

Statocysts of cephalopods can function with asymmetric statoliths: on the discovery of an anomaly in statolith ontogenetic development in squid *Berryteuthis septemdentatus* (Coleoidea: Gonatidae)

V.R. Zimina

Invertebrate Zoology Department, Lomonosov Moscow State University, Leninskie Gory 1-12, Moscow 119234 Russia. E-mail: zimina@vniro.ru
Victoria Zimina: ORCID 0009-0006-4902-5921

ABSTRACT: The article presents a description of the first discovered squid *Berryteuthis septemdentatus* with an asymmetric pair of statoliths. One statolith from a pair had three initial crystallization points, two of which gave rise to the development of two separate paralarval statoliths during squid ontogenesis. These paralarval statoliths were located in one statocyst chamber at the time of hatching, and merged into single statolith only on the fourth day after paralarva hatching. The statolith that appeared after merging, retained an abnormal modified form until the adulthood. The second statolith of the pair had normal shape for this species. The finding suggests the presence of unknown mechanisms that may compensate asymmetry in the statolith-statocyst system functioning and allow a squid with defects in vestibular apparatus to survive and reach adulthood. Apparently, on early stages of paralarvae ontogenetic development the statocysts do not play a vital role, which may be related to rather inactive, planktonic lifestyle of paralarvae. Data on morphometry of statoliths is presented for the commander squid *Berryteuthis magister* from the Bering Sea and Pacific Ocean and the previously considered of its subspecies *B. septemdentatus*, inhabiting the Sea of Japan. Morphometry of adult and paralarval statoliths of these two species is compared. The paralarval statoliths are larger in *B. septemdentatus* than in *B. magister*. This is due to the fact that paralarvae of *B. septemdentatus* hatch from the larger eggs than paralarvae of *B. magister*.

How to cite this article: Zimina V.R. 2024. Statocysts of cephalopods can function with asymmetric statoliths: on the discovery of an anomaly in statolith ontogenetic development in squid *Berryteuthis septemdentatus* (Coleoidea: Gonatidae) // *Invert. Zool.* Vol.21. No.1. P.67–80. doi: 10.15298/invertzool.21.1.03

KEY WORDS: *Berryteuthis septemdentatus*, *Berryteuthis magister*, asymmetric statoliths, paralarval statolith, nuclei, morphometry.

The article presents the materials of the scientific work of V.P. Zimina awarded the “Invertebrata” prize for the best bachelor’s thesis (<http://invert.bio.msu.ru/ru/education-ru/baccalaureate/92-invertebrata-premium>).

В статье представлены материалы работы В.Р. Зиминой, удостоенной премии «Invertebrata» за лучшую бакалаврскую диссертацию (<http://invert.bio.msu.ru/ru/education-ru/baccalaureate/92-invertebrata-premium>).

Статоцисты головоногих моллюсков могут работать с несимметричными статолитами: об обнаружении аномалии онтогенетического развития статолитов кальмара *Berryteuthis septemdentatus* (Gonatidae: Coleoidea)

В.Р. Зими́на

Кафедра зоологии беспозвоночных, Московский государственный университет им. М.В. Ломоносова, Ленинские горы 1-12, Москва 119234 Россия. E-mail: zimina@yuniro.ru

РЕЗЮМЕ: В работе представлено описание впервые обнаруженного кальмара *Berryteuthis septemdentatus* с ассиметричной парой статолитов. Один из статолитов был с тремя точками кристаллизации, две из которых в процессе онтогенеза кальмара дали начало двум независимым статолитам. Эти статолиты личинок-параларв располагались в одной камере статоциста, и лишь на четвертые сутки после выклева слились в один общий статолит, сохранивший у взрослого кальмара уродливую видоизмененную форму. Второй статолит из пары сохранил нормальную форму, присущую кальмарам этого вида. находка позволяет предположить наличие неизвестных пока механизмов компенсации асимметрии в работе системы статолит-статоцист, позволивших выжить кальмару с дефектами в развитии вестибулярного аппарата и достичь дефинитивных размеров и половой зрелости. По-видимому, на ранних стадиях онтогенетического развития личинок-параларв работа статоцистов не играет жизненно важную роль, что может быть связано с их малоподвижным, планктонным образом жизни. Представлены данные морфометрии статолитов кальмаров рода *Berryteuthis*, включающие командорского кальмара *Berryteuthis magister* из Берингова моря и Тихого океана и ранее считавшегося его подвидом *Berryteuthis septemdentatus*, населяющего Японское море. Приведены сравнения морфометрии статолитов взрослых животных, а также статолитов личинок-параларв этих двух видов. Статолиты личинок-параларв на момент выклева у *B. septemdentatus* больше таковых у *B. magister*. Это связано с тем, что личинки-параларвы *Berryteuthis septemdentatus* вылупляются из более крупных яиц, чем у командорского кальмара.

Как цитировать эту статью: Zimina V.R. 2024. Statocysts of cephalopods can function with asymmetric statoliths: on the discovery of an anomaly in statolith ontogenetic development in squid *Berryteuthis septemdentatus* (Gonatidae: Coleoidea) // *Invert. Zool.* Vol.21. No.1. P.67–80. doi: 10.15298/invertzool.21.1.03

КЛЮЧЕВЫЕ СЛОВА: *Berryteuthis septemdentatus*, *Berryteuthis magister*, asymmetric statoliths, paralarval statolith, nuclei, morphometry.

Introduction

Statocysts are the paired equilibrium organs located in cartilaginous cranium of cephalopods (Budelmann, 1988). Serving as acceleration and gravity receptors, the statocysts of squids and other decapod cephalopods (cuttlefishes, bobtail squids and *Spirula*) represent the most complex balance organs of all invertebrates and are similar in structure to the vestibular apparatus of fishes.

The similarity with fishes is due to the similarity in ecology, high maneuverability and the need for complex orientation in space in both squids and fishes (Packard, 1972). Different types of locomotion determine the structure of the statocysts, which reflects animal's way of life (Budelmann, Wolff, 1976; Maddock, Young, 1984).

Basing on morphological differences, the statocysts in cephalopods are classified into three types: nautilus type, octopod type and decapod

type (Budelmann, 1988). Decapod statocysts are the most complexly developed, being an irregularly shaped cavity due to the cartilaginous protrusions called anticristae and containing calcareous statolith. The statocyst cavity is partially separated into three chambers: anterior, posterior and ventral. Within the statocyst there is a sensory epithelium called macula to which the statolith is attached (Young, 1960; Budelmann, 1988). The sensory cells of macula serve as a part of gravitation-reception system, while the sensory epithelium of the crista provides reception of endolymph motion under angular acceleration; each of the receptor areas has its own projection in the animal's brain (Stephens, Young, 1982; Budelmann, 1988, 1990). The structure of the gravitational system consists of the three laminae of the sensory epithelium (macula) and two statoconial layers. These layers are completely adjacent to macula neglecta superior (MNS) and macula neglecta inferior (MNI), while the statolith is attached to maculae statica princeps (MSP) only in the wing area called the spur (Budelmann *et al.*, 1973; Arkhipkin, Bizikov, 2000). Typically, statolith of decapod cephalopods consists of the four main parts: wing, rostrum, dorsal dome and lateral dome.

The angular acceleration reception system in decapod statocysts consists of about a dozen of finger-like cartilaginous processes inside the statocyst cavity (curved hamuli and straight anticristae) as well as low cartilaginous ridges covered with sensitive hair cells called cristae, which run along the anterior, posterior and lateral walls of the statocyst (Dilly *et al.*, 1975; Arkhipkin, Bizikov, 1998). The cristae are divided into 4 sections: the anterior transversal crista (cta), longitudinal crista (cl), posterior transversal crista (ctp) and vertical crista (cv). The finger-like cartilaginous processes protruding into the chamber of statocyst (curved hamuli and straight anticristae) structure the inner statocyst cavity forming channels that direct the endolymph flow. All crista have a transparent sail-like structure called cupula, attached to their ridge. Deviation of the cupula under the endolymph flow may excite hair cells of the sensory epithelium providing reception of angular acceleration (Stephens, Young, 1978; Arkhipkin, Bizikov, 1998, 2000). The most developed, almost completely closed horizontal channel in statocysts is associated with the cristae responsible for reception of angular

acceleration, produced by yawing (Stephens, Young, 1978).

