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The relationship between fine rings in the statolith and growth of the cubomedusa *Chiropsalmus quadrigatus* (Cnidaria: Cubozoa) from Okinawa Island, Japan

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Abstract: Sixty-nine *Chiropsalmus quadrigatus* medusae were collected from Okinawa Island, Japan, in June–August 2000. The bell height ranged from 2.5 to 97.6 mm. The numbers of fine rings on polished statoliths were counted, and the coefficient of variation for the within-individual counts for the four statoliths was 3.3±1.9% (mean±SD, n=17). The slope of the linear regression of the number of rings against collecting date in 61 medusae was near 1.0, suggesting that the statolith rings are daily increments. The relationship between bell height and number of rings fitted a logistic growth curve. And, the relationship between statolith length and number of rings fitted the Gompertz growth curve. A check ring was present at a position of 5–10 rings from the center of each statolith. The back-calculated dates of check ring formation dated mainly from early to mid June, suggesting that the polyp of *C. quadrigatus* finished the metamorphosis to medusa and the medusa was liberated from a substratum during this period.

Key words: cubomedusae; *Chiropsalmus quadrigatus*; statolith; daily increment; growth curve; Okinawa Island; check ring; liberation of medusa

Introduction

The deadly cubomedusa *Chiropsalmus quadrigatus* Haeckel is called "*Habu-kurage*" in Japanese. "*Habu*" comes from the Japanese name for the pit viper, *Trimeresurus flavoviridis* (Hallowell). The sting of *C. quadrigatus* causes severe pain for humans and has resulted in three fatalities in Okinawa since 1961. However, the ecology and life history of this medusa are virtually unknown.

Growth rings in fish otoliths (Williams & Bedford 1974), amphibian bones (Castanet & Smirina 1990), squid statoliths (Kristensen 1980), and gorgonian coral skeletons (Grigg 1974) are reported to be useful for determining age. Daily increments occur in fish otoliths, squid statoliths, and coral skeletons (Pannella 1971, Hurley et al. 1985, Lipinski 1986, Wells 1963). In Cnidarian medusae, Ueno et al. (1995) first found statolith rings from the cubomedusa *Carybdea rastoni* Haacke, and they suggested that these rings were daily increments. The statolith of cubomedusae commences forming during metamorphosis from polyp to medusa (Werner 1975).

In this paper, we report that *C. quadrigatus*, which has four statoliths, has many fine rings similar to those of *C. rastoni* as observed by Ueno et al. (1995). We examined the relationships between these statolith rings and the growth of *C. quadrigatus*. Using a check ring found in the statoliths, we also estimated when the medusae were liberated from a substratum.

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Materials and Methods

From June 19 to August 26, 2000, we collected 69 *Chiropsalmus quadrigatus* medusae at five sites on Okinawa Island (Fig. 1): Nishizaki, Ginowan Port Marina and Ginowan Tropical Beach, Nago Shimin Beach, and Kaganji on the East China Sea, and Awase fishing port on the Pacific coast.

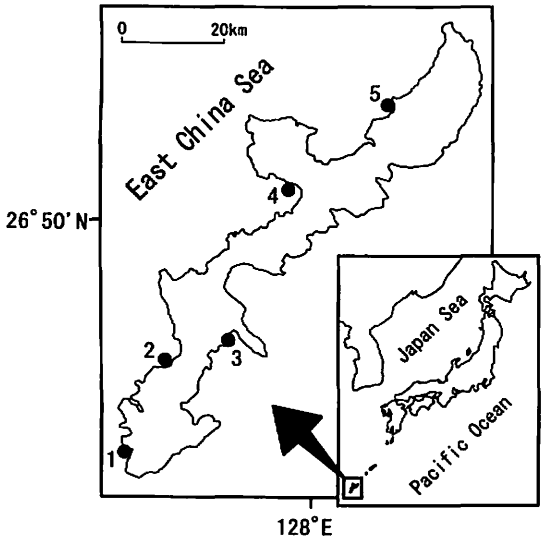

Fig. 1. Sites where *Chiropsalmus quadrigatus* were collected on Okinawa Island, Japan. 1) Nishizaki, 2) Ginowan Port Marina and Ginowan Tropical Beach, 3) Awase fishing port, 4) Nago Shimin Beach, 5) Kaganji.

