Chapter 19
Geographical and Seasonal Variations of the Shell Microstructures in the Bivalve *Scapharca broughtonii*

Kozue Nishida and Takenori Sasaki

Abstract Cyclical ontogenetic changes of shell microstructures have been observed in the subfamily Anadarinae (Mollusca: Bivalvia, Arcidae) including fossil taxa. The changes in the bloody clam *Scapharca broughtonii* are controlled by temperature, which fluctuates seasonally, and can be used to determine the age of the individuals and to reconstruct paleoenvironments. In this study, samples of *S. broughtonii* from eight localities covering broad geographical regions at various latitudes in Japan, Korea, and Russia were examined to assess the utility of time series variations in microstructures for paleoenvironmental and paleoecological studies. All specimens showed cyclical changes in the relative thickness of the composite prismatic and crossed lamellar structures in the outer layer with ontogenetic progression, and thus, this feature can be used as a proxy for water temperature of their habitats. Specimens from southern latitudes showed higher annual shell growth rates than northern specimens, suggesting that low temperatures arrest shell growth in *S. broughtonii* and play a key role in determining the longevity and body size in *S. broughtonii*. In long-lived individuals from the four northernmost localities, the relative thickness of the composite prismatic structure tended to decrease as the individuals aged, which may be a consequence of declining physiological activity, such as organic matrix secretion.

K. Nishida (✉)
Ibaraki College, National Institute of Technology, Ibaraki, Japan

Japan Society for the Promotion of Science (JSPS), Tokyo, Japan
e-mail: nishida@gm.ibaraki-ct.ac.jp

T. Sasaki
The University Museum, The University of Tokyo, Bunkyo-ku, Tokyo, Japan
e-mail: sasaki@um.u-tokyo.ac.jp

19.1 Introduction

Shell microstructures of molluscs are highly diversified (Carter 1990), and the shell microstructures formed by a single individual can differ, depending on phylogenetic (Taylor et al. 1969; Shimamoto 1986; Sato and Sasaki 2015), crystallographic (Ubukata 2001; Checa et al. 2009, 2013), and environmental (Carter 1980; Kennish 1980; Lutz and Clark 1984) factors. Recently, Nishida et al. (2012) reported seasonal changes in the relative thickness of the two microstructures (composite prismatic and crossed lamellar structures) in the outer layer of the bloody clam *Scapharca broughtonii*. The composite prismatic and crossed lamellar structures of the outer layer are formed on the exterior and interior sides, respectively (Fig. 19.1), with the composite prismatic structure being thicker at lower water temperatures (Nishida et al. 2012). Nishida et al. (2015) observed shell microstructures in cultured specimens of *S. broughtonii* reared at five different temperatures, demonstrating experimentally the thermal dependency of the mode of shell microstructural formation in this species. Cyclical changes in microstructures with ontogeny have been observed in the subfamily Anadarinae (Mollusca: Bivalvia, Arcidae), including fossil taxa (Kobayashi and Kamiya 1968; Kobayashi 1976a, 1976b; Nishida et al. 2012), and can be useful for age determination and temperature reconstruction. Knowledge on geographical variations in shell microstructural formation in *S. broughtonii* remains limited (Nishida et al. 2012). Thus, samples of *S. broughtonii* were collected for this study from eight localities at various latitudes in Japan, Russia, and Korea to assess the utility of the cyclic thickness fluctuation in shell microstructures in paleoenvironmental and paleoecological studies.
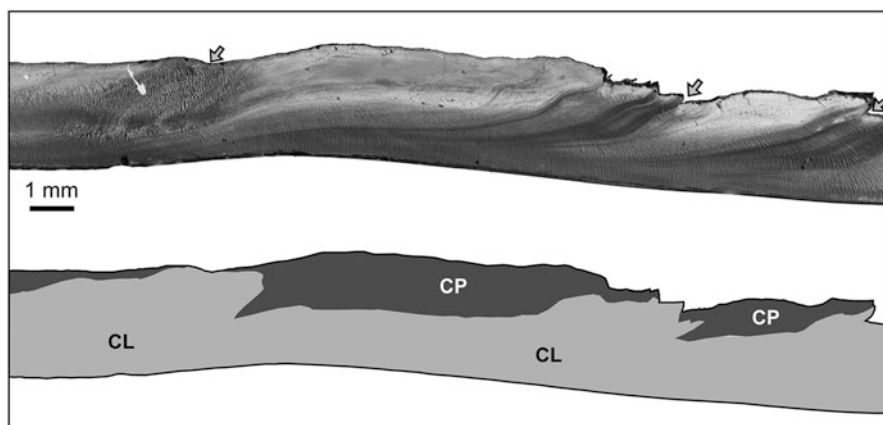