During the animal's movement the statoliths attached to the anterior wall of statocysts by the macula, oscillate on it, generating directed flows of endolymph in the statocysts chambers. The morphology of the statoliths is functionally determined: each part of the statolith while oscillating directs the flow of endolymph to corresponding part of the sensory epithelium (Arkhipkin, Bizikov, 1998).

Thus, morphology of the statolith is determined by the functional role of each of its parts that together with complexly structured inner space of statocysts comprise vestibular apparatus of decapods ensuring them effective direction and acceleration control during jetting swimming. As the morphology of the statolith is closely related to its function, it is important to note that the statoliths of squids with different lifestyles differ. Two types of statoliths are distinguished: demersal (near-bottom) and pelagic. Typical features of demersal statoliths are the presence of large usually paddle-shaped rostrum that is mostly free from wing, and well-developed lateral and dorsal domes separated by a distinct groove. Dorsal and lateral domes of the pelagic statolith are poorly separated from each other and function as a unit; rostrum is short and wing is long, extending almost to the rostrum distal end and overlapping most of the dorsal dome. Statoliths of demersal squids are two or more times larger than those of pelagic squids among animals of the same size class (Clarke, Maddock, 1988; Arkhipkin, 1997; Arkhipkin, Bizikov, 2000). Taking into account the statolith functioning model described above, the more massive and structurally differentiated statolith of demersal type ensures better sensitivity to angular accelerations and provides accurate movements such as roll and yawing, in highly structured space of near-bottom environment, while pelagic type statoliths are more adopted to monitor accelerations in the water column where there are no obstacles for the jetting swimming (Arkhipkin, Bizikov, 2000).

Statoliths are widely used as registering structures to age squids and cuttlefishes. Their slides bear growth marks (increments) with verified daily periodicity (Rosenberg *et al.*, 1981; Dawe *et al.*, 1985; Lipinski, 1986; Jackson, 2004; Arkhipkin *et al.*, 2018). These increments

start to form either during late embryogenesis before hatching (loliginid squids) or at the moment of hatching (oegopsid squids). The first check (hatching mark) is located outside the focus (nucleus), which is the point of statolith growth (Arkhipkin, Bizikov, 1997; Arkhipkin, 2005). Growing throughout the life of an animal, statoliths contain information about individual life events such as stress, mating, spawning and so on. These events may be seen as stress-marks on the statolith sections (Arkhipkin *et al.*, 1999; Arkhipkin, 2005). In general, statolith growth increments consist of crystals of aragonite (CaCO_3) lying in a protein matrix consisting mainly of acidic proteins. Statoliths contain large amount of aspartic acid, which can serve as a catalyst for the precipitation of calcium carbonate (Radtko, 1983). The protein matrix can induce and direct crystallization process (Weiner, Traub, 1984). On the other hand, inhibition of growth may occur through the interaction of proteins with the axes of crystal, so stereochemical control takes place (Addadi, Weiner, 1985). Diurnal increments are related to the peculiarities of the biomineralization process: the calcium-protein ratio in the statocysts endolymph changes during the day. In this case the presence of calcium-binding proteins can block carbonate deposition even after crystallization has begun (Wright, 1991). Thus, variations in the organic/calcium ratio in statocysts endolymph throughout the day produce alternating light and dark growth increments that may be observed on sections of statoliths (Castro, Guerra, 1989; Sauer, Lipiński, 1991). The lighter growth increments contain more calcium and the darker ones contain more protein. The concentration of magnesium ions probably affects the deposition of calcium, as there is more magnesium than calcium in the endolymph during the day and vice versa during night. As a result, more organic matter with less calcification may be deposited in the daytime. The visibility of growth increments on the rostrum is also related to biomineralization: it becomes difficult to distinguish growth lines because less organic matter is deposited as the animals gets older (Bettencourt, Guerra, 2000). It has also been found that statoliths do not start to form at all in the absence of strontium in the water: the presence of this cations is vital for cephalopods normal growth (Hanlon *et al.*, 1989).

Material and methods

COLLECTION DATA AND BIOANALYSIS. Squids *Berryteuthis septemdentatus* Sasaki, 1915 were collected from trawl catches of R/V “Bukhoro” (expedition of “TINRO” — Pacific branch of Russian Federal Research Institute of Fishery and Oceanography (“VNIRO”) carried out on 11.07.2014 and 18.07.2014 at the continental slope in the Peter the Great Bay in the Sea of Japan (Primorsky Krai). The squids were sampled on July, 11, 2014 in position $42^{\circ}24'5''$ N $131^{\circ}34'9''$ E and on July, 18, 2014 in position $42^{\circ}27'8''$ N $132^{\circ}30'0''$ E. A total of 97 squids were collected (51 specimens for July, 11 and 46 specimens for July, 18). Bioanalysis was conducted for all squids with determination of sex, stage of maturity, mantle length and body weight; for females the length of nidamental glands was recorded. In all analyzed squids a pair of statoliths were extracted from the statocysts through a ventral incision at the base of the head behind the funnel. Sampled statoliths were stored in tubes containing 96% ethanol. All in all, the statoliths from 39 squids (19 specimens caught on July, 11 and 20 specimens caught on July, 18) were used in this study. Mantle length of squids ranged from 141 mm to 203 mm, in this area are mainly individuals in 3–5 stages of maturity with domination of 3 stage.

For comparison, 65 pairs of statoliths from squid *Berryteuthis magister* Berry, 1913 (commander squid) were also analyzed: 30 pairs from squids caught in the Bering Sea ($61^{\circ}18'30''$ N; $175^{\circ}11'59''$ E) during TINRO expedition in the end of November 1996 and 35 pairs from squids caught in the Pacific Ocean (Middle Kuril Islands, $49^{\circ}44'42''$ N; $155^{\circ}14'6''$ E) in TINRO expedition in the end of December of 2006. All statoliths of *B. magister* were accompanied by bioanalysis data of the specimens from which they were extracted. Squids from the Bering Sea were 188–258 mm in mantle length and from 2 to 5 maturity stages with domination of stage 2 and Pacific Ocean specimens were 158–231 mm in mantle length and in 2–3 maturity (only one specimen in 4 stage) stages with domination of stage 2. Statoliths from these squids were stored dry, without fixation for a long time (27 and 17 years, respectively).

MORPHOMETRY AND GRINDING. Statoliths were measured similarly to the scheme used in Lishchenko & Lishchenko (2017) (Fig. 1), where the length of dorsal dome is *ddl*, free part of rostrum length is *fp_{rl}*, length of lateral dome is *ldl*, rostrum base width is *rbw*, rostrum length is *rl*; statolith width is *sw*, total statolith length is *tsl* and the wing length is *wl*. Measurements were taken with an ocular ruler, converting the values obtained to millimeters with rounding to the nearest thousandth. A total of 133 statoliths were measured: 77 statoliths of *B. septemdentatus* (38 pairs from specimens sampled 11.07.2014 and 39 pairs from

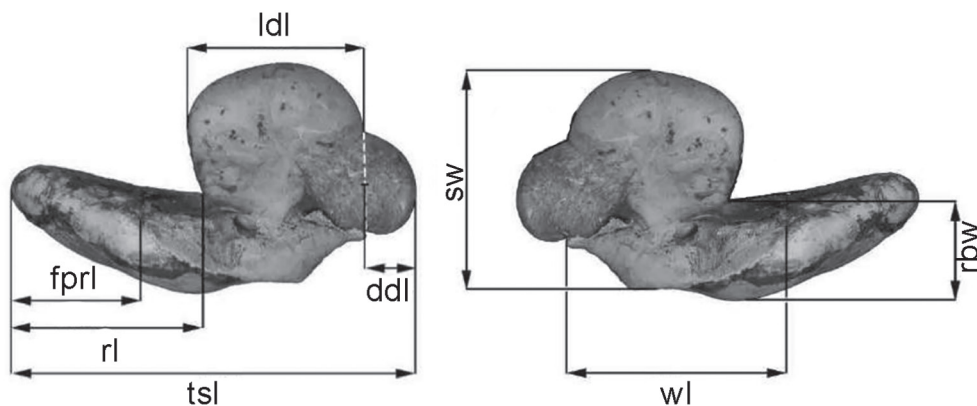


Fig. 1. Scheme of statoliths measurements.

