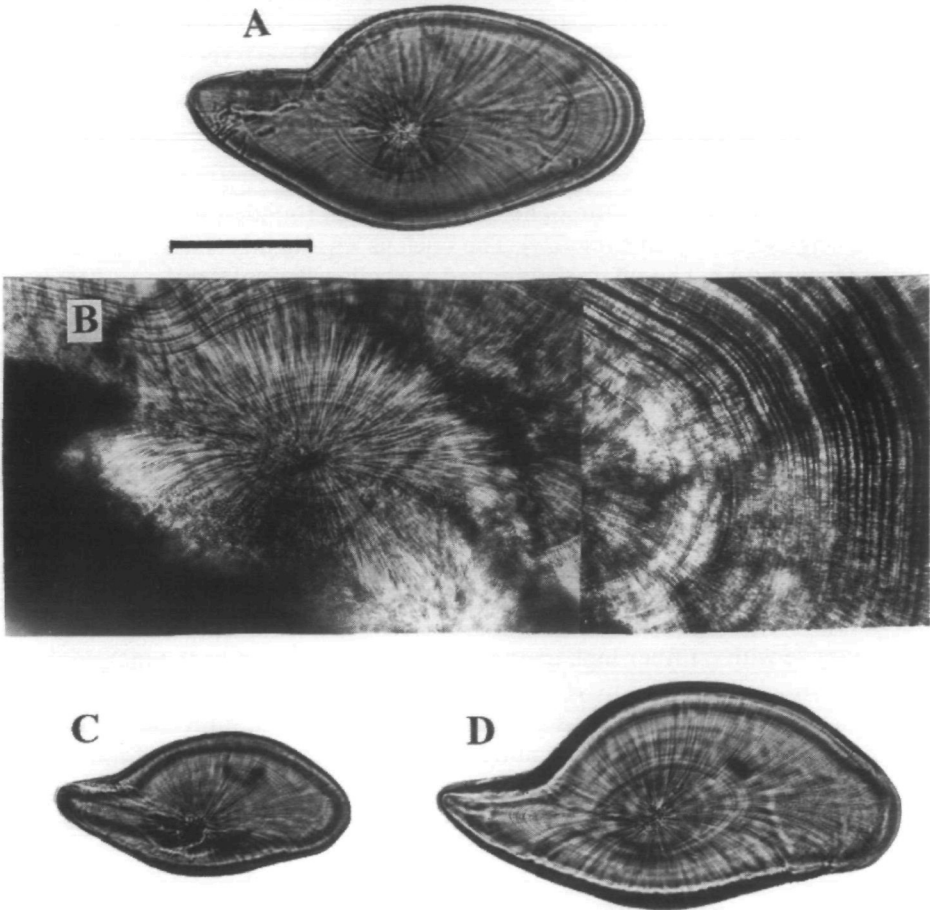
Fig 3. Light photomicrographs of the statolith of *Gonatus berryi* (**A**, **B**) and *Gonatus madokai* (**C**, **D**). (A) Statolith of paralarva (12 mm ML); (B) postnuclear and dark zone of juvenile (134 mm ML); (C) statolith of paralarva (14.8 mm ML); (D) statolith of small juvenile (23.4 mm ML). Scale bar = 100 µm.

postnuclear zone and dark zone were described using statoliths of large juveniles. Paralarvae of *G.makko* were not found in our samples, so the development and microstructure of its paralarval statolith were studied using statoliths of a subadult specimen.

Gonatus berryi *Naef, 1923*

Statolith microstructure. The nucleus was round. From 6 to 16 (mean 9) faint and uniform increments (~5 µm in width) were observed inside the first check (Figure 3A and B). The first check looked like a single or double prominent increment. Between the first and second checks, increments became narrower and more distinct than those in the former zone (4–4.5 µm). Outside the second check, the

increments were well defined and narrow (3–4 µm). In the dorsal dome, narrow and uniform subdaily increments (1.5–1.7 µm in width) were visible sometimes in the inner part of the dark zone. There were regularly spaced distinct checks in the dark zone. From 6 to 11 (mean 7–8) growth increments were observed between each of these two checks.

Statolith development. The first-check statolith was rounded in outline, with a large triangular rostrum (Table IV). The nucleus was slightly shifted to the inner dome (Figure 4A). The lateral dome had a swollen dorso-lateral and flattened ventro-lateral side. The rostrum was wide at its base. At 10 mm ML, statolith proportions remained generally the same, but the dorsal dome started developing as a distinct projection in the upper part of the statolith. The rostrum narrowed and bent laterally. At 15 mm ML, the dorsal dome distinctly separated both from the lateral and inner domes. The lateral dome was wide and low, similar in outline to the inner dome. The rostrum apex became blunt, with the dorsal dome becoming more prominent and separated from the lateral dome, gradually bent to the inner side. The rostrum increased in length.

Age and growth. Relative sizes of statoliths remained almost the same in the paralarvae studied (2.46–2.67% ML). Allometric growth of the TSL versus ML was negative with a rather high coefficient '*b*' (0.668). The power function fitted to the relationship had a low R^2 (0.5057) due to the small number of specimens and the limited ML range. The first check within the statolith microstructure appeared at ML < 5 mm (about 4 mm ML, based on extrapolation of the curve); the second check formed at a mean ML of 18 mm (Figure 5A). Absolute growth of the statoliths was slow (Figure 5B). The youngest *G.berryi* (8.5 mm ML) was 17 days old, while the oldest specimen (11.5 mm ML) was aged 37 days. The relationship between ML and age was best described by the exponential curve (Figure 5C). Instantaneous rates of growth were low ($G = 0.0264$).

Gonatus madokai *Kubodera and Okutani, 1977*

Statolith microstructure. The nucleus was oval (Figure 3C and D). There were from 4 to 11 (mean 7) faint increments within the postnuclear zone (4–5 µm in width) (Table II). The first check was like a single non-prominent increment. Outside the first check, increments were faint, uniform and narrower than those inside it.

Statolith development. The first-check statolith was somewhat elongated (Table IV) with a swollen lateral dome, small sharp rostrum and nucleus situated close to the inner dome (Figure 4C). At 10 mm ML, the lateral dome was flattened dorso-laterally and swollen ventro-laterally. The dorsal dome became more prominent and bent to the inner side of the statolith. At 15 mm ML, the dorsal part of the inner dome became concave. At 25 mm ML, the dorsal dome was prominent, round in outline and distinctly bent to the inner side. The inner dome
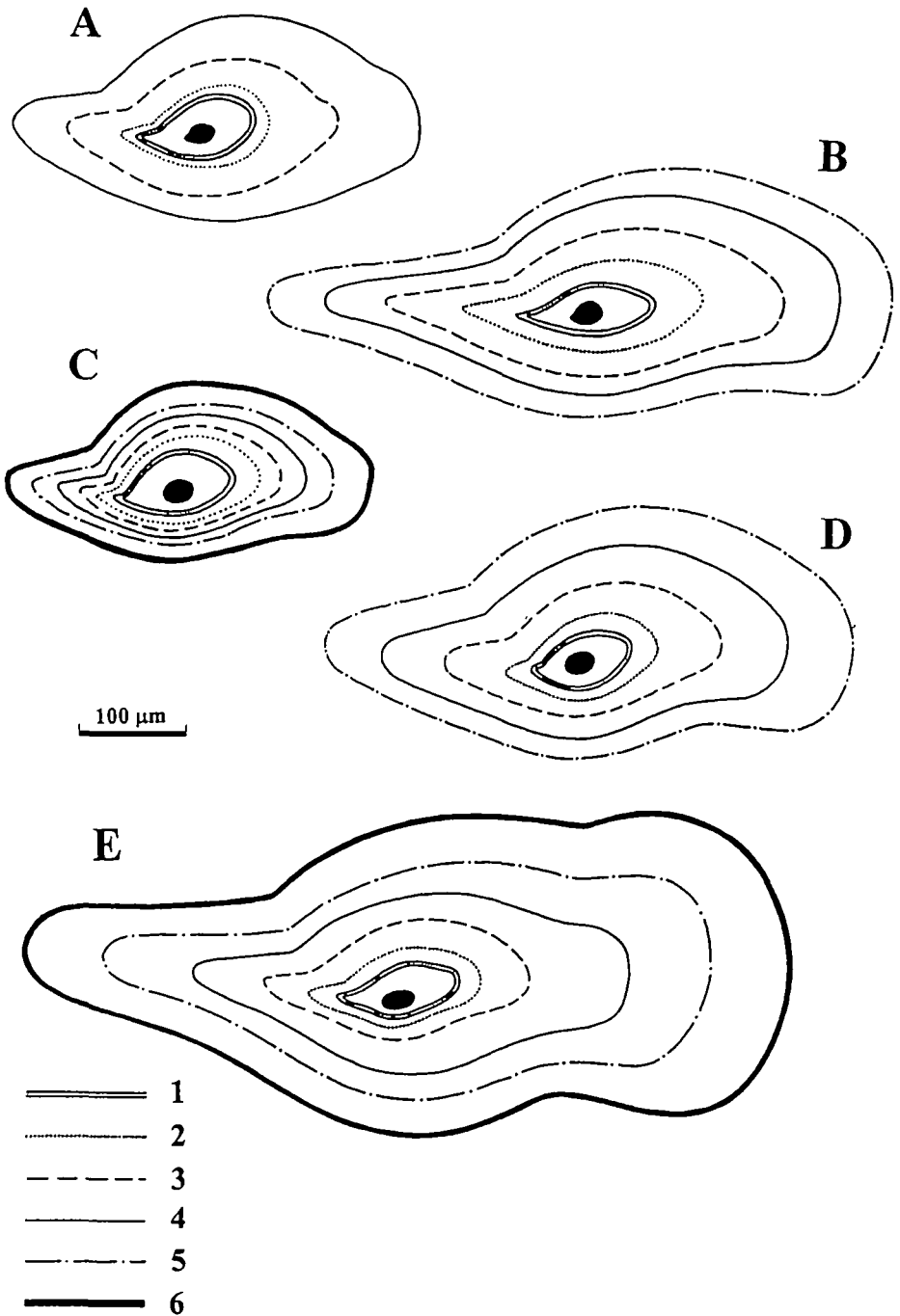
A

B

C

D

100 μm

E

1
2
3
4
5
6

Fig. 4. Schemes of statolith development in the paralarvae of *Gonatus* spp. (**A**) *G.berryi*; (**B**) *G.middendorffi*; (**C**) *G.madokai*; (**D**) *G.pyros*; (**E**) *G.onyx*. Statolith outlines at: the first check (1); 5 mm ML (2); 10 mm ML (3); 15 mm ML (4); 20 mm ML (5); 25 mm ML (6).

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Table IV. Morphometric features of gonatid paralarvae statoliths

	First-check statolith				Statolith at 15–25 mm ML				
	Nucleus position	SRW	RRL	RRW	SRW	RRL	RRW	DDA	RDA
Gonatus berryi	Slightly shifted	58	24–25	120–130	40–54	24–26	120–125	5–7	15
Gonatus madokai	Strongly shifted	52	13	115–135	46–55	20–25	90–95	16	20
Gonatus middendorffi	Strongly shifted	43	22	68–87	36–40	38	66–71	13	6
Gonatus onyx	Strongly shifted	60	16	80–95	40	32	65–75	8	19
Gonatus pyros	Slightly shifted	58	13	120	48	25–30	103–116	18	23
Eogonatus tinro	Center	47	23	95–100	46	30	85–88	19	17
Gonatopsis borealis	Center	48	28	90–105	50	33	80–90	13	20
Gonatopsis makko	Center	50	21	120	54	24	95–105	28	16
Gonatopsis okutanii	Center	50	25	100	50	24	110	11	24
Berryteuthis magister	Center	54	30	95–110	53	41	67–75	22	20

SRW, statolith relative width (%); RRL, rostrum relative length (%); RRW, rostrum relative width (%); DDA, dorsal dome deviation angle; RDA, rostrum deviation angle.

was markedly swollen in the lower part. The rostrum became longer, narrower and bent laterally.