We captured swimming medusae mainly at the surface of the sea at Ginowan Port Marina and Awase fishing port, at night using a handheld flashlight. At Ginowan Port Marina, each week from April 19 to October 18, 2000, we measured water temperature at 0- and 2.5-m depths with a Pettenkofer thermometer (accuracy: 0.1°C), and salinity at 3 m with a salinity refractometer (accuracy: 0.5‰).

After measuring bell height (*BH*) of medusae (accuracy: 0.05 mm), the four statocysts together with the surrounding mesoglea were cut out from each individual and fixed in 70% ethanol. We observed the superior gonads (Southcott 1956) under a microscope to distinguish the sexes. Medusae without superior gonads were regarded as immature. The preserved statocysts were put in distilled water to make it easier to remove the fixed tissue surrounding the statolith. Then, each statolith was removed and placed on a slide glass using needles. We polished the statolith following the method of Ueno et al. (1995) and counted the number of rings observed on the polished statolith using a microscope.

We used the mean value of the long axis lengths of the four statoliths (*SL*, accuracy: 1 μm) and the maximum number of rings on these statoliths (*NR*) as the representative values for each medusa. Then, we tried to fit *BH* and *SL* to four growth curves, the von Bertalanffy, logistic, Gompertz, and Richards curves (Akamine 1995), which are given by the following respective formulae:

$$l_t=L_\infty\{1-e^{-K(t-t_0)}\},\quad l_t=L_\infty/\{1+e^{-K(t-t_i)}\},$$
$$l_t=L_\infty \exp\{-e^{-K(t-t_i)}\},\quad \text{and}\quad l_t=L_\infty/\{1+re^{-K(t-t_i)}\}^{1/r}.$$

In these formulae, l_t is the length at medusan age t (days), L_∞ is the asymptotic length, t_0 is the age at zero length, and t_i is the age at the inflexion point. K and r are the parameters. We estimated these parameters using a Gauss–Newton

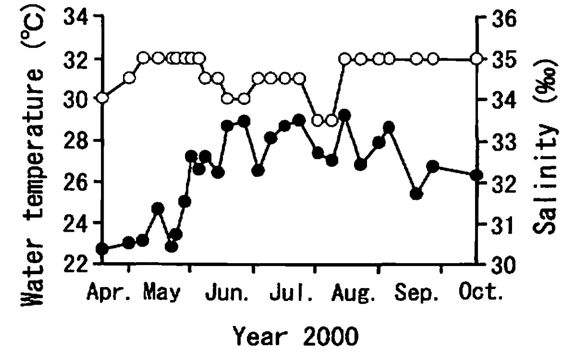

Fig. 2. Surface water temperature (●) and salinity at 3 m (○) at Ginowan Port Marina during the study period.

algorithm in the statistical analysis package "R" (Bates & Watts 1988, Ihaka & Gentleman 1996) and compared for the fitting with Akaike Information Criterion (AIC) (Sakamoto et al. 1986).

Results

Only surface temperature is shown in Fig. 2, because the water temperatures at 2.5 m depth were similar to the surface ones (the difference was <0.7°C). Three seasons were identified from the changes in the water temperature: the ascending (April-mid June), maximum (mid June-late August), and descending (after September) periods. The salinity at 3 m depth ranged between 33.5 and 35.0‰.

The 69 *Chiropsalmus quadrigatus* medusae consisted of 51 immature individuals, 8 males, and 10 females (Table 1). Most of the immature medusae were collected in June and July, while males and females were collected in mid July through August. Bell height (*BH*) ranged from 2.5–63.4, 50.0–83.4, and 47.0–97.6 mm in immature individuals, males, and females, respectively. The *BH* of medusae with superior gonads exceeded 47 mm.

The *C. quadrigatus* statoliths were ellipsoid (Fig. 3A, B) with a shallow V-shaped notch parallel to the short axis. There were many fine concentric rings on the polished statoliths (Fig. 3C). All the statoliths with fine rings had an eye-like structure at their centers and a check ring. The check rings were hexagonal, ranging from elliptical (Fig. 3C) to equilateral (Fig. 3D) hexagons. This check ring diameter averaged 49.2±7.7 μm (mean±SD, n=61) in the long axis and the check ring was between the 5th and 10th ring from the center.