Abbreviations: ddi — length of dorsal dome; fpri — free part of rostrum length; ldl — length of lateral dome; rbw — rostrum base width; rli — rostrum length; sw — statolith width; tsl — total statolith length; wl — wing length (Lishchenko, Lishchenko, 2017 with changes).

Рис. 1. Схема промеров статолитов.

Обозначения: ddi — длина дорсального купола статолита; fpri — длина свободной части роострума; ldl — длина латерального купола статолита; rbw — ширина основания роострума; rli — длина роострума; sw — ширина статолита; tsl — общая длина статолита; wl — длина крыла статолита (Лищенко, Лищенко, 2017, с изменениями).

specimens sampled 18.07.2014); 56 statoliths of *B. magister*: 28 pieces from two TINRO expeditions for 1996 and 2006 years. All statoliths were grinded on both sides according to the standard methodology (Arkhipkin, Shcherbich, 2012; Arkhipkin *et al.*, 2018) (Fig. 2A). Before grinding, the statoliths were mounted on Crystalbond 509 resin. The resin was melted using an electric cooker. Each side of the statolith was grinded with 1000 or 1500 grit sandpaper and then polished with 3000 grit sandpaper. A total of 108 statoliths were grinded. Morphometric data were obtained from 95 statoliths. Low quality or overgrinded slides were not used in the study.

The section with a drop of glycerol was covered with a coverslip and observed at 40X magnification (Fig. 2B). Paralarval statoliths were also morphometrically measured: length and width to the nearest thousandths in micrometers using a measuring ruler in ToupView software. Optical images were obtained using cameras mounted on Olympus microscope (Olympus Corporation, Tokyo, Japan), Zeiss (Zeiss Axioplan2 microscope equipped with an AxioCam HRm digital camera), Leica microscope (Leica Microsystems, Wetzlar, Germany), Olympus binocular and softwares such as ToupView, AxioVision, Helicon Focus and Adobe Photoshop CC 2019. STATISTICS: Statistical data processing was performed in Microsoft Excel and Numbers using a two-sample t-test with different variances, calculating correlation coefficients, coefficients of determination and trend line functions. To analyse how statolith proportions change with growth we studied correlations between different statoliths parameters. In all cases these correlations

could be described in first approximation by a linear regression function ($Y = a + b \cdot X$, where 'a' and 'b' are linear regression coefficients).

Results

STATOLITHS' MORPHOLOGY AND MORPHOMETRY. General morphology of the statolith of *B. septemdentatus* from the Sea of Japan is presented on Fig. 3. Average total length of the statoliths was 2.56 ± 0.11 mm in *B. septemdentatus* from the Sea of Japan; 2.48 ± 0.10 mm in *B. magister* from the Kuril Islands area, and 2.65 ± 0.16 mm in the *B. magister* from the Bering Sea.

Proportions of statoliths parts were slightly different in two species. The ratios sw/tsl , rbw/tsl and rbw/rli in *B. magister* were lower than in *B. septemdentatus* (Table 1). To test the validity of the differences in these three indices, the *B. magister* data from the Kuril Islands and the Bering Sea were combined into one sample and a t-test with unequal two-sided criterion was performed: for sw/tsl the significance level was $p=0.01$, for rbw/tsl $p=0.0003$ and for rbw/rli $p=0.0006$. Thus, differences are reliable and sw/tsl , rbw/tsl and rbw/rli indices can be used as diagnostic features to distinguish these two squid species.

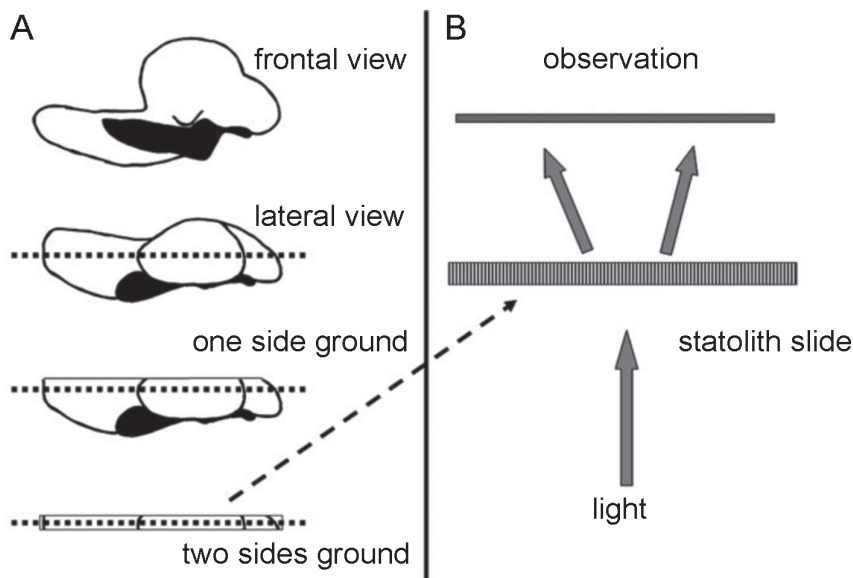


Fig. 2. Scheme of statolith grinding (A) and general principle of section observation (B), (Arkhipkin, Shcherbich, 2012, with changes).

Рис. 2. Схема шлифовки статолита (A) и общий принцип анализа шлифа (B), (Arkhipkin, Shcherbich, 2012, с изменениями).

Table 1. Morphometric indices determining the main proportions of *B. septemdentatus* and *B. magister* statoliths.

Таблица. 1. Морфометрические показатели, определяющие основные пропорции статолитов кальмаров *B. septemdentatus* и *B. magister*.

Index	<i>B. septemdentatus</i>		<i>B. magister</i>	
	Sea of Japan n=39 m±sd	Pacific Ocean n=28 m±sd	Bering Sea n=28 m±sd	
rl/tsl	0,459±0,02	0,470±0,03	0,460±0,04	
sw/tsl	0,527±0,04	0,515±0,03	0,510±0,02	
rbw/tsl	0,259±0,02	0,246±0,02	0,240±0,02	
ldl/tsl	0,443±0,05	0,439±0,03	0,437±0,02	
rl/ldl	1,045±0,11	1,075±0,09	1,053±0,09	
fprl/tsl	0,275±0,05	0,273±0,03	0,279±0,04	
ddl/tsl	0,125±0,02	0,126±0,01	0,128±0,02	
ddl/ldl	0,284±0,04	0,289±0,03	0,288±0,03	
wl/tsl	0,594±0,05	0,599±0,04	0,591±0,03	
rbw/sw	0,492±0,05	0,480±0,05	0,472±0,03	
rbw/rl	0,565±0,05	0,525±0,04	0,526±0,06	

Mantle length of *B. septemdentatus* was 141–203 mm, maturity stage — 3–5; Mantle length of *B. magister* was 158–258 mm, maturity stage — 2–5.

Abbreviations: m — mean value; n — number of statoliths; sd — standard deviation; ddl — length of dorsal dome; fprl — free part of rostrum length; ldl — length of lateral dome; rbw — rostrum base width; rl — rostrum length; sw — statolith width; tsl — total statolith length; wl — wing length.