Age and growth. Relative sizes of statoliths decreased during the ontogenetic period studied from 2.83% ML in paralarva of 6 mm ML to 1.4–1.45% ML in paralarvae of 18–19 mm ML. The relationship between TSL and ML was linear. The first check formed at ML < 5 mm (extrapolated value was ~3 mm ML); the second check was not observed in the largest paralarvae (19 mm ML) and probably developed at greater sizes (Figure 5D). Absolute growth of the statoliths was very slow (Figure 5E). The youngest specimen (6.0 mm ML) was 14 days old; the oldest paralarva (19.0 mm ML) was aged 33 days. Paralarvae had rapid exponential growth and high instantaneous growth rates ($G = 0.0407$). At 20 days, there was considerable variation in ML (from 10 to 16 mm) (Figure 5F).

Gonatus middendorffi *Kubodera and Okutani, 1981*

Statolith microstructure. The nucleus was almost round with a knob-like primordial rostrum (Figure 6A). There were from 5 to 13 (mean 6) faint increments within the postnuclear zone (~5 μm in width). The first check was a single prominent increment. Outside the first check, increments became distinct and uniform (4–5 μm) (Figure 6C).

Statolith development. The first-check statolith was slender (Figure 4B), with a narrow dorsal dome and long straight rostrum (Table IV). The nucleus was shifted to the inner dome. At 10 mm ML, the statolith became more elongated. The dorsal dome was also elongated, round in outline and bent to the inner side. The rostrum became longer and beak-shaped. At 15 mm ML, the dorsal part of the inner dome outline became concave, the dorsal dome distinctly bent to the inner side. The rostrum increased in length, its axis was close to the longitudinal axis of the statolith. The rostrum tip became round.
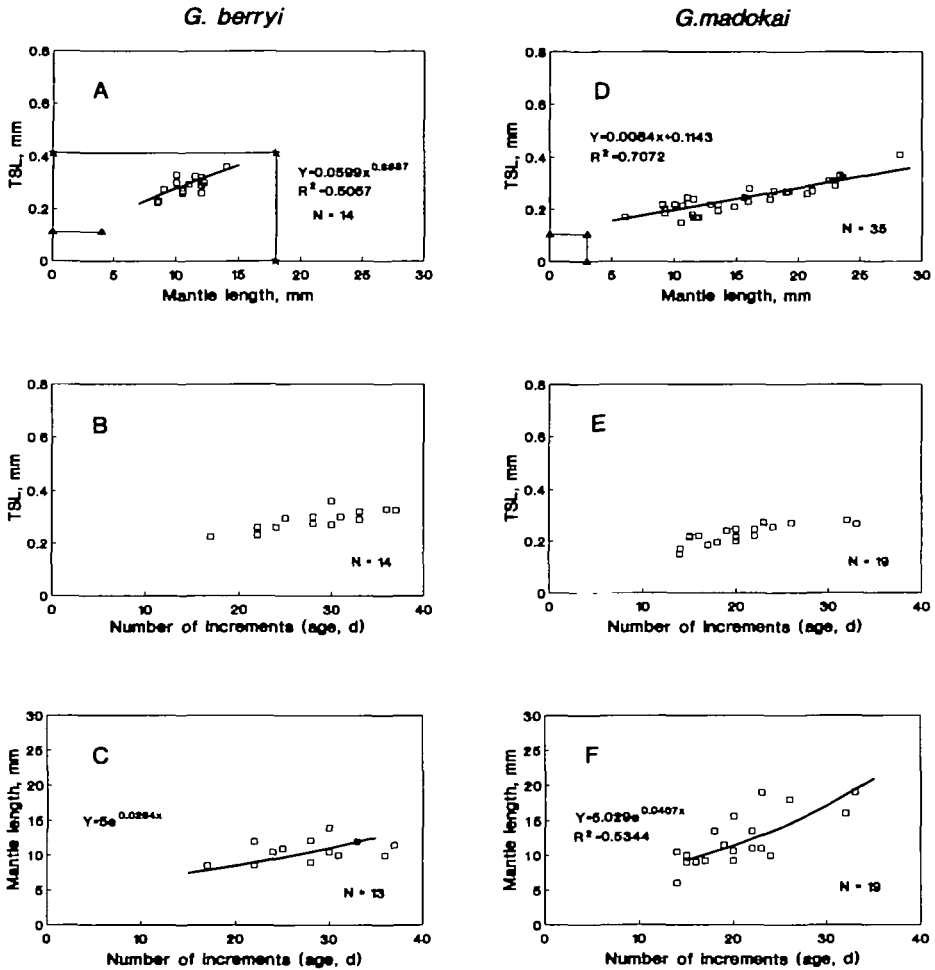
Fig. 5. Relationships between mantle length and total statolith length (**A, D**), number of increments and total statolith length (**B, E**) and number of increments and mantle length (**C, F**). *Gonatus berryi*, left; *Gonatus madokai*, right. Triangles, mantle length at appearance of the first check; stars, mantle length at appearance of the second check.

Age and growth. Statoliths were relatively long, their TSL decreased from 4.3–4.5% ML in small squids (6.9–7.1 mm ML) to 2.8–2.9% ML in large para-larvae (18–18.2 mm ML). The relationship between TSL and ML was best described by the logarithmic function. The first check formed at an extrapolated value of ~4 mm ML; the second check appeared rather early (at 7 mm ML) (Figure 8A). Absolute growth of statoliths was fast; they increased in TSL almost twice in squid aged between 13 and 32 days (Figure 8B). The youngest paralar-vae (7.5 and 9 mm ML) were 13 days old; the oldest paralarvae (15.9 and 18.2 mm ML) were aged 32 days. Paralarval growth was exponential with high instant-aneous rates of growth ($G = 0.0433$) (Figure 8C).
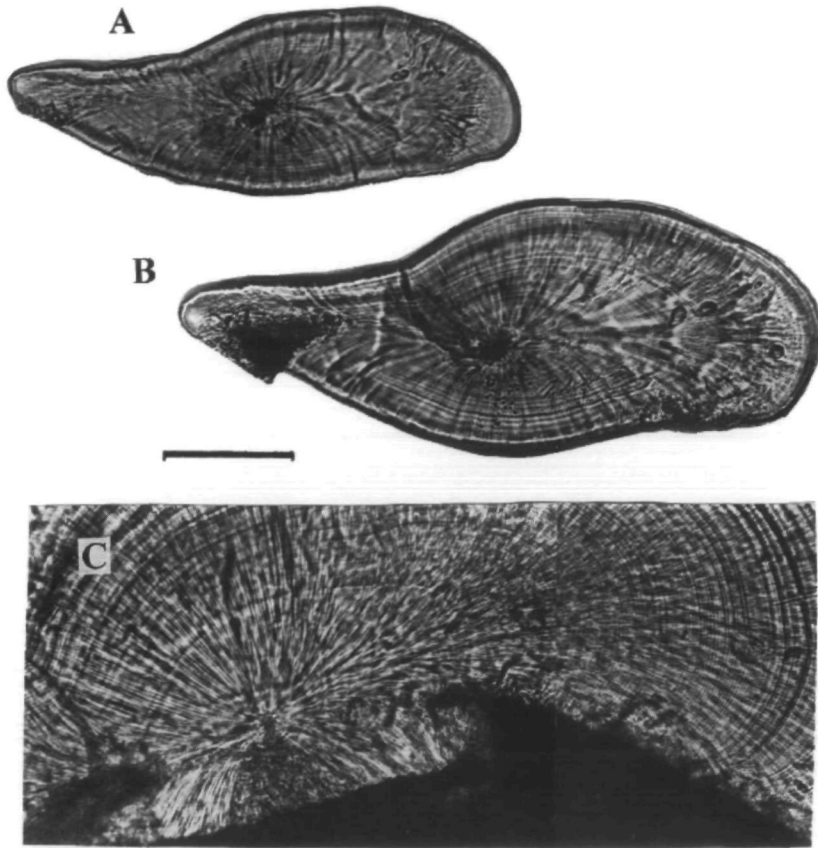
Fig. 6. Light photomicrographs of the statolith of *Gonatus middendorffi*. (**A**) Statolith of paralarva (11.3 mm ML); (**B**) statolith of paralarva (16.3 mm ML); (**C**) postnuclear and dark zone of immature female (415 mm ML). Scale bar = 100 µm.

Gonatus onyx *Young, 1972*

Statolith microstructure. The nucleus was round with a knob-like primordial rostrum (Figure 7A). Inside the first check, increments were faint and wide (4–5 µm in width), becoming more distinct to the first check. Their number varied from 4 to 14 (mean 8) (Table II). The first check looked like a double prominent increment (Figure 7B). Between the first and second checks, increments were similar to those inside the first check. Outside the second check, the increments became distinct and wide, especially in the dorsal dome (6–7 µm). Sometimes narrow and uniform subdaily increments (1–2 µm in width) were observed in the dorsal dome of the statolith. They appeared outside the first check and became more prominent in the midpart of the dark zone. In the dark zone, the increment width gradually decreased to 4–5 µm. Several irregularly spaced checks were observed in the dark zone (Figure 7C).
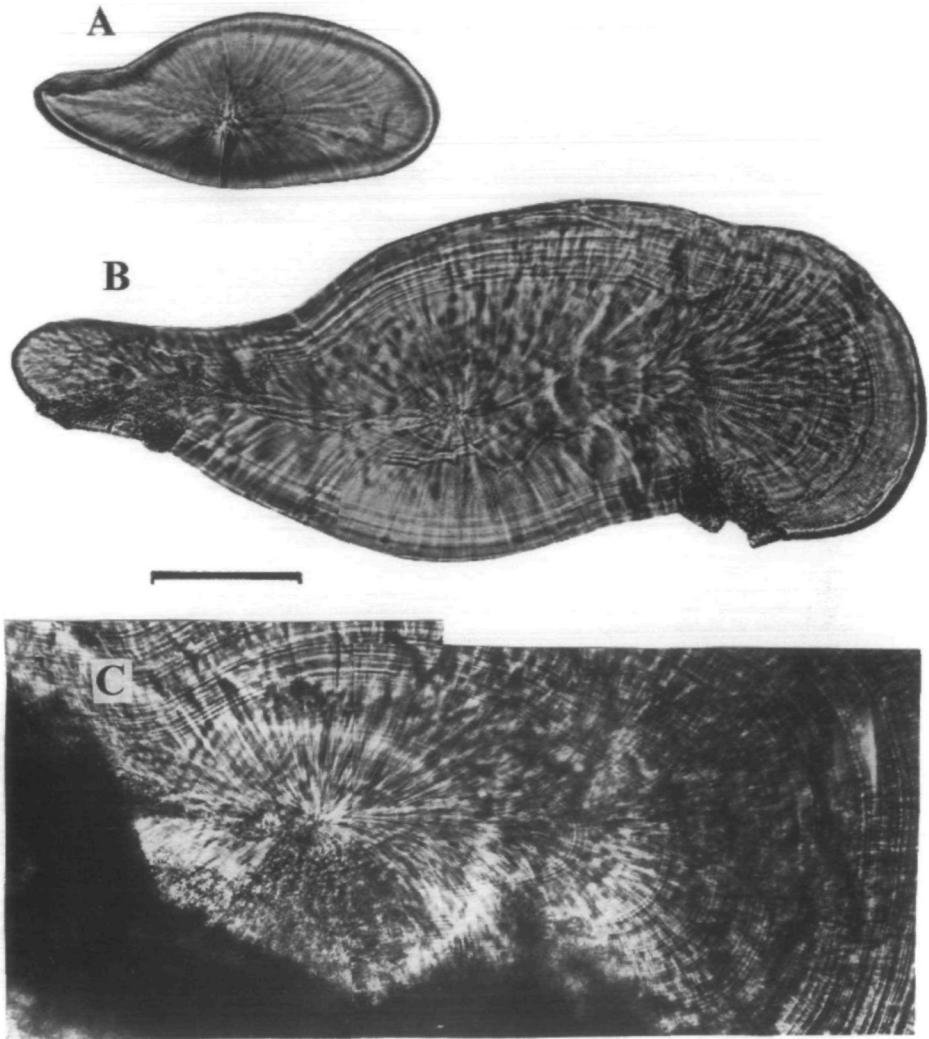
Fig. 7. Light photomicrographs of the statolith of *Gonatus onyx*. (**A**) Statolith of paralarva (9.5 mm ML); (**B**) statolith of small juvenile (23.0 mm ML); (**C**) postnuclear and dark zone of large juvenile (95 mm ML). Scale bar = 100 μm.

