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Application of fluorescent substance to the analysis of growth performance in Antarctic bivalve, *Laternula elliptica*

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***Abstract*:** The shells of the Antarctic bivalve, *Laternula elliptica*, contain considerable information about their growth history and environmental changes. Growth rate determination in *Laternula elliptica* was attempted using a fluorescent substance, tetracycline, as a growth marker. The specimens were exposed to tetracycline solution of 200 mg/*l* for periods of 18 to 30 h at about 0–2°C without food. A distinct and narrow yellow fluorescent line was identified from the umbonal part to the ventral margin in all animals examined. This is the first success of fluorescent substance marking under the condition of low temperature and in Antarctic species. In the umbonal region, especially in the chondrophore, relatively rapid growth was observed. In the central part, a lower growth rate was observed. The growth rate of this species is not necessarily low in comparison with the temporal or tropical species. The combination of fluorescent marker and growth increment analyses will provide a powerful tool in estimating the benthic animal production, which is important information to understand the Antarctic ecosystem.

key words: fluorescent substance, shell, growth rate, bivalve, *Laternula elliptica*

Introduction

Biomineralization research is one of the more important research subjects from both a fundamental and applied point of view. The hard tissues of marine organisms contain considerable information about their own growth history including the changing condition of mineralization and environmental stress or disease (Okoshi and Sato-Okoshi, 1996). The molluscan shells also contain a record of both their life history and environmental changes. This information is preserved as structural, morphological, and chemical changes within the shell. Analysis of this information is a factor of great importance in marine biology and ecology as well as paleontology (Rhoads and Lutz, 1980).

Low temperature slows many biological processes. Many ecological processes take place slowly in Antarctica. The growth rate is significantly lower in Antarctic benthic marine invertebrates in comparison with temporal or tropical species. In the Antarctic region, the embryonic stages also show striking differences from warmer or lower latitudes. The early ecological studies of Antarctic benthos showed that most species grew slowly and tended to have long life-spans (Clarke, 1996). There are, however, a number of interesting exceptions to this general rule. Some sponges and ascidians grow

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rapidly (Dayton *et al.*, 1974; Dayton, 1989; Rauschert, 1991). A relatively fast growth rate was also observed for the Antarctic limpet, *Nacella concinna* by a mark/recapture experiment at Palmer Station (Shabica, 1976).

In the cases of gastropods and bivalves, the benthic Antarctic molluscan fauna has been well researched. About 80 gastropods and 30 bivalves are known in Enderby Land (Powell, 1958; Numanami *et al.*, 1996). To this date, over 100 gastropods and over 40 bivalves are known from the Davis Sea (Thiele, 1912; Egorova, 1982; Tucker and Burton, 1987; Numanami *et al.*, 1996). Thirteen gastropods and 6 bivalves were collected in the areas of Lützow-Holm Bay, Casey Bay and Prydz Bay, and 7 gastropods and 3 bivalves were recorded around Syowa Station by the Japanese Antarctic Research Expedition (JARE) in 1992–1994 (Numanami *et al.,* 1996).

Although many molluscs were collected and observed, there are few analytical studies on the growth performance or production of Antarctic species in comparison with temporal or tropical ones. Age and growth rate determinations in Antarctic bivalves have been attempted by analysis of shell morphology and by cohort analysis. Ralph and Maxwell (1977) estimated the growth rates and ages of two Antarctic bivalves, *Adamussium colbecki* and *Laternula elliptica*, using growth increments on the shell surface. It has been confirmed that the growth increments are laid down annually in some temporal and tropical species such as *Mya arenaria* (Newcombe, 1936) and *Spisula solidissima* (Jones *et al.*, 1983). However, there was no evidence that these growth increments were laid down annually either in *A. colbecki* or *L. elliptica*.

