Daily Growth Lines of the Clam, *Meretrix lusoria*

— A Basic Study for the Estimation of Prehistoric Seasonal Gathering.

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Abstract The growth line of shells showing yearly and daily periodicities of deposition is a useful characteristic for reconstructing prehistoric seasonality of shell gathering. For such investigations, basic studies in recent molluscs are required. This report discusses the results of vital staining and planting (16 days) of the clam, *Meretrix lusoria*. In the scanning electron photomicrographs, growth lines of variable thickness were observed. They were classified into 5 types (A-E) according to the combination of the thicknesses along the surface of maximum growth ($\geq 3\mu, 3-1\mu, <1\mu$), and those in the “inner zone” of outer layer ($\geq 1\mu, <1\mu$). Among them there were 2 categories: the growth line of the primary order (Types A and B) well discernible in the “inner zone” and more or less conspicuous at the surface of maximum growth, and the secondary one (Types C-E) barely visible or invisible in the “inner zone” and fainter at the surface of maximum growth. In the 2/3 of the specimens, the primary line included only the Type A, the Type B being absent, and in the rest, the Type A formed the great majority in it, a few of the Type B being included. In both cases, the totals of the frequency of these primary lines (only A or A+B) in each specimen were always exactly 16, coinciding the number of days of emplanting. The primary and the secondary growth lines are considered as that of daily and subdaily formation, respectively. The growth increment between the growth lines was seen to be composed of an alternation of lighter and darker zonules.

INTRODUCTION

The seasonal patterns of exploitation by hunter-gatherers have recently become one of the main concerns for anthropologists interested in reconstruction of prehistoric economic life. Animal bones and shells discovered in prehistoric sites have been used to infer hunting and gathering seasons. For example, the presence of bones of migratory animals have used to presume the season of occupation from a knowledge of animal ecology. The direct estimation of the season of death of animals based on the life histories of the species is a valid method. However, few other characteristics useful for seasonal dating are known except for the yearly growth bands of shells (GORMAN, 1971) and of animal teeth (SAXON and HIGHAM, 1969).

When the cross-section of a shell is observed under a microscope, fine striations called growth lines are detectable. WELLS (1963) has suggested that a fossil with
Daily Growth Lines of the Clam, *Meretrix lusoria*

both daily and yearly records of deposition could be used as a "Paleontological Clock" discussing the results of counting of the growth rings between annual growth bands on corals. Barker (1964) reported a tidal cluster of fifteen growth laminae in a recent species of *Chione*. Growth lines can also be applied to seasonal dating. Coutts and Higham (1971) estimated the average number of growth lines from the last winter band to the shell margin in an intertidal bivalve (*Chione stutchburyi*) discovered in prehistoric settlements in New Zealand and suggested seasonal occupation for those coastal sites. However, as Runcorn (1968) pointed out, application of these data to geological as well as prehistorical estimations requires basic studies of the daily and tidal formations of growth lines in living materials. A modern bivalve, *Mercenaria mercenaria*, showed the same number of growth increments as of days from marking to killing (Pannella and MacClintock, 1968). House and Farrow (1968) compared the variation of daily growth increments with tidal and weather records.

In this report, the daily formation of growth lines was studied with experimental method, in order to establish a sound base for the seasonal dating of shell gathering. Alizarin red S has been used in the vital staining of both vertebrate bone and molluscan shell, and no striking inhibition of growth after staining was reported (Hudi and Hanks, 1968). Vital staining by alizarin solution was carried out to mark the initial date of experiment, and the number of growth lines from the stained line to the shell margin was examined in each specimen.

**MATERIALS AND METHODS**

The vital staining experiment was carried out on a clam species, *Meretrix lusoria* (Röding), in the tidal flat in the Ariake Sea, about 1.5 kilometers west of the mouth of the Midori River in Kumamoto Prefecture, on the western coast of Kyushu (Fig. 1). The stained bivalves were planted in a central part of the privately owned culturing field, which is located within a natural habitat of the species, to prevent them from accidental collecting by local fishermen. This area is exposed at spring lowest tides, while during neap tides it is always covered with seawater.