Statolith development. The first-check statolith was elongated with a large beak-like rostrum. The inner dome had a swollen midpart (Figure 4E). The nucleus was shifted to the inner dome. At 10 mm ML, the dorsal dome began to develop. The outline of the inner dome became concave in its dorsal part. The rostrum increased in length and curved laterally. At 15 mm ML, the statolith became elongated (Table IV). The dorsal dome became tongue-shaped, separated from the lateral and inner domes by shallow grooves. At 20–25 mm ML, the dorsal dome became more prominent, round and slightly bent to the inner side. The inner
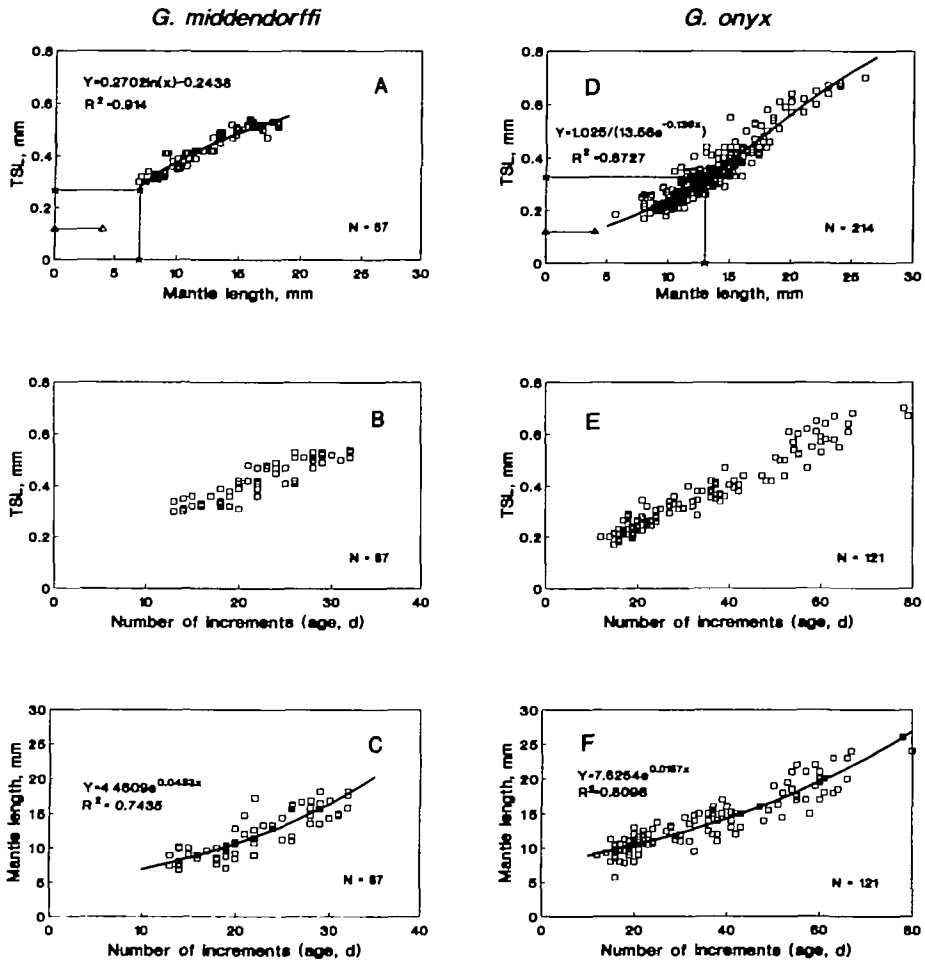
2007

G. middendorffi   G. onyx

Fig. 8. Relationships between mantle length and total statolith length (A, D), number of increments and total statolith length (B, E) and number of increments and mantle length (C, F). *Gonatus middendorffi* (left) and *G.onyx* (right). Symbols are the same as in Figure 5.

dome was swollen; the lateral dome was flat, with a convex ventral side. The rostrum increased in length and curved laterally.

Age and growth. The relative sizes of statoliths decreased slightly within the ML range studied: from 3.2% ML in the smallest paralarva (5.7 mm ML) to 2.7–2.8% ML in paralarvae of 24–26 mm ML. The relationship between TSL and ML was best described by a logistic function. The first check formed at smaller sizes than those of paralarvae studied (extrapolated ML was ~4 mm); the second check developed at a mean ML of 13 mm (Figure 8D). Absolute growth of the stato-liths was fast. TSL increased more than three times in paralarvae aged between 17 and 70 days (Figure 8E). In our samples, the youngest paralarva (9.0 mm ML) was 12 days old; the oldest paralarva (24 mm ML) was 80 days old. Paralarval
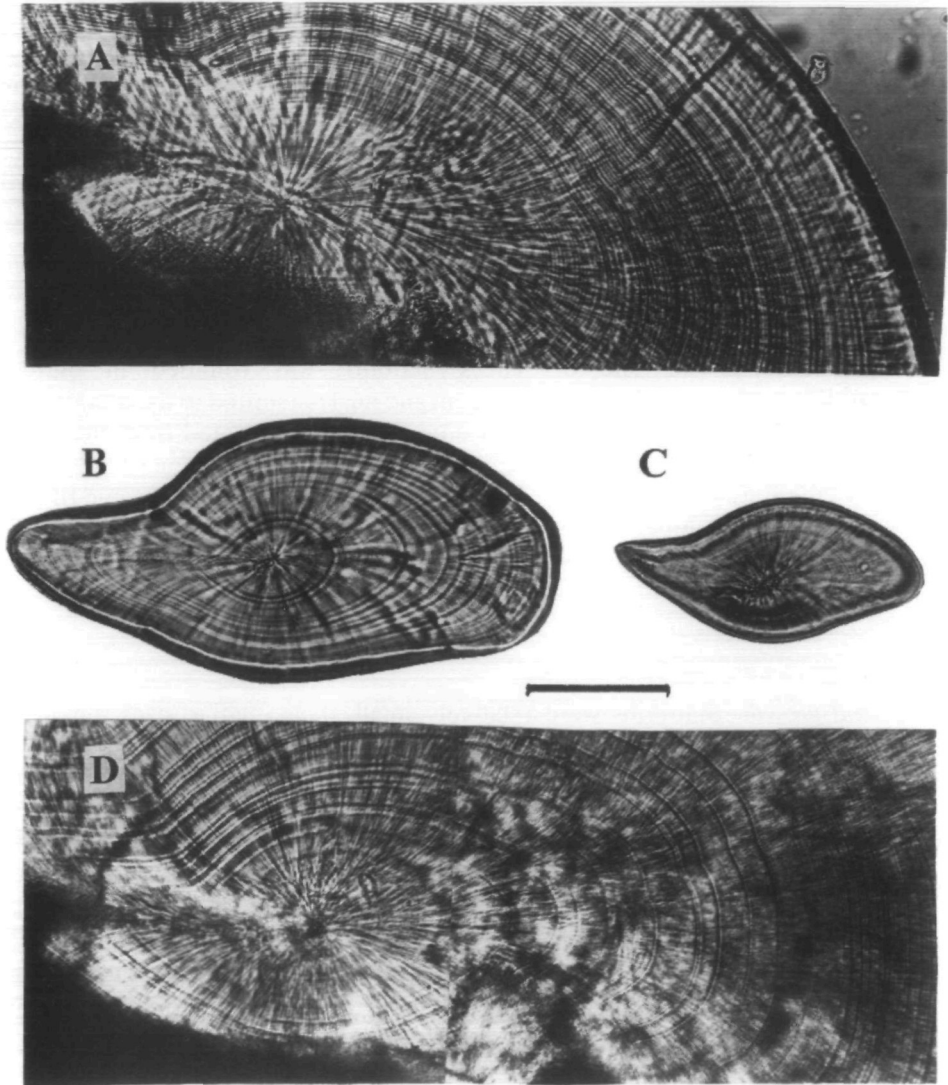
2008

Fig. 9. Light photomicrographs of the statolith of *Gonatus pyros* (**A**, **B**) and *Gonatopsis okutanii* (**C**, **D**). (A) Postnuclear and dark zone of a juvenile (97 mm ML); (B) statolith of paralarva (16.0 mm ML); (C) statolith of paralarva (10.6 mm ML); (D) postnuclear and dark zone of an immature female (208 mm ML). Scale bar = 100 μm.

growth was exponential with low instantaneous rates of growth (G = 0.0157, Figure 8F).

Gonatus pyros *Young, 1972*

Statolith microstructure. The nucleus was round (Figure 9A). Increments inside the first check were distinct and narrow (3–4 μm in width). Their number varied

from 5 to 12 (mean 9) (Table II). The first check looked like a single prominent increment. Between the first and second checks, the first 4–5 increments were distinct (Figure 9B). The rest were faint and broader (5–6 µm). Sometimes there was a faint additional check approximately in the middle between the first and second checks. Growth increments outside this additional check became more distinct and narrow (4–5 µm). Outside the second check, the increments became well resolved and wide, especially in the dorsal dome (6–7 µm). Their width gradually decreased towards the statolith edge up to 5–6 µm. Several irregularly spaced checks were situated in the dark zone (Figure 9B).

Statolith development. The first-check statolith was rounded in outline (Figure 4D), with a small knob-like rostrum and nucleus shifted to the inner dome (Table IV). At 10 mm ML, the dorsal dome was not separated from the inner dome. The rostrum became longer and bent laterally, its tip became round. At 15 mm ML, the statolith was elongated (Table IV). The dorsal dome became round, separated from the inner dome by a wide shallow groove. At 20 mm ML, the dorsal dome became more distinct and bent to the inner side. The inner dome was more swollen than the lateral dome. The rostrum became longer and curved laterally.

Age and growth. Statoliths were relatively small, their relative sizes remained similar over the size range of the paralarvae studied (2.5–2.53% ML). Allometric growth of the TSL versus ML was slightly positive (coefficient 'b' = 1.1). Despite the small number of specimens studied, the fitted power function had a high R^2 (0.909). The first check formed at an extrapolated value of ~4.5 mm ML; the second check developed at a mean ML of 13.5 mm (Figure 11A). The statolith increased in TSL more than two times in paralarvae aged between 9 and 42 days (Figure 11B). The youngest specimen of *G.pyros* (7.3 mm ML) had an age of 9 days; the oldest specimen (16.0 mm ML) was 42 days old. Growth of paralarvae was best described by an exponential function. Instantaneous rates of growth were low (G = 0.0212). Individual growth rates varied insignificantly (Figure 11C).