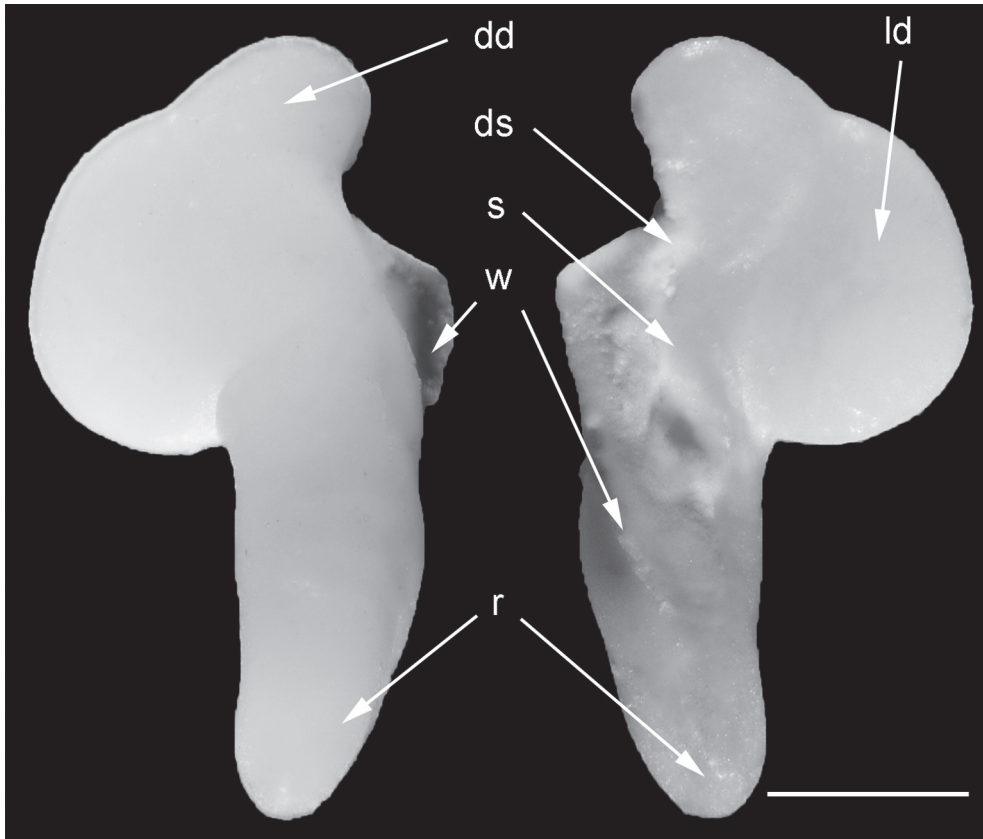


Fig. 3. Structure of *B. septemdentatus* statolith, light microscopy. Left — view from the posterior side, right — view from the anterior side.

Abbreviations: dd — dorsal dome; ds — dorsal spur; ld — lateral dome; r — rostrum; s — spur; w — wing. Scale bar: 500 μm .
Рис. 3. Строение статолита кальмара *B. septemdentatus*, световая микроскопия. Слева вид с задней стороны, справа вид с передней стороны.

Обозначения: dd — дорсальный купол; ds — дорсальная шпора; ld — латеральный купол; r — роstrum; s — шпора; w — крыло. Масштаб: 500 μm .

Regression coefficients for different statoliths indices were processed for the purpose how statoliths change during its growth. Low negative correlation was found between the growth of rbw/tsl , rbw/rl indices with the growth of the statolith for both squid species indicating that the statoliths become more streamlined as the squid grows. Low positive correlation of growth indices in all samples was observed for $fpri/tsl$, rl/dl and rbw/sw indicating to a change in shape of the statolith that becomes more elongated and slenderer as it grows. On the contrary, proportions of the wing (wl) and the lateral dome (ldl) do not change during the growth of statolith. In two of the three cases, a low positive correlation is noted for the sw/tsl and rl/tsl indices, confirm-

ing “stertching” of the statolith, while the ddl/tsl and ddl/dl indices in two of the three samples do not change with statolith growth, indicating that the dorsal dome grows proportionally with the growth of the statoliths.

PARALARVAL STATOLITHS’ MORPHOMETRY. Statolith nucleus and first check mark are distinguished clearly on the grinded statolith (Fig. 4) The morphometrics of paralarval statoliths were measured according to scheme on Fig. 5. In average, the paralarval statoliths of *B. septemdentatus* were 242.3 μm in length and 121.9 μm in width. The width to length ratio was 0.5. Morphometry was performed for 38 specimens. In *B. magister*, the following values were obtained: average length of the paralarval

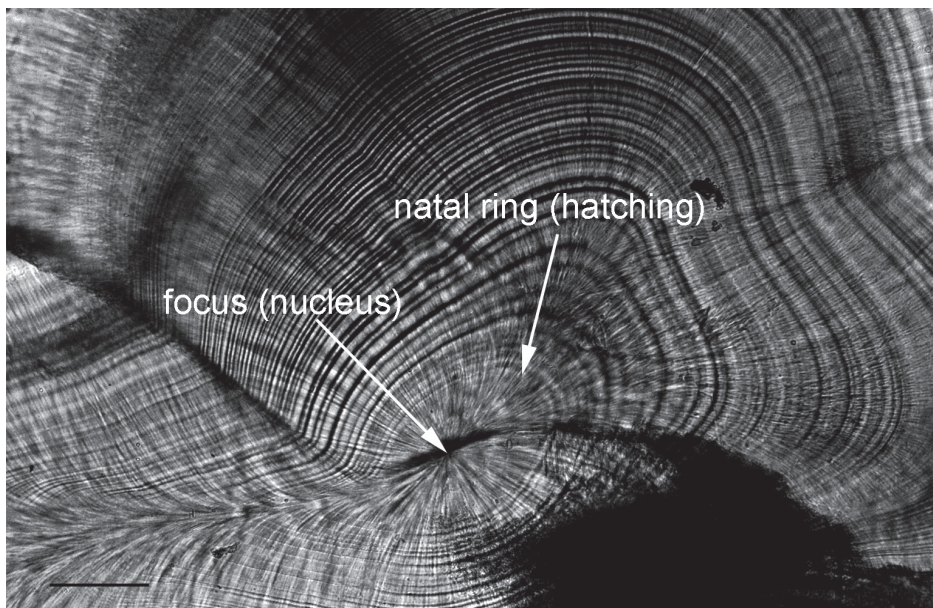


Fig. 4. Bilateral section of *B. septemdentatus* statolith body, light microscopy. Scale bar: 100 μm .

Рис. 4. Двухсторонний шлиф тела статолита кальмара *B. septemdentatus*, световая микроскопия. Масштаб: 100 μm .

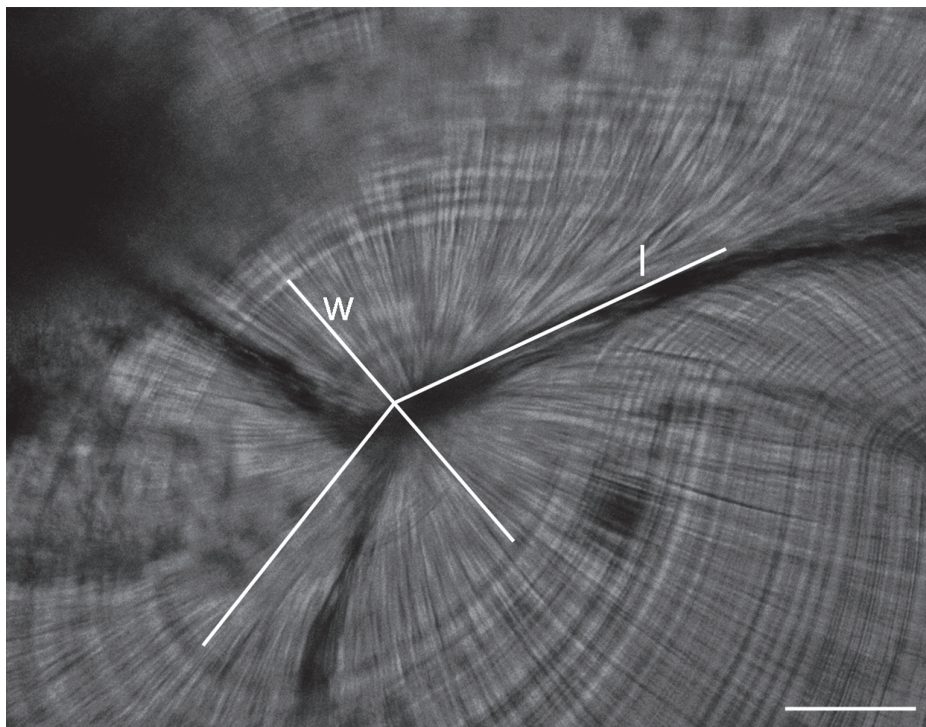


Fig. 5. Scheme of paralarval statolith morphometry, light microscopy.