Of the 69 medusae examined, there was no statolith in five statocysts from five medusae, abnormally small ones in 12 statocysts from 10 medusae, and obscure rings on 10 statoliths from eight medusae. Forty-six statoliths from 36 medusae were broken in preparation. Ultimately, 171 statoliths from 61 medusae were analyzable.

Statolith length (*SL*) ranged from 127–732 μm, and the number of rings (*NR*) ranged from 15–82. We obtained data for all four statoliths from 17 medusae. Within an individ-

Table 1. Collection data of *Chiropsalmus quadrigatus* medusae from Okinawa Island, Japan.

Date	Locality[1)]	Number of individuals collected			Range of bell height (mm)		
		immature	male	female	immature	male	female
June 19	2	3	0	0	2.8–5.2	–	–
June 27	2	12	0	0	2.5–20.2	–	–
July 4	2	3	0	0	5.7–8.3	–	–
July 10	2	9	0	0	7.2–52.8	–	–
July 17	2	1	0	0	44.5	–	–
July 18	3	17	2	4	37.9–58.5	62.5–68.2	57.0–68.0
July 24	2	3	1	1	53.0–53.9	72.6	77.1
July 24	5	0	1	0	–	50.0	–
Aug. 2	2	0	1	1	–	65.0	66.4
Aug. 2	4	0	1	0	–	60.0	–
Aug. 6	2	0	2	1	–	56.0–83.4	97.6
Aug. 9	2	1	0	0	63.4	–	–
Aug. 24	1	0	0	1	–	–	79.0
Aug. 26	2	2	0	2	41.4–49.8	–	47.0–60.0
All comb.	1–5	51	8	10	2.5–63.4	50.0–83.4	47.0–97.6

[1)] Collecting site numbers as in Fig. 1.
–: no data

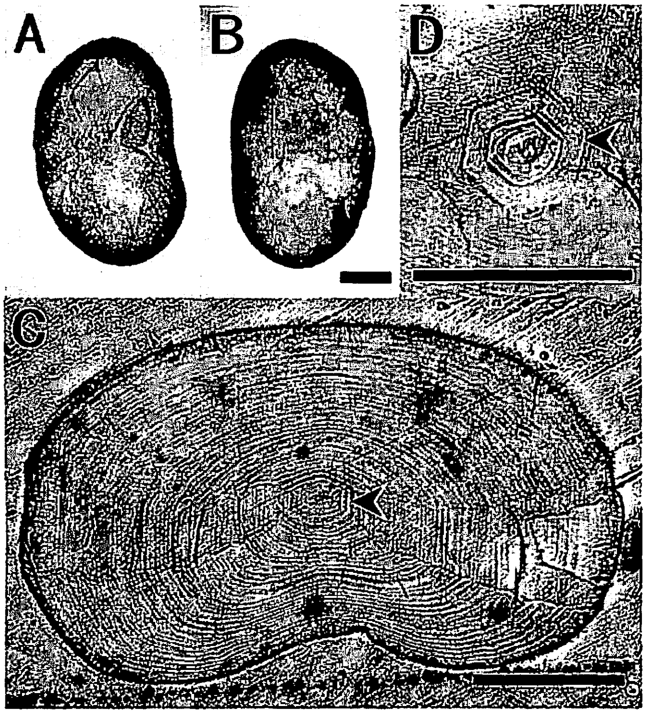

Fig. 3. *Chiropsalmus quadrigatus* statoliths. The interior (A) and exterior (B) sides of a statocyst. (C) A polished transverse section. (D) An enlargement of the center of another statolith. The bars are 100 μm. The arrows in C and D show check rings.