The application of fluorescent substances to the analysis of growth performance and hard tissue formation is popular in medical and dental sciences. Fish otoliths can also be marked by immersion in some fluorescent substances. In recent years, some fluorescent substances have been tested as growth markers in a variety of marine animals (Kaehler and McQuaid, 1999; Okoshi, 2000, 2001, 2002). Little is known about their usefulness for marking bivalves except for the Pacific oyster *Crassostrea gigas*, pearl oyster *Pinctada fucata* (Okoshi, 2000, 2001, 2002) and brown mussel *Perna perna* (Kaehler and McQuaid, 1999). These fluorescent substances were also used for mass marking for hatching stages of fishes (Tsukamoto, 1985) and gastropods (Moran, 2000). This becomes a powerful tool in understanding the population dynamics and life histories of marine organisms which have calcareous hard tissues.

The object of this study is to develop a method of marking the shells of Antarctic benthic molluscs with a fluorescent substance in order to obtain effective information on shell formation and growth. This study investigates the effectiveness of tetracycline in marking shells of Antarctic bivalves, *Laternula elliptica* and investigates the potential as a growth marker. The results of this study will lead to estimating the benthic animal production, which is one of the most important information to understand the characteristics of the Antarctic ecosystem.

Materials and methods

Sampling of specimens

Dredge sampling was carried out at Torinosu Cove, Skarvsnes, on the east coast of Lützow-Holm Bay (69°28.95′S, 39°34.68′E) in January 8–12, 2001 during the field

operation of the 42nd Japanese Antarctic Research Expedition (JARE) (Figs. 1, 2). The net size was 40 cm in width, 15 cm in depth and 70 cm in length. The mesh size was

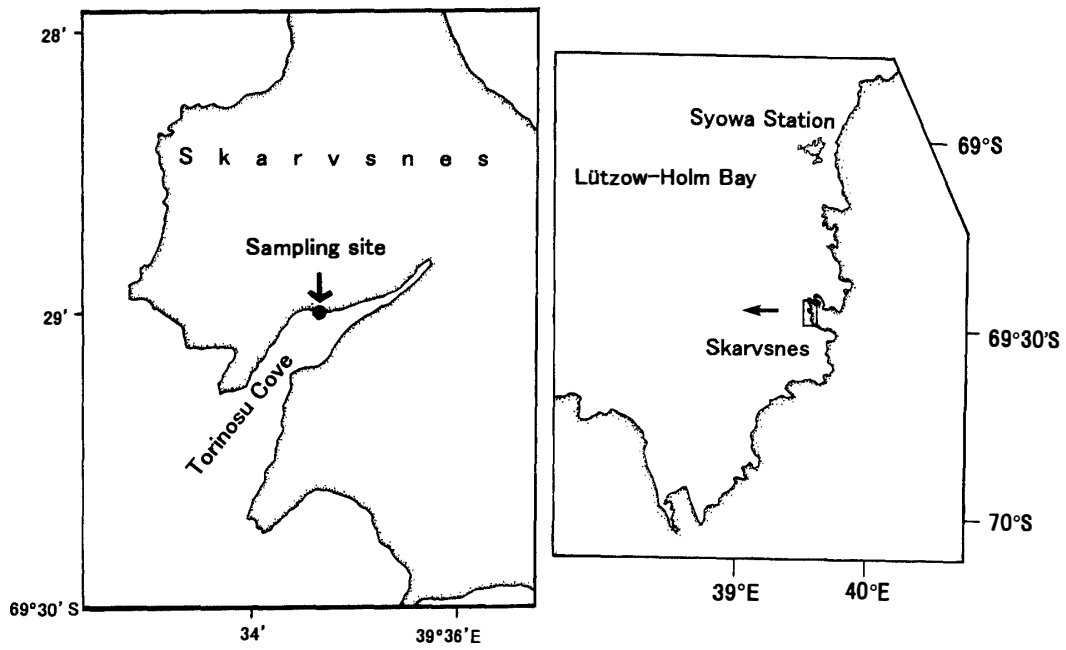

Fig. 1. Map showing location of the sampling site in Torinosu Cove, Skarvsnes, on the east coast of Lützow-Holm Bay, Antarctica.

Fig. 2. Landscape of sampling site.

1.0 cm. The water depth of the sampling site was 3–5 m. The sandy and/or muddy bottom was observed, and numbers of polychaetes and amphipods were collected simultaneously.