Eogonatus tinro *Nesis, 1972*

Statolith microstructure. The nucleus had a tear-drop shape (Figure 10A). There were from 6 to 18 (mean 12) faint increments visible inside the first check (5–6 µm in width). The first check looked like a narrow prominent line with sharp borders (Figure 10B and C). Sometimes one or two faint checks were revealed inside the first check. Between the first and second checks, increments became somewhat more distinct than those inside the first check. Outside the second check, the increments were more prominent and wider (7–8 µm), and gradually decreased up to 4–5 µm and then increased again near the statolith edge (7–8 µm). There were not any distinct checks in the dark zone (Figure 10C).
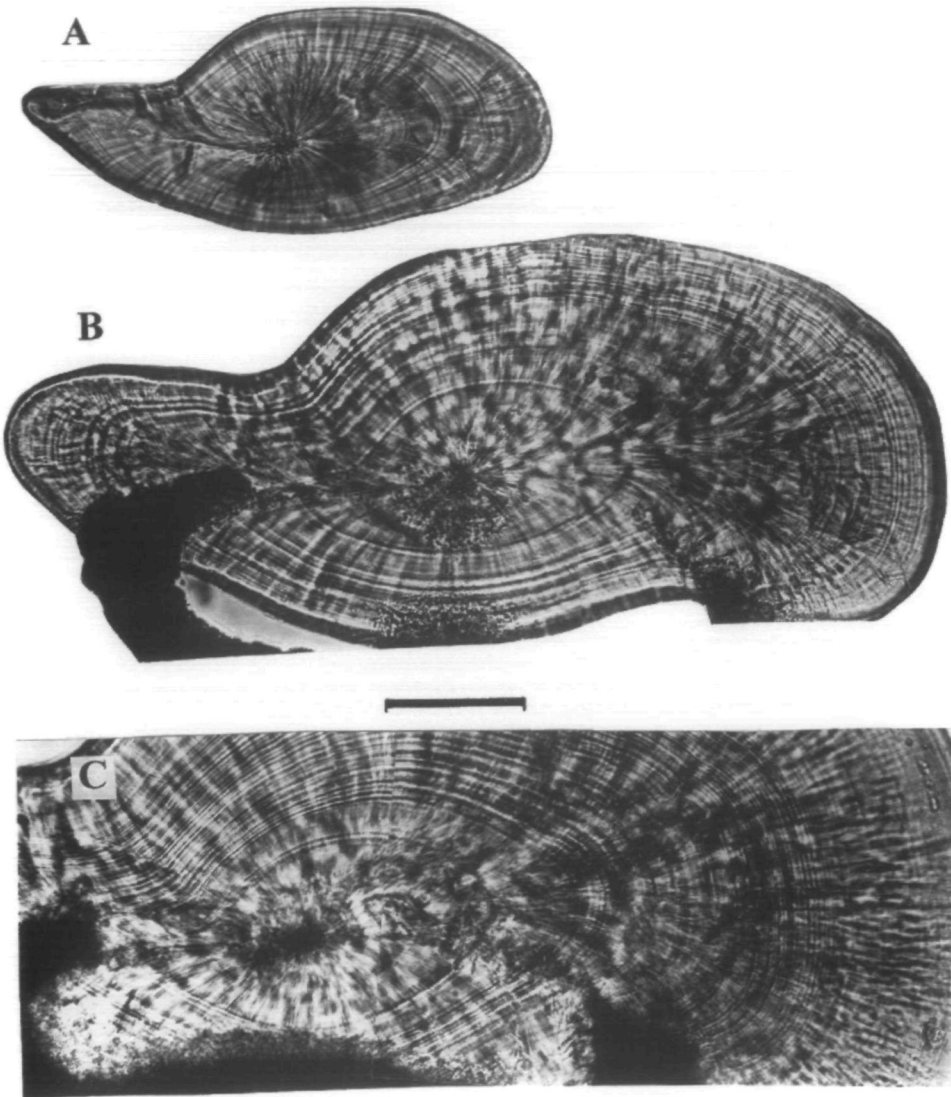
Fig. 10. Light photomicrographs of the statolith of *Eogonatus tinro*. (**A**) Statolith of paralarva (11.5 mm ML); (**B**) statolith of small juvenile (24.0 mm ML); (**C**) postnuclear and dark zone of large juvenile (48 mm ML). Scale bar = 100 μm.

Statolith development. The first-check statolith was elongated, with a convex inner dome and large triangular rostrum (Table IV). The nucleus was in the center of the statolith (Figure 12B). At 10 mm ML, the dorsal dome started to develop and became round. The rostrum slightly increased in length, its tip became round. At 20 mm ML, the dorsal dome became well distinguished, wide, distinctly bent to the inner side. The inner dome and lateral dome were swollen in their ventral parts. The rostrum became large, blunt and bent to the lateral side.
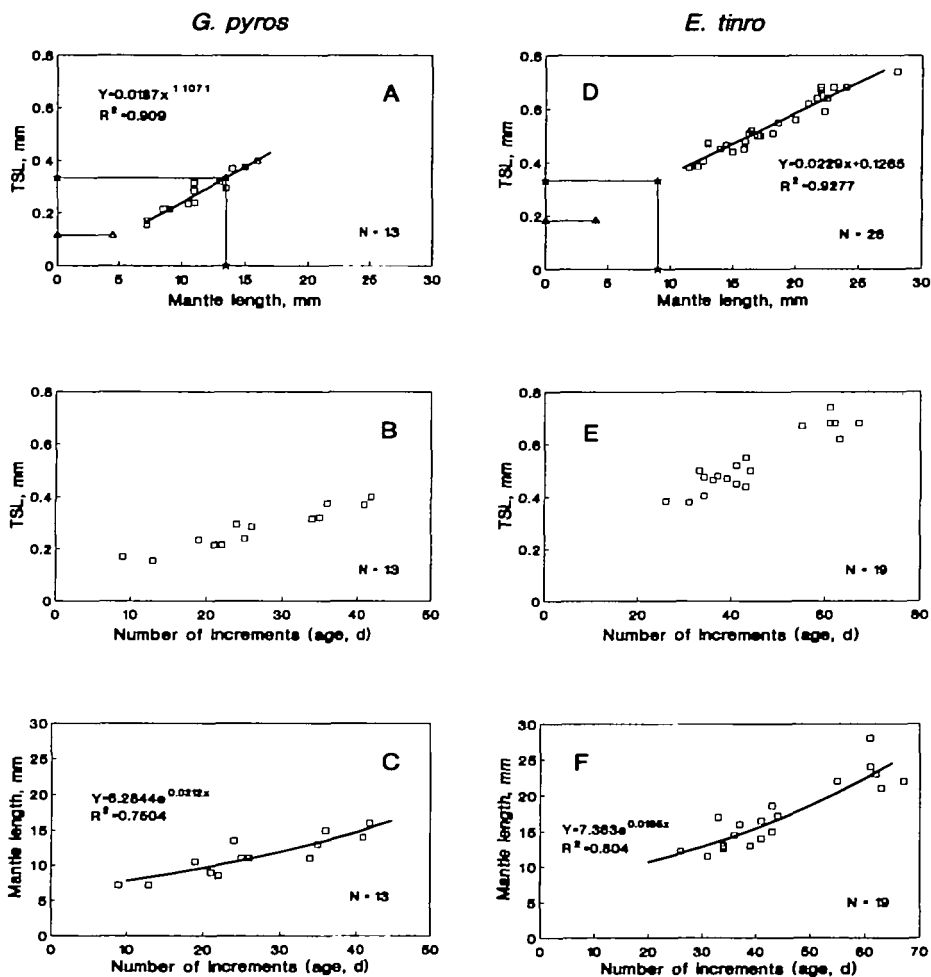
2011

G. pyros

E. tinro

Fig. 11. Relationships between mantle length and total statolith length (A, D), number of increments and total statolith length (B, E) and number of increments and mantle length (C, F). *Gonatus pyros* (left) and *E.tinro* (right). Symbols are the same as in Figure 5.

Age and growth. The relative sizes of statoliths decreased during the ontogenetic period studied from 3.16–3.3% ML in small paralarvae (11.5–12.0 mm ML) to 2.6–2.8% ML in large paralarvae (24–28 mm ML). The relationship between TSL and ML was linear with a high R^2 (0.927). Both the first and second checks appeared at smaller sizes than those of sampled paralarvae (extrapolated values of ML being 4 and 9 mm, correspondingly) (Figure 11D). Absolute growth of statoliths was fast (Figure 11E). The youngest paralarva (12.2 mm ML) was 26 days old, while the oldest paralarva (22.0 mm ML) was 67 days old. Growth of the paralarvae was best described by an exponential function. Instantaneous rates of growth were low ($G = 0.0185$, Figure 11F).
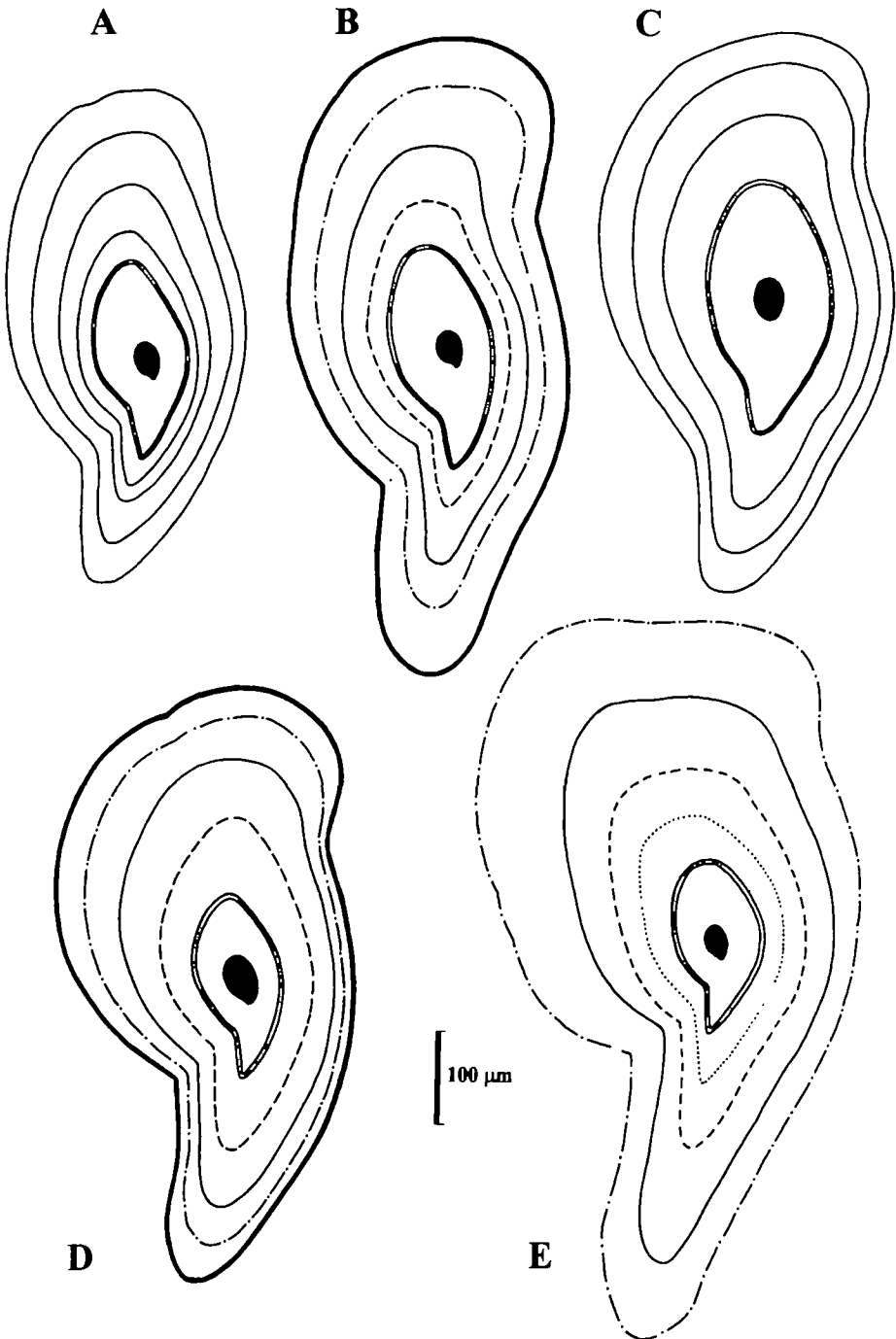
Fig. 12. Schemes of statolith development of gonatid paralarvae. (**A**) *Gonatopsis okutanii*; (**B**) *Eogonatus tinro*; (**C**) *Gonatopsis makko*; (**D**) *Gonatopsis borealis*; (**E**) *Berryteuthis magister*. Symbols are the same as in Figure 4.
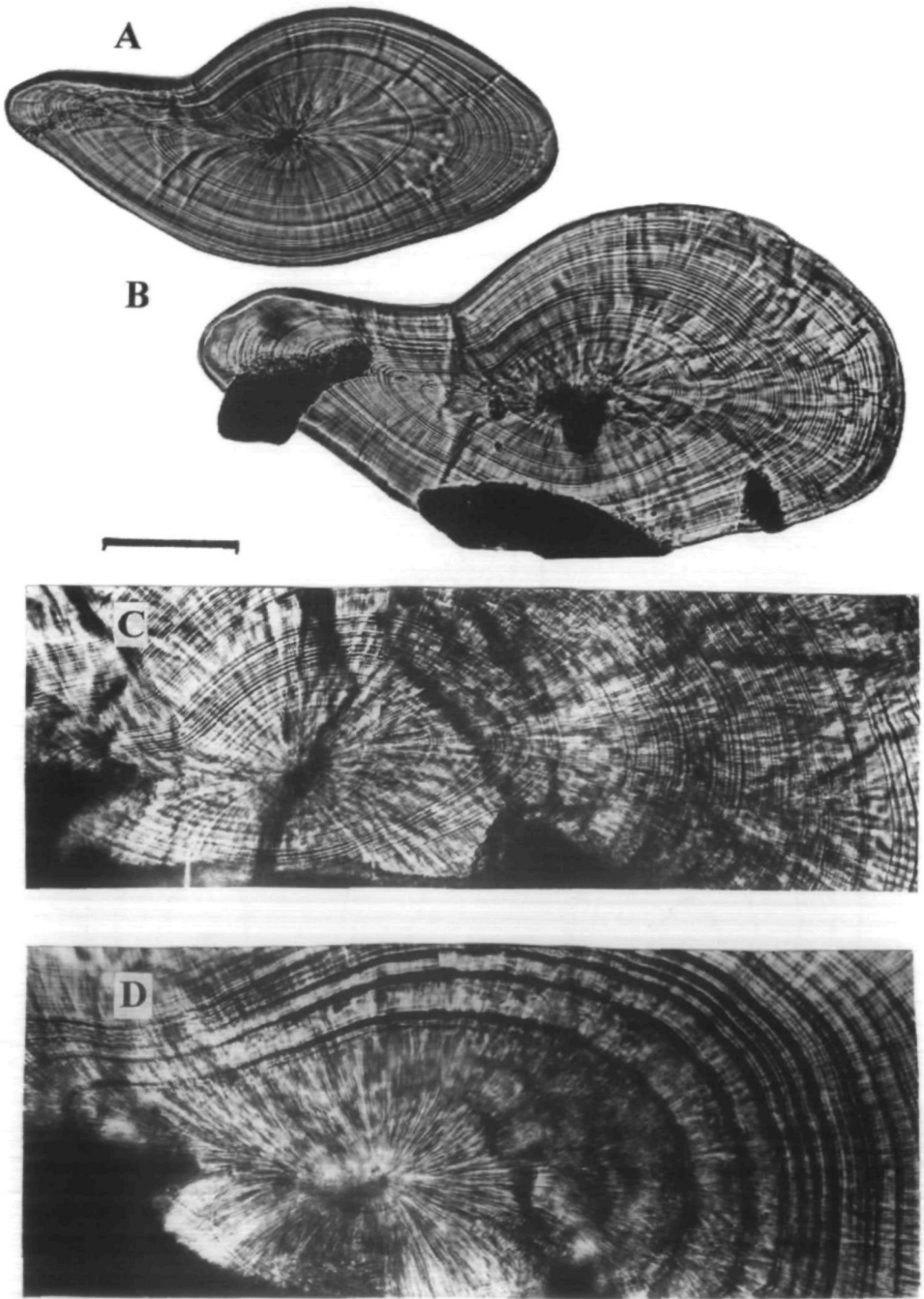
Fig. 13. Light photomicrographs of the statolith of *Gonatopsis borealis* (**A–C**) and *Gonatopsis makko* (**D**). (A) Statolith of a paralarva (13.1 mm ML); (B) statolith of a small juvenile (19.2 mm ML); (C) postnuclear and dark zone of a large juvenile (85 mm ML); (D) postnuclear and dark zone of immature female (380 mm ML). Scale bar = 100 μm.