Fig. 19.1 An optical micrograph of the acetate peel of radial section of the outer layer near the outer shell margin in the specimen SB-IN3-01 collected at locality 4. With the growth toward to the right, fluctuations are observed in the relative thickness of the composite prismatic and crossed lamellar structures of the outer layer. Gray arrows indicate growth breaks. Abbreviations: CL, crossed lamellar layer; CP, composite prismatic layer

19.2   Materials and Methods

We examined *S. broughtonii* shells collected from six sites in Japan (Localities 1, 2, 4, 5, 7, 8), one site in Russia (Locality 3), and one site in Korea (Locality 6) (Table 19.1, Fig. 19.2). Of the 12 specimens collected from Localities 2–6 and 8, 9 were collected by dredge operations, and the remaining 3 specimens were likely collected also by dredging. The specimens at Locality 1 were cultured in a net, and the specimens at Locality 7 were cultured in a cage. Shell microstructures of those 14 specimens were prepared by the acetate peel method (Kennish et al. 1980), and then the thickness of the composite prismatic and crossed lamellar structures and the total thickness of the outer layer were measured at approximately 1-mm intervals following Nishida et al. (2012) with ImageJ/NIH image analysis software (version 1.45; http://imagej.nih.gov/ij/). Data of three specimens from Localities 1, 2, and 7 (SB-MT3, SB-YR-101-1, and SB-KM10b-2, respectively) reported in Nishida et al. (2012) were used for comparison with the data obtained in this study. According to Nishida et al. (2012), the age of each individual could be estimated by the number of the growth break (summer break) intervals and the positive peaks observed in the relative thickness of the crossed lamellar structure.

19.3   Results

The relative thickness of the composite prismatic and crossed lamellar structures in the outer layer of each specimen changed cyclically with ontogeny (Figs. 19.2 and 19.3). The ratio of the composite prismatic structure thickness to the total outer layer thickness was 0% at the minimum and had a maximum value as high as 58–80% (Figs. 19.2 and 19.3). For all specimens, the intervals between the cycle of relative thickness fluctuation of the two structures shortened with ontogeny, and the range of fluctuation in the relative thickness of the composite prismatic structure decreased in specimens older than 4 years (Figs. 19.2 and 19.3). In the specimens from Vladivostok (SB-RU11–01, SB-RU11-02; Fig. 19.2), the relative thickness of the composite prismatic structure fluctuated seasonally during earlier growth stages, while the fluctuations became smaller at later growth stages until the cyclic changes in the relative thickness became almost indiscernible.

To examine the variations in annual growth of individuals from the same localities, we compared at least two specimens each for four localities (Localities 1–3, 7; Figs. 19.3 and 19.4). Individuals cultured in the same cage at Locality 7 showed a similar pattern of microstructural changes (Fig. 19.3c, d). In contrast, growth patterns of the individuals cultured at Locality 1 showed considerable variations (Fig. 19.3a, b).