Abbreviations: l — length; w — width. Scale bar 100 μm .

Рис. 5. Схема морфометрии личиночного статолита, световая микроскопия.

Обозначения: l — длина; w — ширина. Масштаб: 100 μm .

Table 2. Morphometry of paralarval statoliths of squids *B. septemdentatus* and *B. magister*.
Таблица 2. Морфометрия личиночных статолитов кальмаров *B. septemdentatus* и *B. magister*.

	w, mm (average)	l, mm (average)	w/l	n
<i>B. septemdentatus</i>	0,122±0,01	0,242±0,01	0,503±0,03	38
<i>B. magister</i>	0,106±0,01	0,197±0,01	0,540±0,04	40
t-statistics	7,842	14,733	5,16	
P(T<=t) two-sided	2,38*10 ⁻¹¹	7,68*10 ⁻²⁴	2,39*10 ⁻⁶	

Abbreviations: l—mean length of paralarval statolith; w—mean width of paralarval statolith; n—number of statoliths.

statolith was 196.5 µm; average width: 106 µm. The width to length ratio was 0.54. All in all, thin sections of 40 *B. magister* statoliths were analysed. Results and t-statistics are presented in Table 2.

ABNORMAL STATOLITH. During our study one pair of statoliths extracted from maturing female *B. septemdentatus* with a mantle length of 198 mm and age of 191 days was found to be asymmetric: the left statolith had normal shape specific for this species, while the right statolith had abnormal shape due to development anomalies: it initially appeared as three crystallization points (Fig. 6A) located at 169, 46 and 215 µm apart from each other. First two crystallization points (Fig. 6A) represent the nuclei of two anomalous embryonic statoliths. In the larger embryonic statolith (Fig. 6A) the first growth mark corresponding to paralarva hatching is located at 49, 100, 74, 53 µm from the nucleus (measurements 104 clockwise on Fig. 6A). In the smaller embryonic statolith the distances from the nucleus to the first (hatching) growth mark are 47, 61, 23 and 45 µm (clockwise). The larger embryonic statolith is 169 µm in length and 120 µm in width, for smaller embryonic statolith similar meanings are 107 and 72 µm. Width to length ratios were 0.71 and 0.67 for both statoliths respectively. At the moment of hatching, each of these statoliths did not have tear-drop shape that is characteristic for the genus *Berryteuthis*. The third crystallization point (Fig. 6A) formed a bubble-like structure on the smaller embryonic statolith (Fig. 6B). The two separate paralarval statoliths merged into a single statolith only on the fourth day after hatching (Fig. 6B), the length of this statolith was 337 µm. Merging of two paralarval statoliths resulted in formation of a single anomalous dumbbell-shaped statolith. It

is important to note that this anomaly resulted in the formation of an ugly statolith in adulthood with an altered general shape (Fig. 7B). In adult statolith, an abnormal convexity is visible in the area of transition of the statolith's body into rostrum, while in normal statoliths of this species such convexity is absent. This modified zone is marked by 3 longitudinal sutures lines originating from the boundary of two paralarval statoliths, the distance between these sutures is 274, 565 and 136, 177 µm for larger and smaller statoliths respectively (Fig. 6C). Thus, anomaly in the appearance of three growth points and two paralarval statoliths of modified shape induced to the change in the crystallization process for this defective statolith as a whole. As can be seen from the growth pattern of abnormal statolith, this squid for the first 4 days after hatching had two statoliths in the right statocyst chamber and one statolith in the left chamber. Such anomaly resulting in appearance of asymmetric statoliths within one pair has never been described yet in coleoid cephalopods.

The statolith in the left chamber of statocyst had no anomalies in its development (Fig. 7A), but at first sight it seems that paralarval statolith in this specimen was more rounded than usual for *B. septemdentatus* (Fig. 8). The length of this paralarval statolith was 242 µm and the width was 131 µm. Thus, the width to length ratio was 0.54, while in the rest 38 *B. septemdentatus* specimens studied the width to length ratio averaged 0.5. Distances from nucleus to first-check mark were 49, 125, 58 and 118 µm. It can be noted that the distance from the crystallization point to the hatching line in direction to lateral dome in all there paralarval statoliths from this pair of statocysts is almost identical: 49 µm and 47 µm for the defective one and 49 µm for the normal.

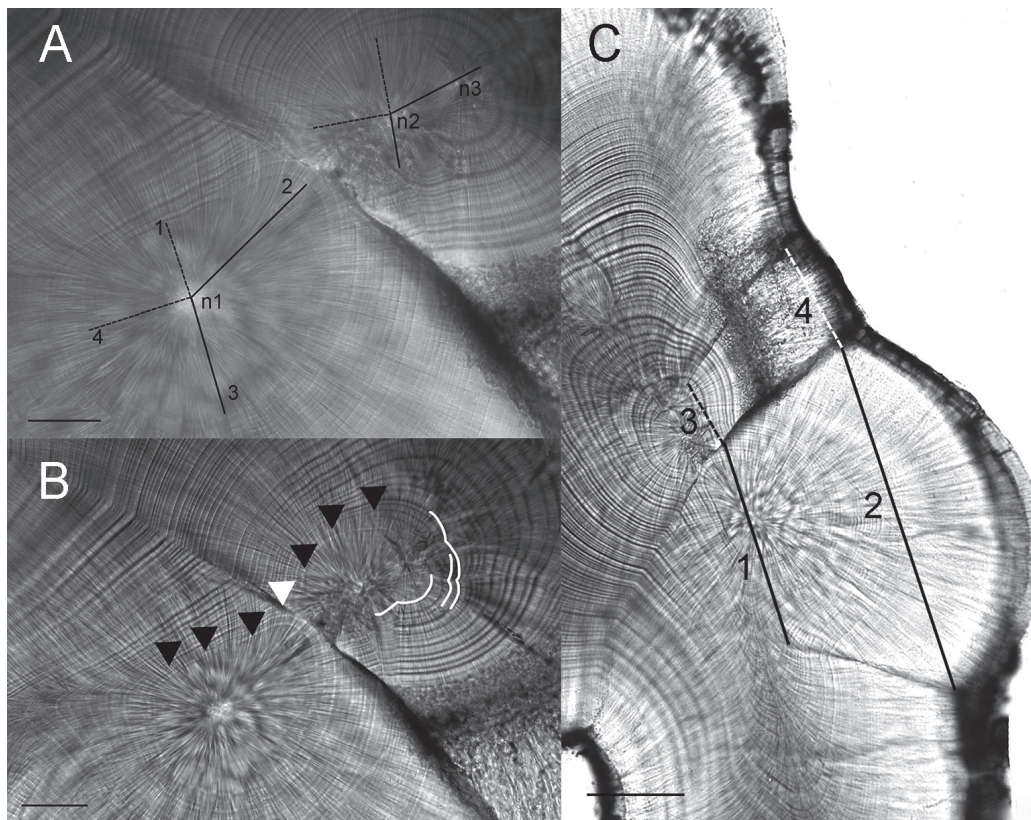


Fig. 6. Nuclei of abnormal *B. septemdentatus* statolith, light microscopy. A — three crystallization points (also called nuclei), lines radiating from the nucleus (marked from 1 to 4 clockwise) indicate the measurements of embryonic statolith (distances between nucleus and first check mark); B — dumbbell-shape paralarval statolith, black tips indicate to the first three growth lines after hatching when paralarval statoliths were separate: white tip indicates to the formation of the first common growth increment (on the fourth day after hatching) when two separate paralarval statoliths merged into a single dumbbell-shaped structure, lines show bubble-like structure; C — longitudinal sutures lines running from the boundary of two paralarval statoliths, lines show distances between sutures lines: 1 and 2 are 274 and 565 μm , respectively; 3 and 4 are 136 and 177 μm , respectively.