Gonatopsis borealis *Sasaki, 1923*

Statolith microstructure. The nucleus was a rounded oval with a tiny knob-like primordial rostrum (Figure 13A). Increments inside the first check were distinct (4–5 µm in width). Their number varied greatly (from 5 to 18), mean 13 increments (Table II). The first check consisted of two or three pronounced increments (Figure 13B). Between the first and second checks, there were faint but wide increments (5–6 µm). Outside the second check, the increments became more resolved and narrower than the inner increments (~3 µm). Their width gradually increased towards the statolith edge (4–5 µm). Regularly spaced prominent checks were observed in the dark zone. From 6 to 8 (mean 7) growth increments were situated between each two checks (Figure 13C).
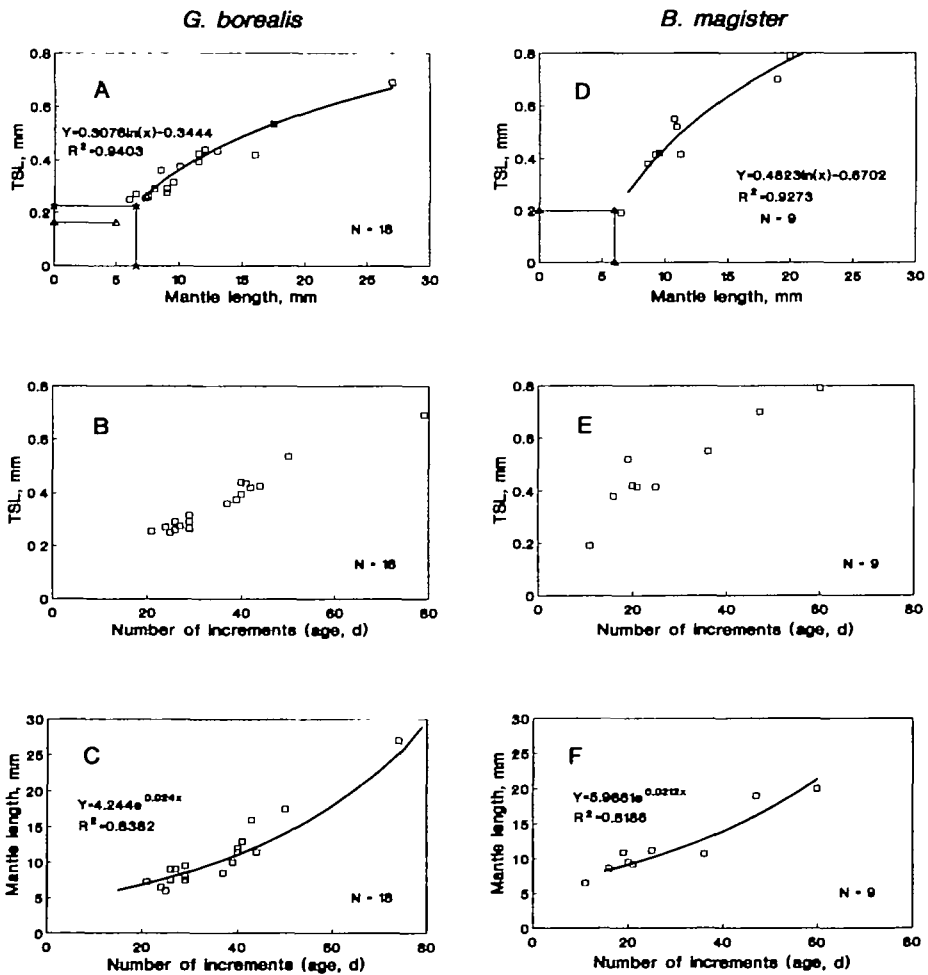
Fig. 14. Relationships between mantle length and total statolith length (**A, D**), number of increments and total statolith length (**B, E**) and number of increments and mantle length (**C, F**). *Gonatopsis borealis* (left) and *Berryteuthis magister* (right). Symbols are the same as in Figure 5.

Statolith development. The first-check statolith was elongated (Figure 12D), with a large beak-like rostrum (Table IV). The lower part of the lateral dome was not swollen. The nucleus was situated in the center of the statolith. At 10 mm ML, the dorsal dome became tongue-like. The lateral dome was distinctly swollen in the midpart. The rostrum tip bent laterally. At 25 mm ML, the dorsal dome was well developed, round and bent to the inner side. It was separated from the inner dome by a distinct groove. The rostrum was large, bent to the lateral side.

Age and growth. Statoliths were relatively large with the TSL decreasing from 4.1% ML in small squids (6.0–6.5 mm ML) to 2.56% ML in a large specimen (27.0 mm ML). The relative growth of TSL versus ML was logarithmic. The first check formed at an extrapolated value of ~5 mm ML; the second check developed soon after the first one (at 6.6 mm ML) (Figure 14A). Absolute growth of statoliths was rapid; they increased in TSL almost three times during the period of 34 days (age ranged from 21 to 55 days) (Figure 14B). The youngest specimen of *G.borealis* (7.3 mm ML) was 21 days old; the oldest specimen (27.0 mm ML) was 79 days old. Paralarvae grew exponentially with low instantaneous rates of growth ($G = 0.024$; Figure 14C).

Gonatopsis makko *Okutani and Nemoto, 1964*

Statolith microstructure. The nucleus was egg shaped. Inside the first check, there were 15 very faint wide increments (7 µm in width) (Table II). Between the first and second checks, there were 15 faint narrow increments (4.5 µm). In the dark zone, increments became more resolved and progressively increased in width toward the statolith margin (from 4.5 to 5.6 µm). There were numerous and irregularly spaced checks in the dark zone. The resolution of these checks varied from sharp narrow lines to highly pronounced wide dark bands. From 2 to 11 growth increments were observed between each two checks. The increment width between several checks decreased sharply to 3 µm (Figure 13D).

Statolith development. The first-check statolith was elongated (Table IV), with the nucleus being in its center. The rostrum was wide with a rounded tip (Figure 12C). The inner dome was swollen. During statolith growth, the dorsal dome bent markedly to the inner side. The lateral dome became swollen in its central part. The rostrum elongated and bent laterally.

Age and growth were not studied due to a lack of *G.makko* paralarvae in our samples (Table II).

Gonatopsis okutanii *Nesis, 1972*

Statolith microstructure. The nucleus was round, with a knob-like rostrum. Inside the first check, increments were very faint and poorly visible (5–6 µm in width) (Figure 9C). The first check looked like a wide prominent increment with sharp borders. Between the first and second checks, increments were faint, being similar to those inside the first check. Outside the second check, the increments were well

resolved and narrow (4–5 µm). Numerous checks were irregularly spaced in the dark zone (Figure 9D). From 4 to 10 increments were visible between two adjacent checks.

Statolith development. The first-check statolith was elongated (Table IV), with a large beak-shaped rostrum, markedly bent to the lateral side. Both lateral and inner domes had swollen lower parts (Figure 10A). The nucleus was in the center of the statolith. During statolith growth, the dorsal dome remained not separated from the lateral dome and later slightly bent to the inner side. The lateral dome became somewhat flattened in its central part. The rostrum was markedly bent laterally.

Age and growth. Statoliths were relatively small, their TSL ranging from 1.52% ML (in paralarva of 9.2 mm ML) to 2.1% ML (11.5 mm ML). The youngest paralarva (9.2 mm ML) had an age of 10 days; the oldest paralarva (11.5 mm ML) was 15 days old. Curves were not fitted to different relationships between ML, TSL and age due to a shortage of available specimens (four specimens; Table I).