Rearing conditions

After the net retrieval, the bivalves were immediately sorted and stored in plastic bottles filled with seawater and kept at the temperature of 2–5°C. These were quickly transferred to a plastic water tank in a cold-storage room at 0°C on the icebreaker *Shirase*. Seawater temperature was kept between about 0 and 3°C during the experiment. Sand from the sampling site was collected and transferred to the bottom of the tank as *in situ*, and the water in the tank was circulated by using an electric pump with filter. The experiment was started 3 days after capture to allow for adjustment of physiological conditions. During the experiment, bivalves were fed twice a day on phytoplankton, mainly diatoms, and detritus except in the period of marking with fluorescent substance.

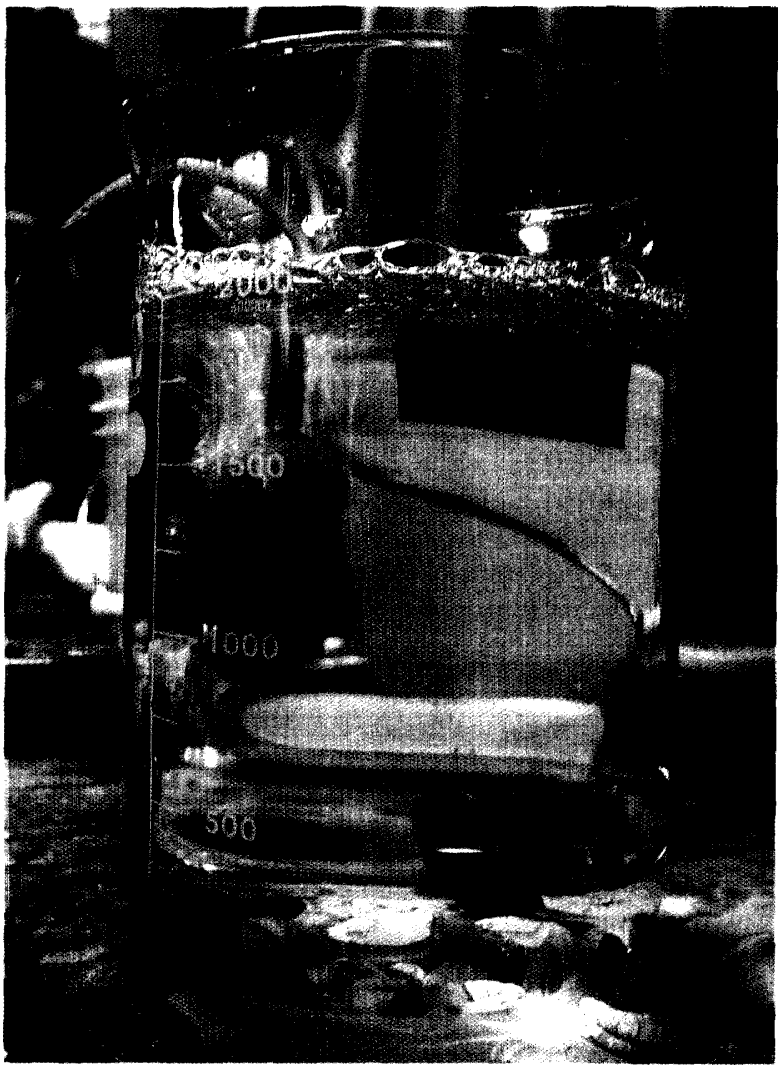

Fig. 3. *Laternula elliptica* exposed to tetracycline marking solution.

The rearing experiment was conducted for 90 days.

Tetracycline marking solution

Tetracycline (TC) hydrochloride (Wako Chemical) was mixed thoroughly with a small volume of filtered seawater until completely dissolved. Filtered seawater was added to make a marking solution containing a total TC concentration of 200 mg/*l*. Twenty to 30 specimens were exposed to marking solutions for periods of 18 to 30 h at about 0–2°C without food (Fig. 3). After this period they were returned to a flow through aquarium. Immersion experiments were carried out during January and February 2001.