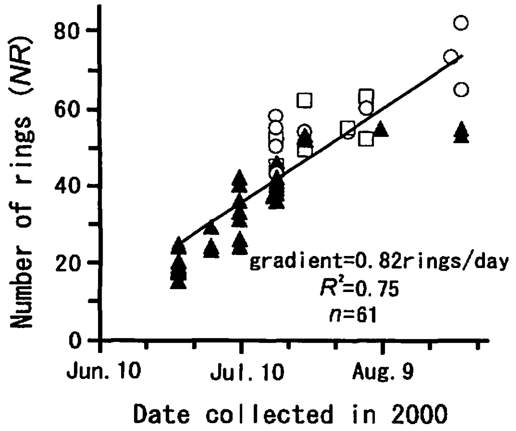

Fig. 4. The numbers of rings in *Chiropsalmus quadrigatus* statoliths (▲: immature, □: male, ○: female).

ual, the coefficients of variation of statolith length and number of rings were very small, 1.6±1.0% and 3.3±1.9% (mean±SD, $n=17$), respectively.

The *NR* increased throughout the collecting period (Fig. 4). The slope of regression of *NR* to the collecting date was not significantly different from 1.0 ($p>0.05$, Student's *t*-test), thus indicating that one ring is formed each day.

Assuming that the rings are daily growth rings, we examined four regression curves of *BH* against *NR* (Table 2). We adopted the logistic curve with the minimum AIC (Fig. 5A). The formula was as follows:

$$BH_{NR}=63.5/\{1+e^{-0.19(NR-33.7)}\} \quad (n=61, R^2=0.86).$$

This growth curve fitted the sigmoid growth of *BH* of *C. quadrigatus* medusae well (maximum growth rate: 3 mm/ring at $NR=34$ and $BH=33$ mm). But the asymptotic *BH* (63.5 mm) was much smaller than the observed maximum *BH* (97.6 mm). Five individuals (open diamonds in Fig. 5A) collected on August 24 and 26 tended to have a smaller *BH*

Table 2. Parameters of four growth curves fitted for the growths of the bell height and the statolith length of *Chiropsalmus quadrigatus* medusae from Okinawa Island, Japan.

Data	Growth curve	Parameter				AIC[2)]
		L_∞	K	t_0 or t_i	r	
Bell height (*BH*)	Bertalanffy	84.8	0.04	18.44	–	465.8
	Logistic	63.5	0.19	33.73	–	451.7
	Gompertz	66.0	0.12	30.48	–	452.0
	Richards	64.2	0.16	32.79	0.63	453.5
Statolith length (*SL*)	Bertalanffy	807.2	0.03	10.62	–	644.4
	Logistic	661.0	0.09	29.44	–	646.3
	Gompertz	700.8	0.06	24.13	–	643.4
	Richards	725.0	0.05	21.03	−0.35	645.2

[2)] Akaike Information Criterion, See Sakamoto et al. (1986)