Berryteuthis magister *(Berry, 1913)*

Statolith microstructure. The nucleus was tear-drop shaped with a distinct pointed primordial rostrum (Figure 15A). There were about 11 faint uniform growth increments (4–6 µm width) inside the first check. The first check had an appearance of a well-resolved single or double narrow growth increment (Figure 15B). Outside the first check, growth increments became distinct and wide, especially in the rostrum. Their maximum width varied from 7 µm in the dorsal dome to 12 µm in the proximal part of the rostrum. Sometimes 4–6 faint narrow increments (= subdaily increments) were visible (Arkhipkin *et al.*, 1996) within each growth increment in the inner part of the dark zone. Well-defined stress checks were irregularly spaced within the dark zone (Figure 15C). There were from 5 to 16 growth increments between two adjacent checks.

Statolith development. The first-check statolith was comma-shaped (Figure 12E), with a large pointed beak-like rostrum (Table IV). The inner dome had a swollen middle part. The nucleus was in the center of the statolith. At 10 mm ML, the lateral dome became swollen in its upper part. The dorsal part of the statolith was wide and round. The rostrum became longer, distinctly curved laterally. The dorsal part of the statolith became flat (at 15 mm ML) and later subdivided into dorsal and lateral domes by a wide groove. The dorsal dome was separated from the inner dome by a distinct groove at 20 mm ML and markedly bent to the inner side. The lateral dome had swollen dorsal and ventral parts. The outline of its lower part formed a right angle with the rostrum. The rostrum was long, finger shaped and bent to the lateral side.

Age and growth. Statoliths were relatively large. Their TSL increased from 2.9% ML in a small paralarva (6.5 mm ML) to 4–4.5% ML in large paralarvae (19–20.0

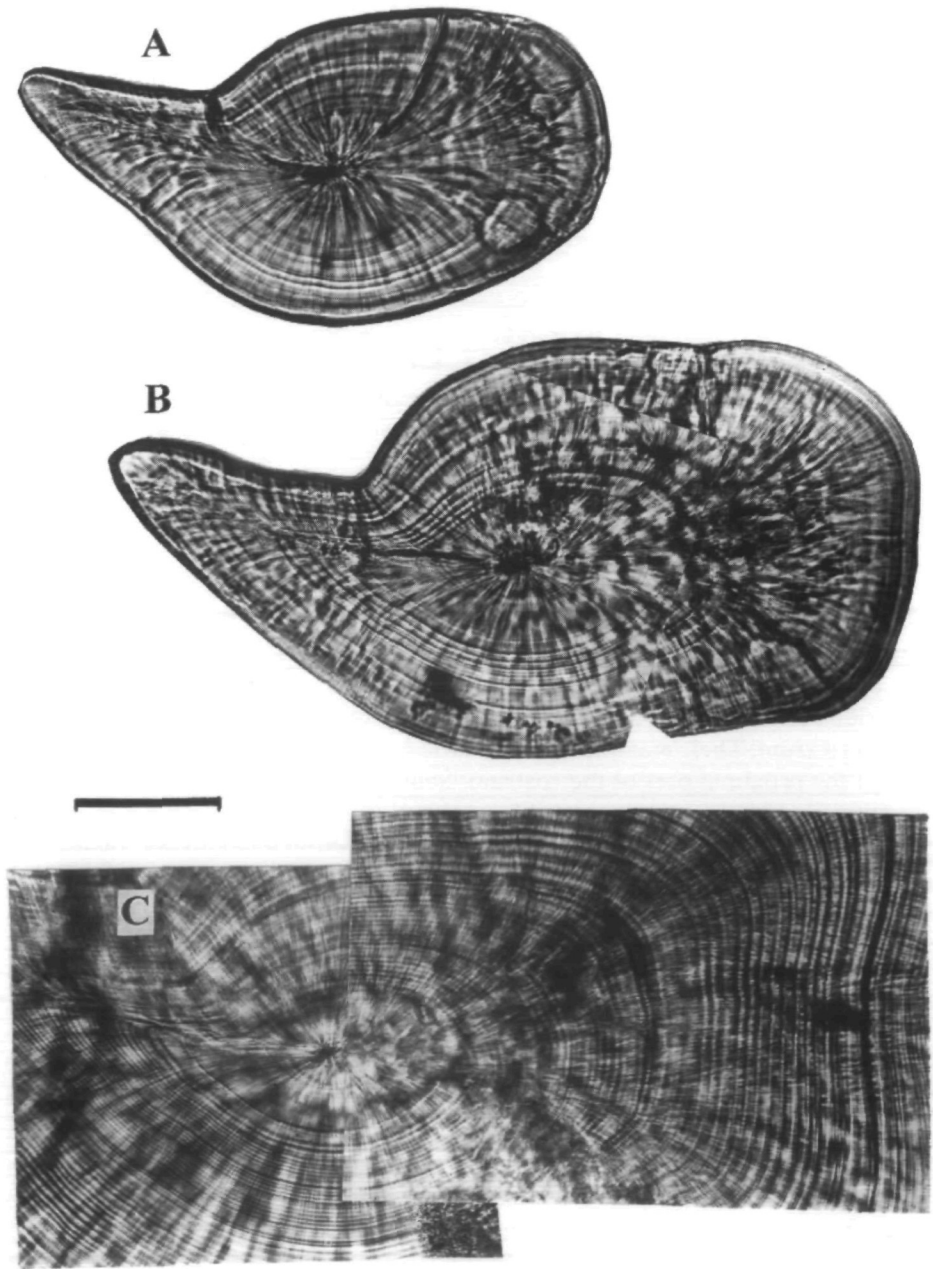**Fig. 15.** Light photomicrographs of the statolith of *Berryteuthis magister*. (**A**) Statolith of a paralarva (9.5 mm ML); (**B**) statolith of paralarva (10.7 mm ML); (**C**) postnuclear and dark zone of a juvenile (122 mm ML). Scale bar = 100 μm.

mm ML). The relationship between TSL and ML was logarithmic with a high R^2 (0.927) despite a small number of paralarvae studied. The first check had just formed in our smallest paralarva at 6.5 mm ML; the second check was never developed (Figure 15). Absolute growth of statoliths was very fast. TSL increased almost four times in squid aged between 17 and 60 days (Figure 14E). The youngest paralarva of *B.magister* (6.5 mm ML) was 16 days old; the oldest para-larva (20.0 mm ML) was aged 60 days. Paralarval growth was best described by an exponential function. Instantaneous rates of growth were low ($G = 0.0212$; Figure 14F).

Discussion

Statolith microstructure

The statolith microstructure of gonatids has several specific features, namely the size and shape of the nucleus and bipartite postnuclear zone.

Nucleus. All gonatids studied have a large nucleus. Its maximum diameter is the largest in *B.magister*, *E.tinro* and *Gonatopsis* spp. (42–46 µm), whereas in *Gonatus* spp. it is consistently smaller than in the above species (32–35 µm). Squids of other families have nuclei 1.5–4 times smaller than those of gonatids, ranging from 10–12 µm in enoploteuthids (*Abralia trigonura*; Bigelow, 1992) to 20–22 µm in ommatsrephids (*Illex illecebrosus*; Balch *et al.*, 1988) and 25–30 µm in loliginids (*Photololigo edulis*; Natsukari *et al.*, 1988). Moreover, the nuclei were either oval or round in all other families studied, whereas in gonatids the nucleus is droplet shaped with a knob-like primordial rostrum.

In enoploteuthids and ommastrephids (Balch *et al.*, 1988; Bigelow, 1992), the nucleus is in fact the statolith of a hatchling, while in loliginids it is formed during late embryogenesis (Lipinski, 1993). It has been found in various enoploteuthid species that the largest hatchlings have the largest nuclei in their statoliths (Arkhipkin, 1996). If the nucleus is formed in gonatids during hatching, its size should also correspond with hatchling size. Unfortunately, neither hatchlings nor mature females of the Pacific gonatids have been found yet, except for mature females of *B.magister* and *G.borealis* (Okutani *et al.*, 1988). Taking into account the large diameter of ripe eggs in the former species (3.5–4 mm) compared to *Gonatus fabricii* (3 mm) (Kristensen, 1977), it is possible that *B.magister* and *G.borealis* hatchlings are larger than those of *Gonatus* spp.

Postnuclear zone. The zone between the nucleus and the first stress mark has been termed the postnuclear zone of the statolith (Lipinski *et al.*, 1991). This zone develops either during embryogenesis (Loliginidae; Natsukari *et al.*, 1988) or during the paralarval phase (Ommastrephidae and Enoploteuthidae; Arkhipkin, 1989; Bigelow, 1992). Thus, the first check appears either at hatching in the former case (and is called a 'natal ring') or during the transition from paralarval to juvenile phases (as in ommastrephids; Arkhipkin, 1989). Among the gonatids studied, only *B.magister* has such a typical postnuclear zone with faint narrow increments, outlined by the first check and followed with broad prominent

increments of the next, dark zone. Previously, it was assumed that the postnuclear zone within a *B.magister* statolith was an embryonic zone and consequently the first check was thought to be a natal ring (Natsukari *et al.*, 1993). In our samples, however, the statoliths of the smallest *B.magister* paralarva (6.5 mm ML) were somewhat smaller in size than the first check seen in adult statoliths which had not yet formed. Thus, unlike the previous assumption of Natsukari *et al.* (1993), the postnuclear zone within the statolith of *B.magister* develops after hatching.

In all other gonatid species studied, growth increments lying between the first and second check were similar in appearance (faint) and width to those lying inside the first check (i.e. in the postnuclear zone, *sensu* Lipinski *et al.*, 1991). Outside the second check, growth increments become well resolved and prominent, as in the typical dark zone of the ommastrephid statoliths (Arkhipkin, 1989). Moreover, regions inside the first check and between the first and second checks are translucent (as in the postnuclear zone of ommastrephids), whereas the region outside the second check is opaque (as in the dark zone of ommastrephids). This suggests that the region outlined by the second check is the postnuclear zone within statoliths of *Gonatus* spp., *Gonatopsis* spp. and *E.tinro*. Thus, all these species have a bipartite postnuclear zone, with one prominent check lying inside it. This phenomenon has also been noted for Atlantic *Gonatus fabricii* (Kristensen, 1980). The size at which the first check occurs is species specific. In *Gonatus* spp., the first check appears early and at a small statolith size. In *Gonatopsis* spp. and *E.tinro*, the first check appears late and at a large statolith size. On the contrary, the second check usually appears at a greater statolith size in *Gonatus* spp. than in *Gonatopsis* spp. and *E.tinro*.

Development of both stress checks and growth zones within teleost otoliths and squid statoliths is usually associated with some physiological event or transition period in the life of fish (Campana and Neilson, 1985) and squids (Arkhipkin and Murzov, 1990; Bigelow, 1992; Jackson, 1993). The appearance of the first check within the statolith microstructure of the gonatids studied (except *B.magister*) takes place at ML < 5 mm. Unfortunately, neither we nor Kubodera and Okutani (1981) have collected such small gonatid paralarvae. It would be possible to assume that the first check appeared at hatching. However, one fact might contradict this assumption. In gonatids, growth increments inside the first check and between the first and second checks are practically similar, unlike those of loliginids (Natsukari *et al.*, 1988). In loliginids, growth increments inside the first check (embryonic) are faint and narrow, whereas those outside the first check (post-embryonic) are prominent and wide. For the final solution of this problem, however, data on small paralarvae are still needed.