Growth curves for the specimens from the eight localities are shown in Fig. 19.2. The annual shell growth rate was higher in the specimens from southern localities than in those from northern localities, corresponding to the general increase in water

Table 19.1 Specimens of *S. broughtonii* examined in this study. All specimens are registered at the Department of Historical Geology and Paleontology, University Museum, The University of Tokyo (UMUT). Asterisks indicate specimens reported by Nishida et al. (2012); specimens 1, 2a, 2b, 3a, and 3b in Nishida et al. (2012) are identified as specimens SB-MT3, SB-YU101–1, SB-YR102–4, SB-KM10b-2, and SB-KM10b-3, respectively, in this study

Locality number	Sampling site	Sampling method	Depth	Collection date	Number of specimens	Specimen number	Collection number
Locality 1	Mutsu Bay, Aomori Prefecture	Cultured in net	5–10 m	20 September 2010	N = 2	SB-MT3*	UMUT RM31012
						SB-MT4	UMUT RM32670
Locality 2	(2–1) at 38°05' N, 140°58′ E, Miyagi Prefecture, in the Pacific Ocean	Dredge	22–23 m	28 December 2010	N = 3	SB-YR101–1*	UMUT RM31013
						SB-YR101–4	UMUT RM32671
						SB-YR101–11	UMUT RM32672
	(2–2) at 38°09' N, 140°59′ E, Miyagi Prefecture, in the Pacific Ocean	Dredge	22–23 m	28 December 2010	N = 3	SB-YR102–2	UMUT RM32673
						SB-YR102–4*	UMUT RM31014
						SB-YR102–9	UMUT RM32674
Locality 3	Off Vladivostok, Sea of Japan	Dredge?	–	July 2011	N = 2	SB-RU11–01	UMUT RM32675
						SB-RU11–02	UMUT RM32676
Locality 4	Nanao Bay, Ishikawa Prefecture, Sea of Japan	Dredge	30 m	01 November 2011	N = 1	SB-IN3–01	UMUT RM32677
Locality 5	Kohama Bay, Fukui Prefecture, Sea of Japan	Dredge	4–5 m	24–27 February 2011	N = 2	SB-FK1	UMUT RM32678
						SB-FK2	UMUT RM32679
Locality 6	Jinhae-gu, Sea of Japan, Korea	Dredge?	–	29 June 2011	N = 1	SB-KOT-3	UMUT RM32680

Table 19.1 (continued)

Locality number	Sampling site	Sampling method	Depth	Collection date	Number of specimens	Specimen number	Collection number
Locality 7	At 33°58' N, 131°50' E off Kudamatsu city, Yamaguchi Prefecture, in the Seto Island Sea	Cultured in cage	10 m	22 December 2010	$N = 2$	SB-KM10b-2*	UMUT RM31015
						SB-KM10b-3*	UMUT RM31016
Locality 8	Tachibana Bay, Nagasaki Prefecture	Dredge	22–23 m	11 January 2011	$N = 1$	SB-NT1	UMUT RM32681

temperature (Figs. 19.1, 19.4 and 19.5). Nishida et al. (2012) reported that shell growth of the field-collected specimens of *S. broughtonii* was probably arrested at temperatures below 12 °C. The length of time in a year when the water temperature was above 12 °C was longer in the south than in the north (Fig. 19.2b).

19.4   Discussion

All specimens showed cyclical ontogenetic changes in the relative thickness of the two structures (composite prismatic and crossed lamellar structures) in the outer shell layer. Thus, this character of shell microstructure in this species can be applied as a proxy of water temperature in different geographic regions. The annual shell growth rate was higher in southern specimens than in northern specimens (Fig. 19.5), probably due to the shorter duration of temperatures below 12 °C, a temperature range in which shell growth is reported to be arrested (Nishida et al. 2012). The specimens from Locality 8 (water temperature range 16–26 °C) probably grew all year round. On the other hand, the specimens from Locality 4 (0–25 °C) may form shells only for a period of approximately 4 months. Thus, low temperatures below 12 °C are suggested to play a key role in the longevity and shell size in *S. broughtonii*.