Sample preparation and detection of TC

Fifteen specimens whose shell length varied from 8 to 17.5 mm were detected this time. After removal of the flesh and part of the periostracum, the shells were rinsed with pure water several times and dried at 40°C. Then the shells were filled with resin. After hardening of the resin, the cubes with shells were cut into many thin sections with a diamond cutter. The thin dorso-ventral sections of the shell were rinsed again with pure water to remove small pieces of resin.

Detection of TC in the shell was carried out by viewing the shell under a compound microscope (Nikon OPTIPHOTO with EFD 2 system) fitted with an ultra-violet (UV) light source using a V-2 B excitation filter cassette (Nikon).

Results and discussion

Only a small number of attempts have been made at incorporating fluorescent substances into bivalve hard tissues, and the results have varied. Two treatment methods for administering three to four fluorescent substances have been tested. One is an injection method and the other is an immersion one. Early attempts with analogues of the antibiotic TC by the injection TC were seldom successful (Nakahara, 1961). Of the two methods used to administer calcein solutions to the brown mussel *Perna perna*, only the injection technique proved successful (Kaehler and McQuaid, 1999). Recent studies, however, using the tetracycline and the alizarin complexone on the Pacific oyster *Crassostrea gigas* and the pearl oyster *Pinctada fucata*, yielded good results, with clear fluorescent marks being incorporated into the shells and pearls (Okoshi, 2000, 2001, 2002).

To determine the optimum TC concentration and treatment time for marking the shells of *C. gigas* and *P. fucata,* various combinations of concentration and treatment time have been tested (Okoshi, 2000, 2001, 2002). In conclusion, immersion in 100–300 mg/*l* for 12–48 h was recommended for marking molluscan shells and pearls. The optimum condition for treatment was 200 mg/*l* for 12–24 hr at 22°C for oysters. Therefore, we employ these treatment conditions except for those of temperature. The result of this study shows the first success of fluorescent substance treatment at low temperature and in Antarctic species.

TC presented a clear yellow fluorescent line in the dorso-ventral section of bivalve shells under UV light (Fig. 4). A distinct and narrow yellow line was identified from the umbonal part to the ventral margin in all animals observed by fluorescent microscope. A