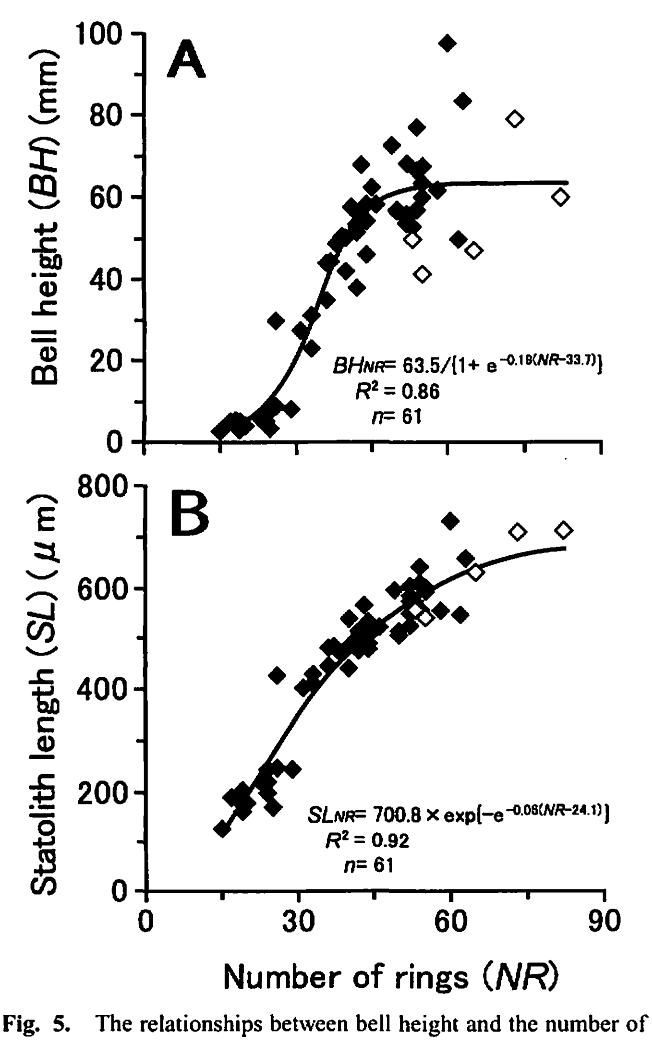

Fig. 5. The relationships between bell height and the number of rings in a statolith (A), and between statolith length and the number of rings (B) in *Chiropsalmus quadrigatus* from Okinawa Island, Japan (◇: five individuals collected on August 24 and 26. These deviated from the regression curve in A, in contrast with those in B.).

than that of other individuals with the same *NR*. For statolith length (*SL*) (Table 2), the Gompertz curve fitted well (Fig. 5B). The formula was as follows:

$$SL_{NR}=700.8\times\exp\{-e^{-0.06(NR-24.1)}\}\quad (n=61, R^2=0.92).$$

The coefficient of determination (R^2) was higher than that in *BH* ($R^2=0.86$). Five individuals (open diamonds in Fig. 5B) did not deviate from the regression curve as they did when plotted against *BH*.

Discussion

Comparison of the statoliths of *Chiropsalmus quadrigatus* and *Carybdea rastoni*

We observed statolith rings in *C. quadrigatus* that were similar to those of *C. rastoni* (Ueno et al. 1995, 1997). The *C. quadrigatus* that we examined ranged from very small to large (2.5 mm≦*BH*≦97.6 mm), while Ueno et al. (1995) studied medium to large *C. rastoni* (21.2 mm≦*BH*≦41.4 mm) (Table 3). The differences between the statoliths of the two species were the longer long axis and shallower V-shaped notch in *C. quadrigatus*. In addition, this species had a single check ring near the center of the statolith, while *C. rastoni* often had several that formed on a semilunar cycle (Ueno et al. 1997).

In *C. rastoni* (Ueno et al. 1995), the range of *NR* concentrated around large numbers, and *SL* was also large because November 4, when *C. rastoni* were collected, was at the end of the period in which *C. rastoni* occurred at Shimonoseki, Japan (Ueno et al. 2003). Also, *C. quadrigatus* with many statolith rings tended to appear in August in contrast with June (Fig. 4), which may suggest that the life history of the two species is similar.

Statolith rings (NR) as a measure of age

Three of our results imply that the statolith rings can be used as a measure of age: 1) One ring was estimated to be

Table 3. Comparison of statoliths between *Chiropsalmus quadrigatus* and *Carybdea rastoni* from Japan.

	Chiropsalmus quadrigatus	*Carybdea rastoni*
Locality	Okinawa Island	Shimonoseki, Yamaguchi Prefecture[3),4)]
Bell height (mm)	2.5–97.6	26.7–41.1[3)]
Statolith		
Shape of notch	shallow	deep[3)]
Check ring	only one near center	some cyclically formed[4)]
Range of *SL* (μm)	127–732	443–554[3)]
Range of *NR*	15–82	76–119[3)]

[3)] Ueno et al. (1995), [4)] Ueno et al. (1997)
SL: statolith length (long axis), *NR*: number of rings

formed per day from the slope of the regression line (Fig. 4). 2) Within individual medusae, *NR* did not differ greatly among the four statoliths. 3) The relationship between *BH* and *NR* fitted the logistic curve, and that between *SL* and *NR* fitted the Gompertz growth curves well (Fig. 5). It is normal that these growth curves are fitted to the relationship between body size and the number of rings when measuring age in other animals (Chadwick-Furman et al. 2000, Francis et al 1999, Grigg 1974, Mistri & Ceccherelli 1994).