The first set of 140 clams was stained on May 24, 1971, and planted in a selected section of the field. On July 9, 45 days after planting, 40 of these marked clams were collected from the site and stained again. Another 50 new clams were additionally stained. On July 25, 16 days after the second planting, 74 marked clams were collected and killed. Only two dead shells were found, so the low recovery rate is probably a result of migration of some clams rather than death from staining. It has been reported that this species is very active in migration especially during the late spring (Uchida, 1941; Hamada and Ino, 1954).

38 specimens were studied by replica technique for the examination of the growth lines. The shells were embedded in polyester resin, and cut along the line
To observe the finer details of the growth lines, a scanning electron microscope was also used, and the thicknesses of the growth lines were measured on the electron-photomicrographs.

RESULTS

Shell structure

The basic shell layers in *Meretrix lusoria* are composed of the inner- and the outer layers (Fig. 2-B), as in the other molluscan shells. The outer layer grows additionally at the shell margin. According to the classification proposed by PANNELLA and MacCLINTOCK (1968: 66), the growth pattern at the shell margin in this species belongs to the "reflected type", which was said to be produced by the reflected mantle. Therefore, growth lines form a series of "semicircular" lines (KOBAYASHI, 1967), and the growth increments are thickest near the middle of the thickness of the shell. Passing through these points of thickest growth increments on each growth line, there can be supposed a hypothetical surface (Fig. 2-C), defined as the "surface of maximum growth" by PANNELLA and MacCLINTOCK (1968: 66—after MacCLINTOCK, 1967: 53-54). The outer layer composed of crossed lamellae structure (MacCLINTOCK, 1967: 19-27; KOBAYASHI, 1971: 37-38). Crossed lamellae run intersecting to the growth lines, and show a fountain
Fig. 2. Cutting method and cross-section of *Meretrix lusoria*.

A—Top view showing cutting line connecting the umbo and the central point of the ventral margin.

B—Cross-section showing the inner layer (IL), outer layer (OL) and the “inner zone” of outer layer (iOL).

C—Cross-section at the shell margin. Growth lines (GL) form semicircular lines. Crossed lamellae (CL) intersect the growth lines, radiating from the surface of maximum growth (SMG).

D—Schematic representation of the outer layer at the shell margin. A growth line changes its direction at point A toward an inner point B, instead of point C on the simple extension. A hypothetical line X-Y connecting the reflected point such as point A is a boundary of the “inner zone” of outer layer.

The growth line curves rather regularly around the surface of maximum growth toward the outer- and inner surfaces (Fig. 2-C).

At a certain point in the inner part of outer layer, however, the line becomes more or less straight, as shown in the schematic representation of the outer layer at the shell margin (Fig. 2-D: point A). At the same point it also changes its direction, running towards an inner point B, instead of C which is on an imaginal extention of growth curve. As a result,
the growth lines slant more or less parallel to the inner shell surface. Therefore, stratification of the growth line changes from the reflected type at the very edge of shell margin, to the slanting type in the peripheral of the inner shell surface. Connecting these points of reflection of each growth line (point A), an imaginal line (X-Y) can be drawn, and inside of this hypothetical line X-Y, a slightly different zone can be recognized. In this zone, growth increments are thin and growth lines are arranged more closely to each other than in the rest of the outer layer. Crossed lamellae run more or less vertically to the inner shell surface and become obscure in this zone. This zone is called, in this paper, the "inner zone" of the outer layer to be distinguished from the outer part of the same layer.