Nishida et al. (2015) suggested that the faster growth at lower temperatures is achieved by dominantly building the composite prismatic structure, probably as an adaptive strategy to precipitate shells under cold water environments. However, as the composite prismatic structure is physically weaker than the crossed lamellar structure (Taylor and Layman 1972; Currey 1976), it is disadvantageous for maintaining the shell mechanical strength. Thus, a trade-off between growth and physical characteristics (e.g., strength) should be considered in investigations of thermal
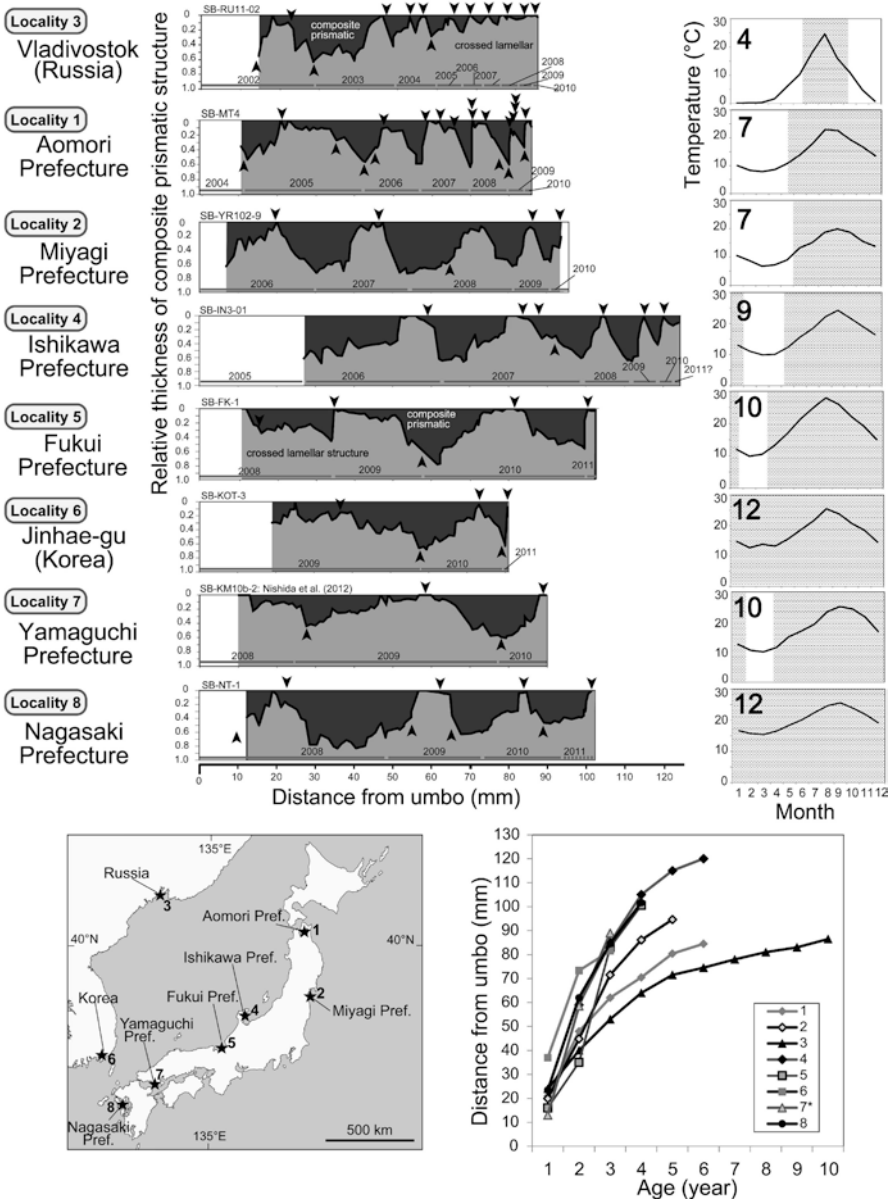
Fig. 19.2 The growth curves and changes in the relative thickness of the two structures in the outer layer at eight localities arranged from north to south along the coasts of Japan, Russia, and Korea. Arrow heads indicate growth breaks; black, gray, and white areas indicate composite prismatic structure, crossed lamellar structure, and missing sections of the outer layer, respectively, and the growth years are indicated by horizontal bars. The water temperature data are from the Japan Oceanographic Data Center (JODC, http://www.data.jma.go.jp/obd/stats/etrn/index.php). Water temperature at each of the eight localities is shown with gray shading on months with water temperature above 12 °C and the number indicating the number of months with water temperature above 12 °C. The growth curve of Locality 7 is for the reference specimen cited from Nishida et al. (2012)
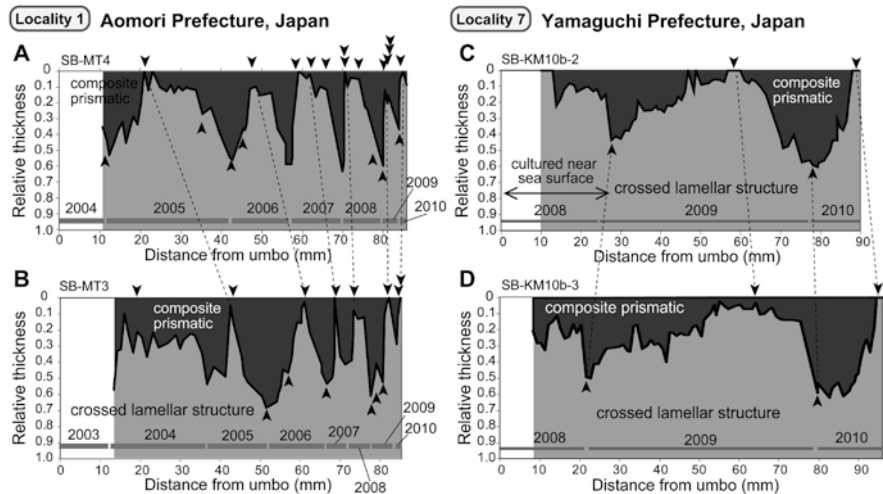
Fig. 19.3 Differences in shell microstructural records between two cultured individuals reared in the same localities. (**a**) Specimen SB-MT4 at Locality 1. (**b**) Specimen SB-MT3 at Locality 1, reported by Nishida et al. (2012). (**c**) Specimen SB-KM10b-2 at Locality 7, reported by Nishida et al. (2012). (**d**) Specimen SB-KM10b-3 at Locality 7. Arrows indicate growth breaks in the outer shell surface; black, gray, and white areas indicate composite prismatic structure, crossed lamellar structure, and missing sections of the outer layer, respectively
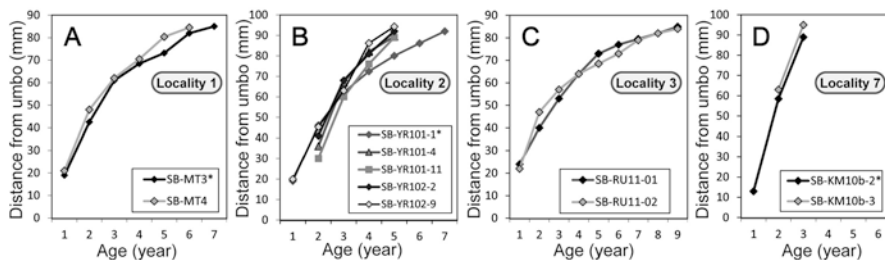
Fig. 19.4 Growth curves of the specimens from Localities 1, 2, 3, and 7 drawn based on the intervals of the summer growth breaks and the positive peaks in the thicknesses of the crossed lamellar structure. Asterisks indicate specimens reported by Nishida et al. (2012); specimens 1, 2a, and 3a in Nishida et al. (2012) are identified as specimens SB-MT3, SB-YU101–1, and SB-KM10b-2, respectively, in this study. (**a**) Specimens SB-MT3 and SB-MT4 from Locality 1. (**b**) Specimens SB-YR101–1, SB-YR101–4, SB-YR101–11, SB-YR102–2, and SB-YR102–9 from Locality 2. (**c**) Specimens SB-RU11–01 and SB-RU11–02 from Locality 3. (**d**) Specimens SB-KM10b-2 and SB-KM10b-3 from Locality 7