Completion of the postnuclear zone (i.e. the appearance of the first check in *B.magister* and the second check in the rest of the gonatids studied) takes place at different paralarval sizes: small (6.5–9 mm ML) in *B.magister*, *G.borealis* and *E.tinro*, and rather large (13–19 mm ML) in *Gonatus* spp. It is not clear what morphological changes or ecological shifts might take place at such a small ML in the three former species due to the lack of available information (Okutani *et al.*, 1988). It is notable that this transition does not correspond with the appearance of the arm hooks in these squids, which takes place at 16–35 mm ML

(Okutani *et al.*, 1988). In *Gonatus* spp., at least in *G.berryi*, *G.pyros* and, perhaps, *G.onyx*, the appearance of the second check coincides with a development of the central hook on the tentacular club (Kubodera and Okutani, 1981). These changes in armature of the capture apparatus probably relate to a diet shift in paralarval *Gonatus* spp. and may account for changes in statolith microstructure. Development of the central club hook in *G.madokai* takes place around 20 mm ML (Kubodera and Okutani, 1981), and may be the reason for the absence of the second check in our paralarvae (<19 mm ML). This is analogous to the change in statolith microstructure in ommastrephids (from postnuclear to dark zones) where these two zones represent a morphological change in the splitting of the proboscis into tentacles (Arkhipkin and Mikheev, 1992).

Dark zone. This zone is situated outside the second check and can be easily distinguished from the postnuclear zone by both the well-resolved growth increments and opaque color of the microstructure. There are not any specific features in the dark zone of paralarval and juvenile gonatids. Growth increments in this zone may be either wide (*G.onyx*, *E.tinro*, *B.magister*, indicating the fast growth of the statolith dorsal dome) or narrow (*G.berryi*, *G.borealis*). In all gonatids studied (except *E.tinro*), growth increments are grouped into distinct growth bands, outlined by well or poorly resolved stress checks. These checks are spaced either regularly with 7–8 increments situated between each pair of checks (*G.berryi*, *G.borealis*), or irregularly with 4–10 increments between them (*G.onyx*, *G.pyros*, the rest of *Gonatopsis* spp. and *B.magister*). Periodic growth bands in the dark zone of *G.berryi* and *G.borealis* statoliths appear to be related to the lunar cycle due to a mean number of growth increments within them. The same periodic lunar bands have been observed in the squids *Gonatus fabricii* (Kristensen, 1980), *Ancistrocheirus lesueurii* (Arkhipkin, 1997) and in juveniles of some marine fish (Campana, 1984). However, as in the rest of our gonatids, no obvious periodicity in the number of growth increments within the second-order bands has been revealed in the statoliths of *Photololigo edulis* (Natsukari *et al.*, 1988) and *Loligo vulgaris* (Natsukari and Komine, 1992).

Periodicity of growth increment deposition in statoliths. In maintenance experiments, it was shown that stress associated with the capture of paralarvae induced the development of a well-defined stress check within their statoliths. The widths of the neighboring growth increments lying inside and outside this check were similar. This does not support the view that the possible origin of the stress check was due to a slight statolith resorption (as in goldfish; Mugiya and Uchimura, 1988) or fusion of neighboring increments. The latter is not likely to be due to the same numbers of growth increments outside the stress check and days elapsed between capture and death. It is likely that this check results from a greater than usual deposition of organic material (responsible for the opaque part of the growth increment) during stress. The daily nature of increment deposition has thus been confirmed for growth increments lying both within the statolith postnuclear and dark zones of gonatid paralarvae and early juveniles. The same

results have been obtained for the post-embryonic period in all squid species studied previously (for a review; see Lipinski, 1993).

Development of statoliths

All gonatids studied can be separated into two distinct groups according to the position of the nucleus in the first-check statolith: species with a central position (*Gonatopsis* spp., *E.tinro* and *B.magister*) and species with the nucleus shifted to the inner side of the first-check statolith (*Gonatus* spp.). In the first group, first-check statoliths grow at the same rate both in lateral and inner directions, while in the second group the increments formed in the lateral part of the statolith are thicker than those formed in the inner part. Therefore, in *Gonatus* spp., statoliths have the nucleus located on its inner (wing) side from the very start of statolith development, while in the rest of the species a shift of the nucleus occurs only during the juvenile phase, i.e. after the formation of a distinct lateral dome. The development of each statolith type can therefore be described separately.

Species with a central position of the nucleus. Early development of statoliths of *Gonatopsis* spp. and *E.tinro* follows similar patterns. At the time of the first check formation, statoliths of these species have an elongated shape with uniformly convex lateral and inner domes and a large triangular rostrum. In *B.magister*, early growth is especially fast in the inner part of the statolith and rostrum, resulting in the characteristic 'comma-shape' first-check statolith with a swollen inner dome and a rather long rostrum (30% TSL).

Later development reveals some variability in the growth patterns of different morphological parts of the statolith. The rostrum bends markedly to the lateral side (RDA 17–24°) in all species. In *B.magister* and *G.borealis*, it reaches the greatest length and becomes narrow, while in *E.tinro* it grows long but somewhat wide with a rounded tip. In *G.makko* and *G.okutanii*, the rostrum grows very slowly and becomes short and wide. The lateral dome grows fast in *B.magister* and *G.borealis*. In *B.magister*, it develops firstly in the dorsal part, then in the ventral part. In *G.borealis*, the lateral dome grows mainly in the midpart. In *G.okutanii* and *E.tinro*, the lateral dome grows very slowly and becomes flattened. The dorsal dome is best developed in *G.borealis* and *E.tinro*. The lateral groove that separates the dorsal dome from the lateral dome appears only in *B.magister* and *G.borealis* juveniles at ML > 20 mm. The inner groove appears in all species at ~15 mm ML. The inner dome is the most prominent in *B.magister* and *G.borealis*.

Comparison of the development of the different morphological parts in the paralarval statoliths makes it possible to distinguish three groups of gonatids based on the central nucleus. In the first group (*B.magister* and *G.borealis*), statoliths are characterized by a long narrow rostrum and well-developed distinctly separated lateral, dorsal and inner domes. In the second group (*G.makko, G.okutanii*), statoliths have a short wide rostrum and less developed domes without the lateral groove separating lateral and dorsal domes. The third group (*E.tinro*)

occupies an intermediate position between the two first groups and is characterized by statoliths with long but wide rostra and well-developed dorsal domes.

Species with a shifted position of the nucleus. At the time of the first check formation, statolith shape is more variable in *Gonatus* spp. than in the species with the central nucleus. *Gonatus middendorffi* and *G.onyx* have slender statoliths with a long and narrow rostrum. The other species in this group have a somewhat rounded statolith with either a large wide rostrum (*G.berryi*) or a very small knob-like (*G.pyros*) or sharp rostrum (*G.madokai*).

During paralarval growth, the rostrum bends to the lateral side (RDA 15–23°) in all species of this group, except *G.middendorffi*. In *G.onyx* and *G.middendorffi*, it grows long and narrow, while in the others it grows slowly, becoming short and wide. The lateral dome develops very slowly and is flattened in *G.onyx* and *G.middendorfi*. The rest of the species have a moderately developed lateral dome. The dorsal dome grows especially fast and becomes long and tongue shaped in *G.onyx* and *G.middendorffi*. In *G.madokai* and *G.pyros* it is rounded. The lateral groove is very distinct in *G.onyx* at ML >15 mm and somewhat less distinct in *G.berryi*. The inner groove appears in all species at 10–15 mm ML. The inner dome is best developed and swollen in the middle part in *G.onyx*.

Two groups may be distinguished within *Gonatus* spp. based on peculiarities of paralarval statolith development. In the first group (*G.onyx* and *G.middendorffi*), statoliths are slender, with a long narrow rostrum and tongue-shaped dorsal dome slightly bent to the inner side (DDA < 10°). In the second group (*G.berryi*, *G.pyros* and *G.madokai*), statoliths are wide, with a short and wide rostrum. The dorsal dome is rounded in outline and either straight (*G.berryi*) or markedly bent to the inner side (*G.madokai*, *G.pyros*).

In general, at the time of the first check formation, all species with a shifted nucleus have a more prominent lateral dome and a low flattened inner dome than species with a central nucleus. The shape and structure of the first-check statoliths are more diverse in species with the shifted nucleus, while later development reveals higher statolith variability in the species with a central nucleus.

Some parallelism can be traced in the early development of statoliths in gonatids with a central and shifted nucleus. Both groups may be further subdivided into subgroups with large (or long) paralarval statoliths characterized by a well-developed long and narrow rostrum and usually prominent lateral dome (*B.magister*, *G.borealis*, *E.tinro*; *G.onyx* and *G.middendorffi*); and species with rather small wide statoliths characterized by a short and wide rostrum and less-developed dorsal dome (*G.makko*, *G.okutanii*; *G.berryi*, *G.madokai*, *G.pyros*).

The diversity of statolith morphology in these gonatids facilitated the construction of keys to their identification. Taking into account that statolith morphology changes considerably after the formation of the first check, a key was constructed firstly for small paralarvae (<10 mm ML) having statoliths before or just after the formation of the first check and, secondly, for paralarvae and early juveniles (from 10 to 30 mm ML) having statoliths with more then one check.

A key to the identification of gonatid paralarvae (<10 mm ML) using their stato-liths (for statoliths with the first check only)

1(2).   Nucleus in the center of statolith or somewhat shifted laterally (Figure 1). . . . . .3.

2(1).   Nucleus shifted to inner side of statolith (Figure 1). . . . . 11.

3(4).   Statolith comma-shaped. Lateral side of rostrum straight or slightly concave.
Rostrum length >30% TSL. . . . . *Berryteuthis magister* (Figure 12E)

4(3).   Statolith not comma-shaped. Lateral side of rostrum convex. Rostrum length less 27% . . . . .5.

5(6).   Rostrum length 20–22% TSL. . . . . *Gonatopsis makko* (Figure 12C)

6(5).   Rostrum length 24–28% TSL. . . . . .7.

7(8).   Dorsal dome rounded in outline (Figure 16B). . . . . *Eogonatus tinro* (Figure 12B)

8(7).   Dorsal dome tongue-shaped (Figure 16A) . . . . .9.

9(10). The greatest width of statolith above the focus (Figure 16A). . . . .
Gonatopsis borealis (Figure 12D)

10(9). The greatest width of statolith at the level or below the focus (Figure 16B) . . . .*Gonatopsis okutanii* (Figure 12A)

11(14). Statolith narrow (SW/TSL < 45%), dorsal dome elongated. . . . . 12.

12(13). Inner dome almost flat in outline. . . . . *Gonatus middendorffi* (Figure 4B)

13(12). Inner dome with distinct swell in the middle part . . . . *Gonatus onyx* (Figure 4E)

14(11). Statolith wide (SW/TSL > 50%), dorsal dome rounded. . . . . 15.

15(16). Rostrum length > 20% TSL. . . . . *Gonatus berryi* (Figure 4A)

16(15). Rostrum length < 15% TSL. . . . . 17.

17(18). Rostrum knob-like, with rounded tip. . . . . *Gonatus pyros* (Figure 4D)

18(17). Rostrum with pointed tip. . . . . *Gonatus madokai* (Figure 4C)

A key to the identification of gonatid paralarvae and early juveniles (10–30 mm ML) using their statoliths (for statoliths with more than one check)

1(2).   Nucleus in the center of the first-check statolith. The first-check statolith is large (its TSL > 160 μm). . . . . .3.

2(1).   Nucleus shifted to inner side of the first-check statolith. The first-check statolith is small (its TSL < 150 μm). . . . . 11.

3(4).   Statolith comma-shaped. Dorsal side of statolith wide and flat. Lower part of lateral dome swollen.
Rostrum length > 33% TSL. . . . . *Berryteuthis magister* (Figure 12E)

4(3). Statolith not comma-shaped. Dorsal side of statolith rounded or tongue-shaped. Lower part of lateral dome poorly developed, smooth. Rostrum length < 33% TSL. . . . . .5.

5(6). Rostrum length from 26 to 33% TSL. Rostrum narrow (ratio rostrum width/rostrum length < 90%). . . . . .7.

6(5). Rostrum length from 20 to 24% TSL. Rostrum wide (ratio rostrum width/rostrum length > 100%). . . . . .9.