Effects of vial staining

The observation of the cross-sections of the marked shells under a stereoscopic microscope showed that the vital staining had produced a red striation running along a growth line. Outward from this red striation the shell was colorless, representing additional growth of the shell after the clam was returned to its natural habitat. Thus the sharp red band produced by the alizarin treatment indicates the exact date of shell planting and makes this method useful at least for a short ranged experiment of shell growth.

The stained growth line was thickened abnormally and more easily seen than normal ones (Pl. 1-A). The arrangement of crossed lamellae was often interrupted at the stained growth line. In addition to this, the direction of maximum growth slipped off inward of the normal surface. The identification of the stained growth line in the colorless replica film was facilitated by these distinctive features.

Growth lines in scanning electron photomicrographs

In the scanning electron photomicrographs of the etched surfaces of cross-sections, the growth line is observed as a bright fine striation on a dark ground. A number of such lines can be seen in a photomicrograph (Pl. 1-A). Closer examination of these lines reveals that some of them are fainter than the others even at the surface of maximum growth. The more conspicuous lines are rather uniform in thickness and brightness, while the fainter lines are not only subdued in brightness but also variable in thickness, ranging to barely visible ones.

The finer details of the area indicated in Pl. 1-A were shown in Pl. 1-B. Two conspicuous lines are seen in the both ends of the field. They are very bright, and are even in thickness along their total lengths. Between them, three fainter lines can be observed. Their thicknesses vary from place to place along their length, and at places, they almost lose their continuity.

The profile of scanning electron image is obtained by the intensity of secondary electron ray. The relative height of the profile is thought to reflect the irregularity and ruggedness of the surface of a material. The profile pattern (Pl. 1-C) on the line A-A' in Pl. 1-B, shows that 2 sharp peaks correspond to the location of the conspicuous lines, and weaker peaks to the
fainter lines. Therefore, it can be recognized that growth lines are raised strips of shell matter left by the etching with a diluted solution of HCl, while the dark ground is a surface of easily soluble component.

In a more detailed observation, the dark ground (Pl. 1-A) can hardly be said to be quite homogeneous, and it is composed of an alternation of lighter and darker zonules, running parallel to the growth lines. The weak jagged peaks in Pl. 1-C could be thought to correspond to these lighter zonules.

Although some of the fainter lines are seen to be partly composed of 2 closely adjoining lines in the higher magnification (Pl. 1-B), these lines are seen only as obscure, but rather thicker lines when observed in the replica film under an optical microscope.

**Different sorts of growth lines**

As mentioned above, the conspicuous lines are invariably thick near the surface of maximum growth, and, what is more, they also extend well into the “inner zone” of outer layer. On the other hand, the fainter lines vary in thickness near the surface of maximum growth, and become gradually narrower particularly in the vicinity of the “inner zone” of outer layer. Within the “inner zone”, the conspicuous lines are seen clearly, while the fainter lines are either barely visible or invisible.

In order to examine the variation in thicknesses of growth lines both along the surface of maximum growth and in the “inner zone”, these which are thicker than 1µ were measured on the electron photomicrographs of 2 specimens under a measuring microscope. The rest of the lines which were poorly visible were counted up indiscriminately as those less than 1µ.

A frequency distribution for the thickness of growth lines measured along the surface of maximum growth (Fig. 3-A) showed 3 modes at 3.5–4.0µ, 2.5–3.0µ and less than 1µ. Therefore, growth lines fell into 3 groups —those greater than 3µ, those between 3 and 1µ, and those less than 1µ. Within the “inner zone”, some of the growth lines which were visible and measured along the surface of maximum growth, vanished from the sight, and consequently, they were counted up as invisible ones. Thicknesses of growth lines in the “inner zone” (Fig. 3-B) showed a bimodal distribution having 2 peaks at 2.0–2.5µ and 0µ (invisible). Therefore, growth lines in the “inner zone” were divided into 2 groups—those greater than 1µ and those barely visible (less than 1µ) plus invisible ones (0µ).