adaptation of microstructures in molluscs. The growth strategy of *S. broughtonii* inferred by shell growth patterns and microstructures (e.g., to reach a larger body size and/or maturity faster) might be important in the growth stage before maturity. In long-lived specimens from Localities 1–4, the relative thickness of the composite prismatic structure tended to decrease as the individuals aged (Figs. 19.2 and 19.3).
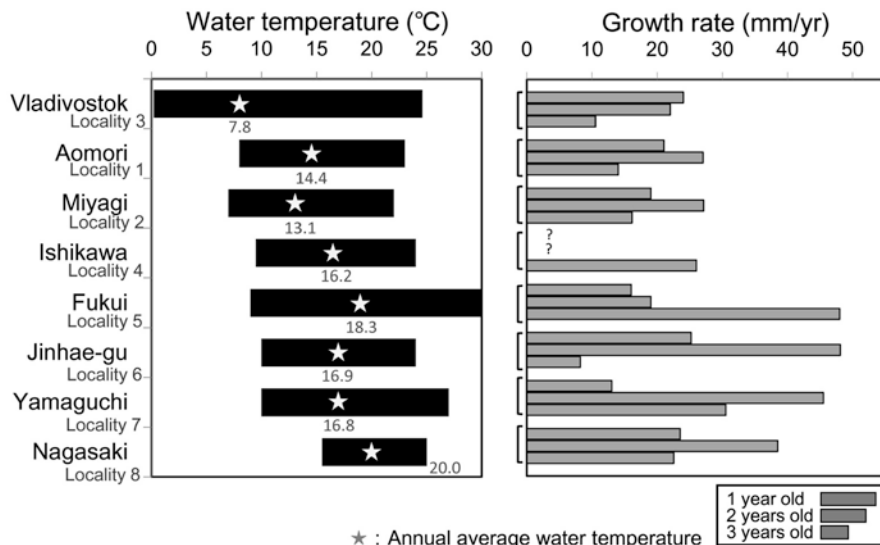
Fig. 19.5 Observed water temperature and estimated annual shell growth rates at eight localities arranged from north to south. The temperature data are from the Japan Oceanographic Data Center (JODC, http://www.data.jma.go.jp/obd/stats/etrn/index.php). The average annual seawater temperature at the eight localities approximately ranges from 7.8 to 20.0 °C. Annual shell growth rate was estimated from growth curves in Fig. 19.2. Annual growth rates of 1- and 2-year-old specimens at Locality 4 were not estimated because no summer growth break was observed in the shell surface of the 1-year-old specimen