Statolith length (*SL*) as a growth character, in comparison with bell height (*BH*)

The *BH* growth showed stronger sigmoidal characteristics than *SL* growth (Figs. 5A, 5B). The concrete cause is not clear, although it is suggested that there was some difference between bell and statolith dimensions in their growth processes.

Some observations deviated markedly from the regression curve for *BH*, but the deviations from the regression curve for *SL* were fairly small. This was ascribed to unstable growth of the soft bell and stable growth of the hard statolith. According to Ichii & Mugiya (1983), the calcium in goldfish otoliths is stable and is unlikely to be resorbed into other tissues. It is likely that *SL* growth and *NR* increase are also steady in *C. quadrigatus*. Therefore, we suggest that *SL* is an excellent indicator of cubomedusan growth and that *NR* is useful as an age character.

Five individuals collected on August 24 and 26 tended to have smaller *BH* than other individuals with the same *NR*, which brought about underestimation in the asymptotic *BH*. One possible reason is that degrowth of the bell may have occurred. In *Aurelia aurita* (Linnaeus), degrowth is observed in nature and potential causes include a shortage of food, low temperatures, and a decline in metabolic activity after sexual reproduction (Lucas & Williams 1994, Möller 1980, Omori et al. 1995). Although we could not identify the factors responsible for reduction in body size in *C. quadrigatus*, the use of statolith rings enabled us to detect the phenomenon.

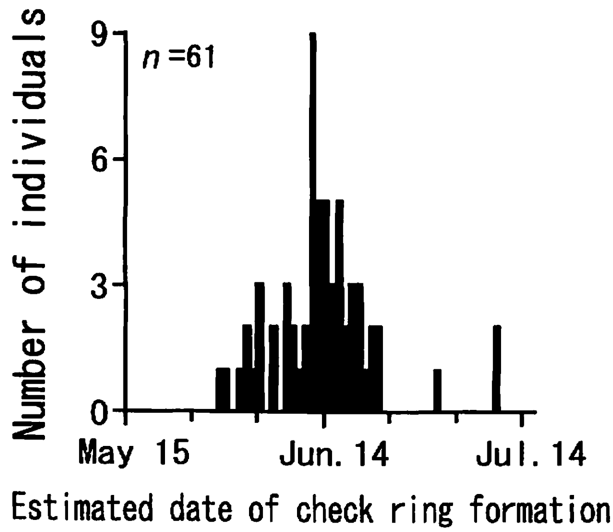

Fig. 6. The distribution of the back-calculated date of check ring formation estimated using the fine rings of *Chiropsalmus quadrigatus* form Okinawa Island, Japan.

Formation of the check ring and estimating the period of liberation

Chapman (1985) reported that the main component of the statolith of *Chiropsalmus* sp. from Puerto Rico was $CaSO_4 \cdot 2H_2O$. Since this crystal has a hexagonal face (Berry & Mason 1983), this might explain the hexagonal shape of the check ring and other rings near the center of the *C. quadrigatus* statolith (Figs. 3C, 3D).

The check rings in fish otoliths are formed as a result of changes of otolith growth rates at hatching, metamorphosis, environmental stresses, or habitat transitions (Morales-Nin 2000). In *C. quadrigatus*, a check ring was located between the 5th and 10th rings from the center of the statolith, independent of the size of the bell or statolith. Check ring formation appears to be a universal event that occurs several days after the commencement of statolith formation in *C. quadrigatus*. Werner (1975) reported that statolith formation commenced a few days before liberation in *Tripedalia cystophora* Conant. Assuming that the statolith rings of *C.*

quadrigatus are daily increments, we attempted to estimate the dates on which the check rings were formed in 61 individuals by back-calculation. The resulting dates were between May 29 and July 10, and most were concentrated between May 29 and June 22 (Fig. 6). If the check ring is a liberation ring, then most cubomedusae are liberated in this period. In recent years, it has been reported that many small *C. quadrigatus* medusae appear in early June (Iwanaga et al. 2001), which is in accord with our hypothesis. In addition, this is the period of ascending water temperature (Fig. 2) and metamorphosis from polyp to medusa may be tied to this temperature change.

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