Abbreviations: n1 — nucleus number one or the first crystallization point; n2 — nucleus number two; n3 — nucleus number 3. Scale bars: A — 50 μm , B — 50 μm , C — 150 μm .

Рис. 6. Ядра аномального статолита *B. septemdentatus*, световая микроскопия. А — три точки кристаллизации (также называемые ядра), отрезками расходящимися от ядра и маркированными по часовой стрелке от 1 до 4, показаны измерения эмбрионального статолита (расстояние от ядра до первой отметки выклева); В — гантелеподобный личиночный статолит, черные стрелки указывают на первые три независимых прироста, белая стрелка указывает на первый общий прирост, сформированный на четвертый день жизни, когда два отдельных личиночных статолита срослись вместе, образовав гантелеобразную структуру, линии показывают пузыревидную структуру; С — микроструктура продольных швов вдоль двух личиночных статолитов, световая микроскопия, линии показывают расстояние между швами. 1 и 2 равны 274 и 565 μm , соответственно; 3 и 4 равны 136 and 177 μm , соответственно.

Обозначения: А — n1 — первое ядро, или первая точка кристаллизации; n2 — второе ядро; n3 — третье ядро. Масштаб: А — 50 μm , В — 50 μm , С — 150 μm .

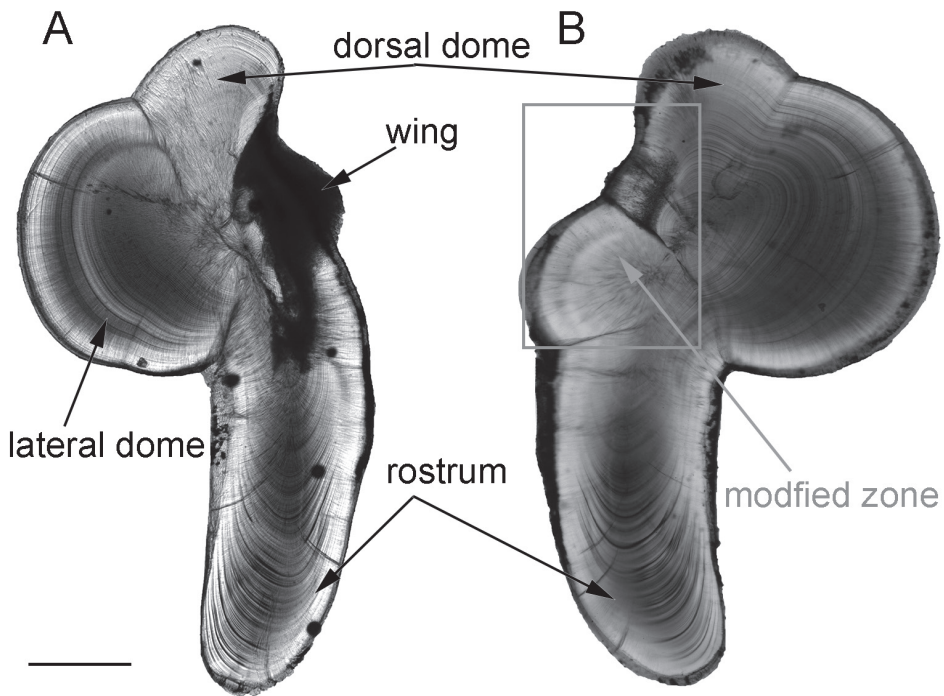


Fig. 7. Comparison of two statoliths, light microscopy. A — general view of normal statolith, light microscopy; B — abnormal statolith with modified zones. Grey rectangle on modified zone. Scale bar: 300 μm .
 Рис. 7. Сравнение двух статолитов, световая микроскопия. А — общий вид нормального статолита; В — измененная форма аномального статолита. Серый прямоугольник показывает зону изменения внешнего облика статолита. Масштаб: 300 μm .

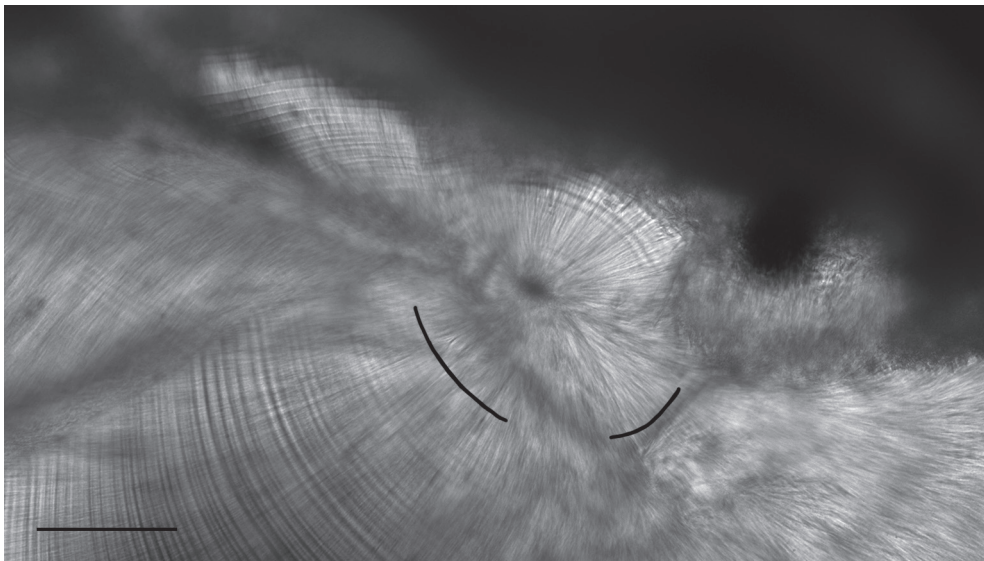


Fig. 8. Normal paralarval statolith *B. septemdentatus*. Lines show approximate boundaries of paralarval statolith. Scale bar: 50 μm
 Рис. 8. Нормальный личиночный статолит *B. septemdentatus*. Линии отображают примерные границы личиночного статолита. Масштаб: 50 μm .

Discussion

STATOLITHS' MORPHOMETRY. *Berryteuthis magister* and *B. septemdentatus* have benthopelagic lifestyle and share similar ecological niche. Both species have morphologically similar statoliths of demersal type, with well-defined rostrum, lateral and dorsal domes (Arkhipkin, Bizikov, 1998, 2000). The differences between total lengths of statoliths in squids from different locations can be explained by the differences in size and ontogenetic stages of the squids sampled. The specimens of *B. septemdentatus* from the Sea of Japan were maturing males and females at stages between 3 and 5 that corresponds to reproductive maturation and readiness for spawning (Alexeyev, 2020). In the Kuril Island area, the specimens of *B. magister* in catches were mainly at maturity stages 2 and 3 that corresponded to immature stages. This pattern is consistent with the conception of the migratory and spatial organization of this species in the Kuril Islands area (Alexeyev, 2022). In the Bering Sea specimens of *B. magister* were at stages of maturity from 2 to 5. Females predominated in the sample (22 females versus 6 males) having higher growth rates and larger body sizes than males (Arkhipkin *et al.*, 1996). Thus, differences in absolute size of statoliths can be explained by differences in body size of animals in our sample. *B. septemdentatus* from the Sea of Japan is generally smaller than *B. magister*. But in the Kuril Islands area the squids in catches haven't reached definitive sizes yet and their statoliths was smaller. Table 1 presents a number of relative values describing the parameters of statoliths of adult squids. It shows that proportions of statoliths between the two species of *Berryteuthis* are very similar. This is consistent with the general conception that the statoliths of squid species with similar lifestyle are similar (Arkhipkin, Bizikov, 2000). It seems that statolith development in the statocyst chamber is strictly determinative and conservative process.