Although the primary factor controlling the relative thickness of the two structures in the outer layer would be the seasonal changes in water temperature, physiological factors related to aging may also control microstructural formation in *S. broughtonii*. Palmer (1983) suggested that the cost of shell production is cheaper in organic-rich shells than in organic-poor shells. Composite prismatic structure in bivalve shells is richer in organics than is the crossed lamellar structure (Taylor and Layman 1972; Nishida et al. 2015) and, thus, after sexual maturity, a decrease in the volume of composite prismatic structure in shells may be accompanied by a decline in physiological activity, such as organic matrix secretion. Age-related changes in shell microstructures may show a trade-off between growth and physiological factors attributable to aging. At later growth stages of the individuals from Locality 3, the relative thickness of the composite prismatic structure became thinner with aging until cyclic changes in the relative thickness were almost indiscernible. Because this region is in the northern limit for this species, energetic cost might be needed not only for shell microstructural formation but also other physiological demands.

Differences observed in cultivation experiments may also have some effect. Patterns of the relative thickness of the two shell structures were more variable in the specimens from Locality 1, where they were cultured in a net hanging in the water column above the seafloor than in those from Locality 7, where they were cultured in a cage resting on the bottom sediment. Yurimoto et al. (2007) reported a

lower monthly shell growth rate in the individuals of *Scapharca kagoshimensis* cultivated in hanging nets than in those cultivated in cages on the seafloor and attributed this difference to buffeting of the suspended individuals by waves. Thus, the specimens from Locality 7 likely experienced less growth stress than those from Locality 1.

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