7(8). Dorsal dome short, flattened in outline. Lateral groove absent. Lateral and inner outline of rostrum straight. . . . . . .*Eogonatus tinro* (Figure 12B)

8(7). Dorsal dome long, tongue-like in outline. Lateral groove present in juveniles at ML > 20 mm. Rostrum inner outline convex, its lateral outline somewhat concave. . . . . *Gonatopsis borealis* (Figure 12D)

9(10). Dorsal dome bent to inner side of statolith (DDA° from 28 to 30°). . . . . *Gonatopsis makko* (Figure 12C)

10(9). Dorsal dome slightly bent to inner side (DDA° from 10 to 12°). . . . .*Gonatopsis okutanii* (Figure 12A)

11(12). Rostrum straight, lies along longitudinal axis of statolith (RDA° < 7°). . . . .*Gonatus middendorffi* (Figure 4B)

12(11). Rostrum bent to the lateral side of statolith (RDA° > 10°). . . . . .13.

13(14). Dorsal dome lies almost along the longitudinal axis of statolith (DDA°< 10°). . . . . .15.

14(13). Dorsal dome bent to the inner side of statolith (DDA > 10°) . . . . .17.

15(16). Dorsal dome wide, round in outline. Lateral and inner grooves distinct and symmetrical. Rostrum long (>28% TSL) and narrow (ratio rostrum width/rostrum length < 80%). . . . . *Gonatus onyx* (Figure 4E)

16(15). Dorsal dome narrow, tongue-shaped. Lateral and inner grooves asymmetrical or lateral groove absent. Rostrum short (24–26% TSL) and wide (ratio rostrum width/rostrum length > 110%). . . . .*Gonatus berryi* (Figure 4A)

17(18). Lower part of lateral dome swollen. Rostrum length from 13 to 25% TSL. Ratio rostrum width/rostrum length < 95%. . . . .*Gonatus madokai* (Figure 4C)

18(17). Lower part of lateral dome smoothened. Rostrum length > 25% TSL. Ratio rostrum width/rostrum length > 100%. . . . . *Gonatus pyros* (Figure 4D)

Age and growth

Statolith growth. Relative growth of different parts of the body is usually best described by an allometric (power) curve. However, in the case of non-proportional development of a given part, the relative growth of this part may be described by another function (Gould, 1966). Different relationships have been observed between TSL and ML for gonatid paralarvae: linear, logarithmic, logistic and power (allometric) proper. Logarithmic and logistic relationships have been observed in *B.magister, G.borealis, G.middendorffi* and *G.onyx*, i.e.

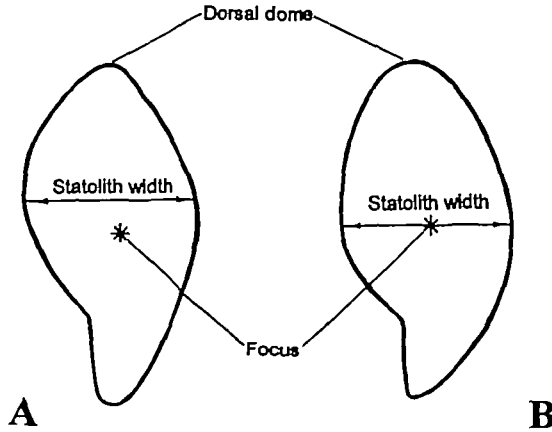**Fig. 16.** Scheme of the first-check statolith with central position of nucleus. (**A**) Tongue-shaped dorsal dome and maximum statolith width above the focus; (**B**) rounded dorsal dome and maximum statolith width at the level of focus.

in paralarvae having statoliths with a rostrum and dorsal dome, that grows quickly and in different axes. After completion of development, specific juvenile shape is achieved and statoliths of these squids start to grow proportionally. Thus, their growth becomes best described by an allometric function (e.g. in *B.magister*, Arkhipkin *et al.*, 1996). Statoliths of other gonatids grow proportionally, so their relative growth is best described by allometric (or isometric) curves.

Small gonatid paralarvae (<10 mm ML) can be grouped into two distinct groups by the size of their statoliths: one group with either large (*B.magister*, *G.borealis* and *E.tinro*) or long statoliths (*G.middendorffi*) and another group with small statoliths (the rest of *Gonatus* spp.). Statoliths of almost all species of the small-sized group grow faster than those of the large-sized group and overtake the latter group at ML of ~20 mm (Figure 17). Only *G.madokai* still have relatively small statoliths at ML > 20 mm. The functional role of statolith size has never been discussed in the literature, so the presence of the two statolith size groups among Pacific gonatids remains unclear.

Growth of paralarvae. Length-at-age data of all gonatid paralarvae are best described by exponential curves. This is consistent with the data on paralarval growth of other squids, e.g. the ommastrephid *Sthenoteuthis pteropus* (Arkhipkin and Mikheev, 1992), the enoploteuthid *Abralia trigonura* (Bigelow, 1992) and various loliginids (Forsythe and Van Heukelem, 1987).

Among the gonatids studied, it is possible to distinguish three groups based on their instantaneous rates of growth (*G*) which are approximately equal to relative daily growth rates (RDGR, %ML per day) at short time intervals (Forsythe and Van Heukelem, 1987).

The first group consists of the fast-growing species of *Gonatus* (*G.madokai* and *G.middendorffi*) (Figure 17). The former species has a very thin mantle wall and
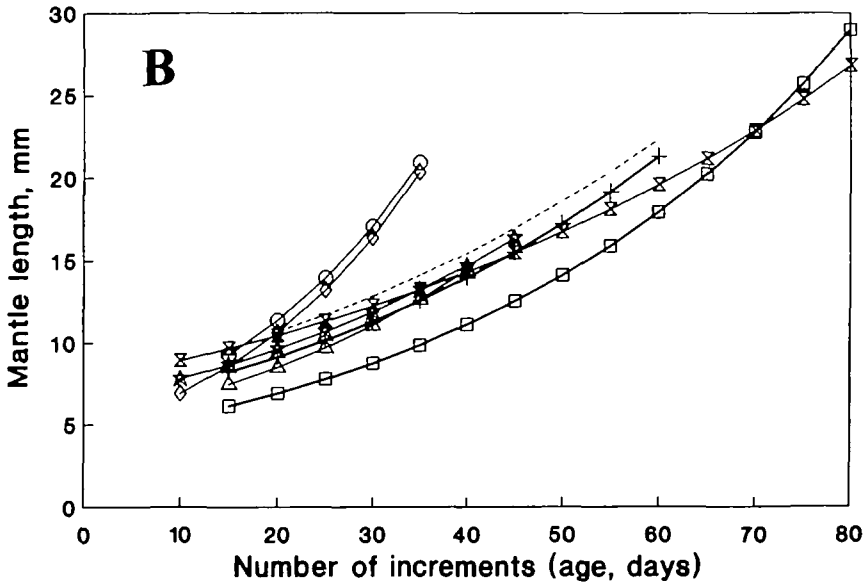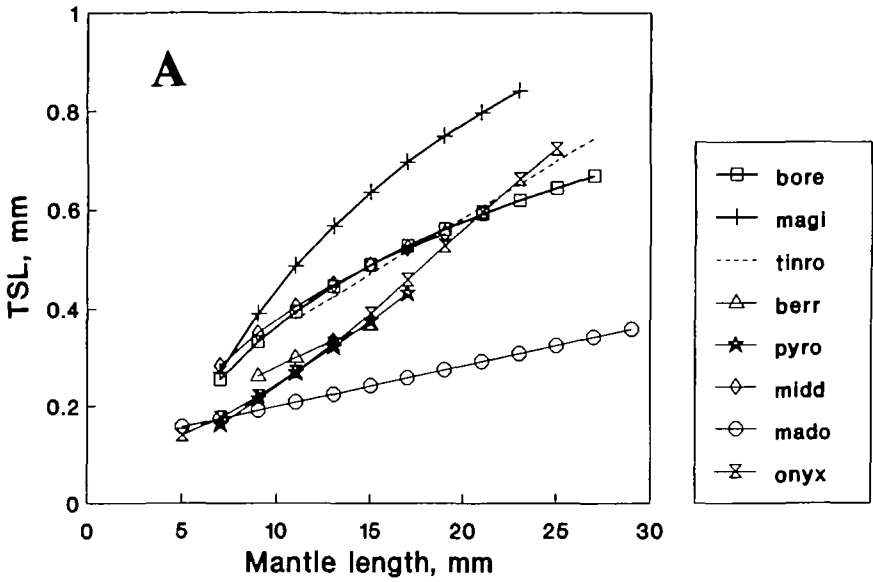
Fig. 17. Relative growth of total statolith length versus mantle length (**A**) and growth curves of mantle length ((**B**) in gonatids from the western Bering Sea. bore, *Gonatopsis borealis*; magi, *Berryteuthis magister*; tinro, *Eogonatus tinro*; berr, *Gonatus berryi*; pyro, *Gonatus pyros*; midd, *Gonatus middendorffi*; mado, *Gonatus madokai*; onyx, *Gonatus onyx*.

at this ontogenetic phase is quite similar to planktonic cranchiids. The latter species has a very long slender mantle (Okutani *et al.*, 1988). Thus, in terms of growth in length, both these species grow fast, while in terms of growth in weight they have comparable growth rates to the other gonatid paralarvae. Kubodera (1982, cited in Okutani *et al.*, 1988) has estimated growth in length in *G.madokai* by length–frequency analysis. His estimation of its growth rate has been low ($G = 0.0133$, or RDGR = 1.33% ML day^{-1}). Our estimations of *G.madokai* growth are three times that of Kubodera (1982). This once again shows that any indirect method of growth rate estimation in squids (i.e. by the length–frequency method) is inappropriate (e.g. Jackson and Choat, 1992).

The second group comprises the rest of the gonatid species studied, excluding *G.borealis*. Paralarvae of these species have ML 6–8 mm at ages of 11–15 days, and medium growth rates (1.5–2.2% ML day^{-1}). At 20–25 mm ML, they acquire body proportions (mainly mantle and fins) like those of juveniles and therefore can be considered as early juveniles (like in Okutani *et al.*, 1988). Paralarvae and early juveniles of the Atlantic *Gonatus fabricii* (7–25 mm ML) had similar growth rates (Kristensen, 1977).

The only squid of the third group, *G.borealis*, is smaller in size than other gonatid paralarvae at ages <60 days. However, at age 70 days (~22 mm ML), this species overtakes gonatids of the second group in length due to higher growth rates (2.4% ML day^{-1}).

It is notable that gonatid paralarvae attain 7–10 mm ML at early ages (15–20 days). Fast-growing paralarvae of tropical ommastrephids (Arkhipkin and Mikheev, 1992) and enoploteuthids (Bigelow, 1992) attain the same ML at ages 30–35 days. However, it does not mean that cold-water gonatids grow faster than tropical species, because their ML at hatching is 3–4 times larger (3–4 mm ML; Kristensen, 1977) than in ommastrephids and enoploteuthids (~1 mm; Nesis, 1979; Bigelow, 1992). Therefore, the starting point of growth in length is different in tropical and cold-water species, showing faster growth rates in ommastrephid and enoploteuthid paralarvae (7–9% ML day^{-1}) (Bigelow, 1992; Laptikhovsky *et al.*, 1993). Nevertheless, cold-water planktonic gonatid paralarvae have relatively fast growth rates in length, attaining the sizes of early juveniles (20–25 mm ML) by 35–70 days.

Statoliths are useful tools for the study of age, growth and possible ecological shifts in gonatid paralarvae from the northern Pacific. Daily periodicity of growth increments within their microstructure enables age estimates growth rates calculation with a high precision. Paralarval statoliths have species-specific characters that allow the identification of gonatid species even at the paralarval phase. These characters are valuable additions to the key of identification of gonatid paralarvae and juveniles (Kubodera and Okutani, 1981), especially as paralarvae are often found partially damaged in planktonic samples. The results of the present paper can be valuable in gut content analysis of oceanic predators. In such studies, it would be possible to identify the species, estimate their size and even assess the age of the prey.

Acknowledgements

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