A possible reason for the differences in the samples noted for the *rl/tsl*, *ddl/tsl*, *ddl/ldl* and *sw/tsl* indices and low correlation coefficients may be due to insufficient material to estimate morphometric and statistical parameters. Considering that statolith structure is conservative and determined by its functional role, we can expect

that their morphometric parameters should be similar (Arkhipkin, Bizikov, 1998, 2000).

PARALARVAL STATOLITHS' MORPHOMETRY. The paralarval statoliths of *B. septemdentatus* from the Sea of Japan are larger than those of *B. magister* from the Kuril Islands area and from the Bering Sea. Larger size of paralarval statoliths in *B. septemdentatus* than in *B. magister* apparently is due to the larger size of eggs and paralarvae (Natsukari *et al.*, 1993 cited from Katugin, 1998, Nazumi *et al.*, 1979 cited from Katugin, 2000) in *B. septemdentatus*. General shape of paralarval statoliths of the two squid species is very similar, but the paralarval statoliths of *B. septemdentatus* are more elongated and this difference is a diagnostic feature of the species. In both species, paralarval statoliths have peculiar 'teardrop' shape with clearly distinguished rostrum and statolith body that is typical for the genus *Berryteuthis* (Arkhipkin, Bizikov, 1997).

ABNORMAL STATOLITH. Development anomalies in gonatids statoliths have never been described yet in literature. Our study represents the first reported case of statolith with three nuclei, which originally gave two paralarval statoliths in one statocyst chamber, merging only on several days after hatching. Formation of the first common growth line indicates to the formation of a single statolith, while the statolith in the second chamber has been developing as usual. Thus, development abnormalities of one statolith from a pair has led to substantial changes in overall shape of the statolith that persisted throughout the squid life.

According to the existing conceptions, asymmetry in the statoliths' structure should lead to disturbance in their vestibular apparatus, because each part of the statolith has its functional importance in determination of angular acceleration and morphologically corresponds to the structure and anatomy of statocyst chamber (Arkhipkin, Bizikov, 1998, 2000). So, we can assume that altered shape of the right statolith may influence the endolymph flows and therefore nerve perception resulting in disbalance in functioning between the right and left statocyst chambers. The fact that the squid survived this disbalance indicates that during first days after hatching the statoliths don't play a vital role in paralarval coordination, as they hover in the epipelagium and have inactive lifestyle (Arkhipkin, Bizikov, 1996, Boletzky,

2003) or the second normal statolith can compensate work of abnormal statolith. Likewise, a key point can be the merger of two paralarval statoliths into one on the fourth day of paralarva life that is quite early during post embryonic period of life. The causes of abnormalities in statolith embryonic development are unclear. There could be mutations in genes responsible for the development of the vestibular apparatus or statoconia formation, disorders of paralarval development or ocean acidification (Maneja *et al.*, 2001, Kaplan *et al.*, 2013).

Nevertheless, the fact that squid with asymmetric statoliths survived and reached sexual maturity indicates the existence of some still unknown mechanisms of compensation for such deformities in the vestibular structure of coleoid cephalopods.

Compliance with ethical standards

CONFLICTS OF INTEREST: The author declares that she has no conflicts of interest.

Acknowledgments. I'd like to express my special gratitude to PhD Oleg Katugin (Russian Federal Research Institute of Fisheries and Oceanography, Pacific branch), who kindly provided material for this work. Special thanks to my supervisor D.Sc. Vyacheslav Bizikov (Russian Federal Research Institute of Fisheries and Oceanography), who taught me how to grind statoliths and read its slides, I also deeply grateful to him for proofreading of the manuscript. Many thanks to D.Sc. Dmitry Alexeyev (Russian Federal Research Institute of Fisheries and Oceanography) for useful consultations, D.Sc. Alexey Buyanovsky (Russian Federal Research Institute of Fisheries and Oceanography) for his consultations in statistical processing of the data, Olga Khoroshutina (Russian Federal Research Institute of Fisheries and Oceanography) for help in choosing mounting resin. I'm very thankful to professor, D.Sc. Elena Temereva (Invertebrate Zoology Department, Lomonosov Moscow State University) for help with LM photography (supported by Russian Science Foundation grant no. 23-14-00020). Three anonymous reviewers are thanked for providing valuable and constructive comments that helped a lot for improvement of this paper.

References

- Addadi L., Weiner S. 1985. Interactions between acidic proteins and crystals: stereochemical requirements in biomineralization // Proceedings of the National Academy of Sciences. Vol.82. No.12. P.4110–4114. <https://doi.org/10.1073/pnas.82.12.4110>
- Alexeyev D.O. 2020. [Spatial biology of commander squid]. [Thesis of Candidate (Ph.D.) of Biological Sci. Degree]. Moscow: Russian Federal Research Institute of Fisheries and Oceanography ("VNIRO"). 237–243 p. [in Russian].
- Alexeyev D.O. 2022. [Spatial and functional structure in populations of squid of the genus *Berryteuthis* in the Russian Far Eastern seas] // Trudy VNIRO. Vol.188. P.13–48. <https://doi.org/10.36038/2307-3497-2022-188-13-48> [in Russian, with English summary].
- Arkhipkin A.I. 1997. Age and growth of the mesopelagic squid *Ancistrocheirus lesueurii* (Oegopsida: *Ancistrocheiridae*) from the central-east Atlantic based on statolith microstructure // Marine Biology. Vol.129. No.1. P.103–111. <https://doi.org/10.1007/s002270050151>
- Arkhipkin A.I. 2005. Statoliths as 'black boxes' (life recorders) in squid. Marine and freshwater research. Vol.56. No.5. P.573–583. <https://doi.org/10.1071/MF04158>
- Arkhipkin A.I., Bizikov V.A. 1996. Possible imitation of jellyfish by the squid paralarvae of the family *Gonatidae* (Cephalopoda, Oegopsida) // Polar Biology. Vol.16. P.531–534. <https://doi.org/10.1007/BF02329074>
- Arkhipkin A.I., Bizikov V.A. 1997. Statolith shape and microstructure in studies of systematics, age and growth in planktonic paralarvae of gonatid squids (Cephalopoda, Oegopsida) from the western Bering Sea // Journal of plankton research. Vol.19. No.12. P.1993–2030. <https://doi.org/10.1093/plankt/19.12.1993>
- Arkhipkin A.I., Bizikov V.A. 1998. Statoliths in accelerometers of squids and cuttlefish // Ruthenica. Vol.8. No.1. P.81–84.
- Arkhipkin A.I., Bizikov V.A. 2000. Role of the statolith in functioning of the acceleration receptor system in squids and sepioids // Journal of Zoology. Vol.250. No.1. P.31–55. <https://doi.org/10.1111/j.1469-7998.2000.tb00575.x>
- Arkhipkin A.I., Bizikov V.A., Doubleday Z.A., Laptikhovskiy V.V., Lishchenko F.V., Perales-Raya C., Hollyman P.R. 2018. Techniques for estimating the age and growth of molluscs: Cephalopoda // Journal of Shellfish Research. Vol.37. No.4. P.783–792. <https://doi.org/10.2983/035.037.0409>
- Arkhipkin A.I., Bizikov V.A., Nesis K.N. 1996. Distribution, stock structure, and growth of the squid *Berryteuthis magister* (Berry, 1913) (Cephalopoda, *Gonatidae*) during summer and fall in the western Bering Sea // Fishery Bulletin. Vol.94. P.1–30.
- Arkhipkin A.I., Jereb P., Ragonese S. 1999. Checks in the statolith microstructure of the short-finned squid, *Illex coindetii* from the Strait of Sicily (Central Mediterranean) // Journal of the Marine Biological Association of the United Kingdom. Vol.79. No.6. P.1091–1096. <https://doi.org/10.1017/S0025315499001344>
- Arkhipkin A.I., Shcherbich Z.N. 2012. Thirty years' progress in age determination of squid using statoliths // Journal of the Marine Biological Association of the United Kingdom. Vol.92. No.6. P.1389–1398. <https://doi.org/10.1017/S0025315411001585>
- Bettencourt V., Guerra A. 2000. Growth increments and biomineralization process in cephalopod statoliths // Journal of experimental marine biology and ecology. Vol.248. No.2. P.191–205. [https://doi.org/10.1016/S0022-0981\(00\)00161-1](https://doi.org/10.1016/S0022-0981(00)00161-1)
- Boletzky S.V. 2003. Biology of early life stages in cephalopod molluscs // Advances in marine biology. Vol.44. P.143–243.