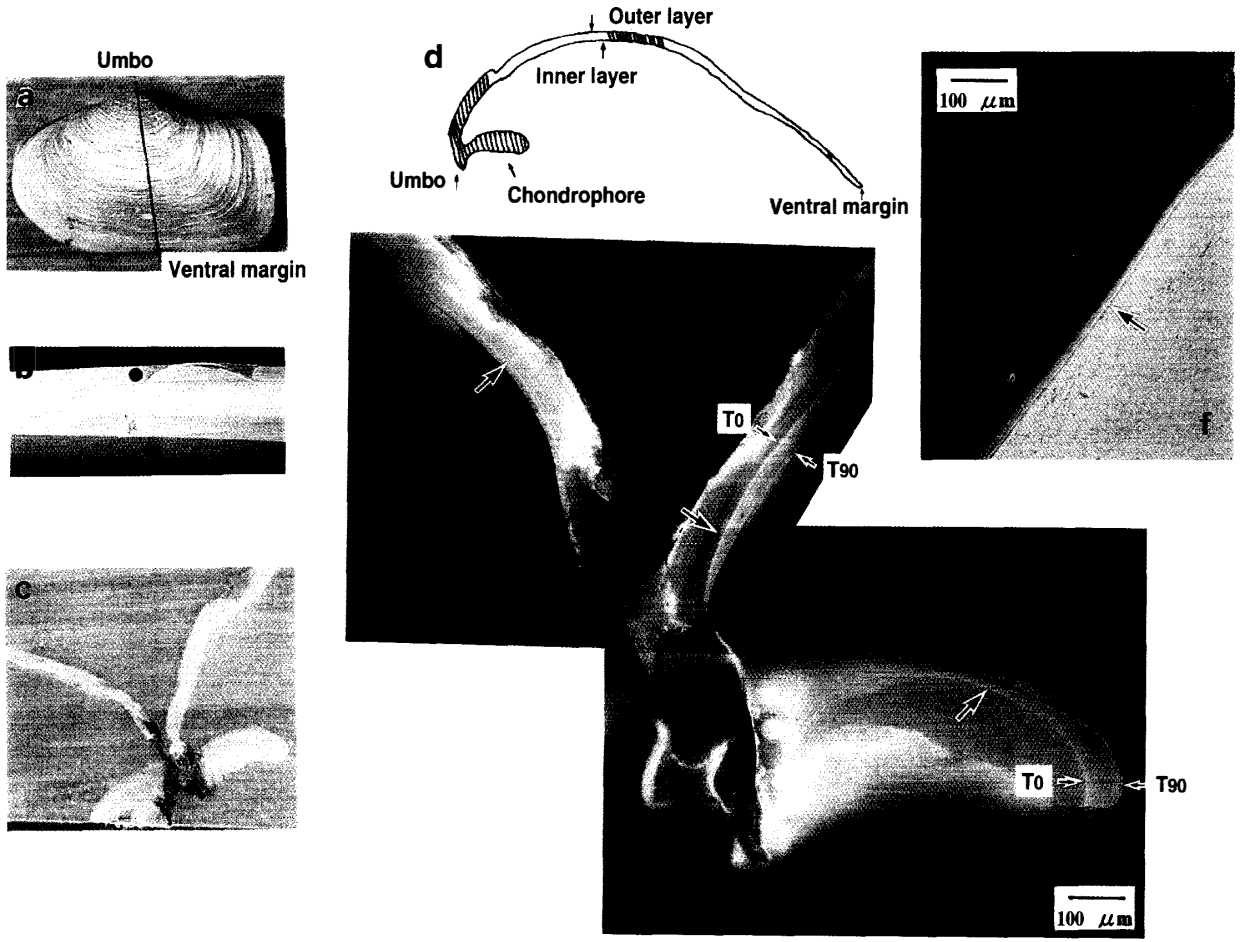

Fig. 4. *Laternula elliptica*. a. *Laternula* shell showing the cutting line from the umbonal part to the marginal edge. b. Photomicrograph of the dorso-ventral section of a *Laternula* shell. c. Higher magnification of the umbonal part of b. d. Line drawing of the dorso-ventral section, indicating the positions of TC line observation (umbonal and central regions of the shell). Photomicrographs of the umbonal region (e) and central region (f) of the shell, 90 days after TC administration. Large arrows show the fluorescent line resulting from TC incorporation. T0 shows the position of the growing edge at the time of TC immersion. T90 shows the position of the growing edge 90 days after immersion. The distance between T0 and T90 represents growth of the chondrophore and growth in thickness of the inner calcareous layer of the shell, respectively.

Fig. 5. *Laternula elliptica*. Well-developed siphons are extended when in seawater.

difference in growth rate was observed among different parts of the shell. *Laternula* shell was elongated, elliptical and thin, inflated with a truncated posterior end (Fig. 5) with resilium under the umbo, and the chondrophore protruding. The distance between the fluorescent mark and the inner surface was measured using the dorso-ventral section of the shell. In the umbonal region, especially in the chondrophore, relatively rapid growth was observed in all individuals examined (Fig. 4). In contrast, in the central part of the dorso-ventral section, a low growth rate was observed. There was a significant difference in growth rate between the umbonal part and the central part. Similarity of growth performance between the left and right valves was also observed. In a small individual (shell length: 8 mm), the distance from the position of the growing edge at the time of TC incorporation to that of the growing edge 90 days after immersion was 70 μm in the chondrophore. The growth rate of this species, which lives in low temperature throughout the year, may not be significantly lower than that of the temporal or tropical species.

Preliminarily, growth increments were also observed in the umbonal and some other regions. The number of growth increments deposited between the fluorescent mark and the valve edge will be discussed in relation to the known time period the bivalve was alive. Age estimation may be possible in calculating the number of growth increments between the fluorescent mark and the valve edge.

Because the treatment had no visible damage on the growth or survival of the animals studied, the application of the method to marine mineralized samples such as coccolithophores, reef-building corals and molluscs should bring progress not only in marine biology but also in aquaculture.

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