- Budelmann B.U. 1988. Morphological diversity of equilibrium receptor systems in aquatic invertebrates // Sensory biology of aquatic animals. P.757–782. https://doi.org/10.1007/978-1-4612-3714-3_30
- Budelmann B.U. 1990. The statocysts of squid // Squid as experimental animals. P.421–439. https://doi.org/10.1007/978-1-4899-2489-6_19
- Budelmann B.U., Barber V.C., West S. 1973. Scanning electron microscopical studies of the arrangements and numbers of hair cells in the statocysts of *Octopus vulgaris*, *Sepia officinalis* and *Loligo vulgaris* // Brain Research. Vol.56. P.25–41. [https://doi.org/10.1016/0006-8993\(73\)90325-9](https://doi.org/10.1016/0006-8993(73)90325-9)
- Budelmann B.U., Wolff H.G. 1976. Mapping of neurons in the gravity receptor system of the Octopus statocyst by iontophoretic cobalt staining // Cell and Tissue Research. Vol.171. No.3. P.403–406. <https://doi.org/10.1007/BF00224665>
- Castro B.G., Guerra A. 1989. Feeding pattern of *Sepia officinalis* (Cephalopoda: Sepioididea) in the Ria de Vigo (NW Spain) // Journal of the Marine Biological Association of the United Kingdom. Vol.69. No.3. P.545–553. <https://doi.org/10.1017/S0025315400030952>
- Clarke M.R., Maddock L. 1988. Statoliths from living species of cephalopods and evolution // Paleontology and neontology of cephalopods. Vol.12. P.153–168.
- Dawe E.G., O'dor R.K., Odense P.H., Hurley G.V. 1985. Validation and Application of an Ageing Technique for Short-finned Squid (*Illex illecebrosus*) // Journal of Northwest Atlantic Fisheries Science. Vol.6. P.107–116.
- Dilly P.N., Stephens P.R., Young J.Z. 1975. Proceedings: Receptors in the statocyst of squids // The Journal of physiology. Vol.249. No.1. P.59–61.
- Hanlon R.T., Bidwell J.P., Tait R. 1989. Strontium is required for statolith development and thus normal swimming behaviour of hatchling cephalopods // Journal of Experimental Biology. Vol.141. No.1. P.187–195. <https://doi.org/10.1242/jeb.141.1.187>
- Jackson G.D. 2004. Advances in defining the life histories of myopsid squid // Marine and Freshwater Research. Vol.55. No.4. P.357–365. <https://doi.org/10.1071/MF03152>
- Kaplan M.B., Mooney T.A., McCorkle D.C., Cohen A.L. 2013. Adverse effects of ocean acidification on early development of squid (*Doryteuthis pealeii*) // PLoS One. Vol.8. No.5. Art.e63714. <https://doi.org/10.1371/journal.pone.0063714>
- Katugin O.N. 1998. [Commander squid *Beryteuthis magister* (Berry, 1913): intraspecific variability, spatial and taxonomic differentiation]. [Thesis of Candidate (Ph.D.) of Biological Sci. Degree]. Vladivostok: Pacific branch of the Federal State Budget Scientific Institution “Russian Federal Research Institute of Fisheries and oceanography” (“TINRO”). 215 p. [In Russian]
- Katugin O.N. 2000. A new subspecies of the schoolmaster gonate squid, *Beryteuthis magister* (Cephalopoda: Gonatidae), from the Japan Sea // The Veliger. Vol.43. No.1. P.82–97.
- Lipinski M. 1986. Methods for the validation of squid age from statoliths // Journal of the Marine Biological Association of the United Kingdom. Vol.66. No.2. P.505–526. <https://doi.org/10.1017/S0025315400043095>
- Lishchenko F.V., Lishchenko A.B. 2017. [Intraspecific variability of statoliths appearance of the Commander squid (*Beryteuthis magister*, Berry 1913)] // Sovremennye problemy i perspektivy-razvitiya rybokhozyaistvennogo kompleksa materialy V nauchno-prakticheskoi konferencii molodykh uchenykh s mezhdunarodnym uchastiem. P.175–178 [in Russian].
- Maddock L., Young J.Z. 1984. Some dimensions of the angular acceleration receptor systems of cephalopods // Journal of the Marine Biological Association of the United Kingdom. Vol.64. No.1. P.55–79. <https://doi.org/10.1017/S0025315400059634>
- Maneja R., Piatkowski U., Melzner F. 2011. Effects of ocean acidification on statolith calcification and prey capture in early life cuttlefish, *Sepia officinalis* // Journal of Shellfish Research. Vol.30. No.3. P.1011. <http://dx.doi.org/10.2983/035.030.0342>
- Natsukari Y., Mukai, H., Nakahama S., Kubodera T. 1993. Age and growth estimation of a gonatid squid, *Beryteuthis magister*, based on statolith microstructure (Cephalopoda: Gonatidae) // Recent advances in fisheries biology. P.351–364.
- Packard A. 1972. Cephalopods and fish: the limits of convergence // Biological Reviews. Vol.47. No.2. P.241–307. <https://doi.org/10.1111/j.1469-185X.1972.tb00975.x>
- Radtke R.L. 1983. Chemical and structural characteristics of statoliths from the short-finned squid *Illex illecebrosus* // Marine Biology. Vol.76. P.47–54. <https://doi.org/10.1007/BF00393054>
- Rosenberg A.A., Wiborg K.F., Bech I.M. 1981. Growth of *Todarodes sagittatus* (Lamarck) (Cephalopoda, *Ommastrephidae*) from the Northeast Atlantic, based on counts of statolith growth rings // Sarsia. Vol.66. No.1. P.53–57. <https://doi.org/10.1080/00364827.1981.10414520>
- Sauer W.H.H., Lipinski M.R. 1991. Food of squid *Loligo vulgaris reynaudii* (Cephalopoda: *Loliginidae*) on their spawning grounds off the Eastern Cape, South Africa // South African journal of marine science. Vol.10. No.1. P.193–201. <https://doi.org/10.2989/02577619109504631>
- Stephens P.R., Young J.Z. 1978. Semicircular canals in squids // Nature. Vol.271. P.444–445. <https://doi.org/10.1038/271444a0>
- Stephens P.R., Young J.Z. 1982. The statocyst of the squid *Loligo* // Journal of Zoology. Vol.197. No.2. P.241–266. <https://doi.org/10.1111/jzo.1982.197.2.241>
- Weiner S., Traub W. 1984. Macromolecules in mollusc shells and their functions in biomineralization // Philosophical Transactions of the Royal Society of London. B, Biological Sciences. Vol.304. No.1121. P.425–434. <https://doi.org/10.1098/rstb.1984.0036>
- Wright P.J. 1991. Calcium binding by soluble matrix of the otoliths of Atlantic salmon, *Salmo salar* L // Journal of fish biology. Vol.38. No.4. P.625–627. <https://doi.org/10.1111/j.1095-8649.1991.tb03149.x>
- Young J.Z. 1960. The statocysts of *Octopus vulgaris* // Proceedings of the Royal Society of London. Series B. Biological Sciences. Vol.152. No.946. P.3–29. <https://doi.org/10.1098/rspb.1960.0019>

Responsible editor E.N. Temereva