Based on the result of measurement in the “inner zone”, growth lines greater than 1µ in this zone were taken up and the thickness of them at the surface of maximum growth were shown in Fig. 3-C. The histogram showed a unimodal distribution with a peak at 3.5–4.0µ. In regard to the rest of the growth, or those lines less than 1µ together with those of invisible ones in the “inner zone”, the thickness of them at the surface of maximum growth were shown in Fig. 3-D. The histogram had a bimodal distribution with 2 peaks at 2.0–2.5µ and less than 1µ. As mentioned above, the thickness at the surface of maxi-
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2 lop-sided peaks of 2.0-2.5μ in Fig. 3-D and 3.5-4.0μ in Fig. 3-C, the second mode being actually situated at 2.0-2.5μ. Consequently, growth lines at the surface of maximum growth can be divided into those greater than 3μ, 3 to 1μ, and less than 1μ. In the “inner zone” of outer layer, growth lines can be assorted into those greater than 1μ, and less than 1μ. The combination of these 3 categories at the surface of maximum growth and 2 categories in the “inner zone” are ought to be 6, but such a combination that less than 1μ at the surface of maximum growth and yet greater than 1μ in the “inner zone” cannot actually be found. Therefore, only 5 types of growth lines could be recognized, and they were indicated as the Types A to E (Table 1).

Table 1. Classification of growth lines.

<table>
<thead>
<tr>
<th>types of lines</th>
<th>growth surface of maximum growth</th>
<th>“inner zone” of outer layer</th>
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<tr>
<td>A</td>
<td>≥3(μ)</td>
<td>≥1(μ)</td>
</tr>
<tr>
<td>B</td>
<td>1~3</td>
<td>≥1</td>
</tr>
<tr>
<td>C</td>
<td>≥3</td>
<td>≤1</td>
</tr>
<tr>
<td>D</td>
<td>1~3</td>
<td>≤1</td>
</tr>
<tr>
<td>E</td>
<td>&lt;1</td>
<td>≤1</td>
</tr>
</tbody>
</table>

These 5 types of growth lines are illustrated in an optical photomicrograph of replica film (Pl. 2), instead of a series of electron photomicrographs, which are somewhat skewed at the edge, and are difficult to join them end to end. The Types A and B are conspicuous and less conspicuous, respectively, at the surface of maximum growth, but both are clearly visible in the “inner zone”. The Type C is also conspicuous at the surface of maximum growth, but barely visible or invisible in the “inner zone”. Some of growth lines (Fig. 3-A) had trimodal distribution. However, it can be known that the 2nd mode at 2.5-3.0μ in Fig. 3-A seems to be a deceptive peak cumulated of tails of
Table 2. Frequencies of five types of growth lines during 16 days after the second staining, and totals of 3 types (A, B, C) in different combinations.

<table>
<thead>
<tr>
<th>specimen</th>
<th>age</th>
<th>types of growth lines</th>
<th>totals of types</th>
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<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
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<td>13</td>
<td>3</td>
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<tr>
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<td>11</td>
<td>5</td>
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<tr>
<td>KM 44 SII</td>
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<td>15</td>
<td>1</td>
</tr>
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</table>
lines such as No. 14 of the Type A and 2 lines of the Type C in the No. 13 and No. 14 increments in Pl. 2, are seen to be composed of closely adjoining 2 lines at the surface of maximum growth. But thinner lines are annexed in a half way to the "inner zone", so these adjoining lines were not measured or counted separately.

**Frequencies of each type of the growth line**

The frequencies of each of the five types of the growth line in each specimen after the second staining were examined in 38 specimens (Table 2).

The Type A appeared relatively constant in number, in the 2/3 of the specimens being 16, and on the remaining 1/3, they were 15 in the greater half and ranged from 14 to 11 in the rest. The Type B were very few, and were absent in nearly half of the specimens. The number of the Type B showed a slight increase with age, being fewer in the 2nd year class and slightly more numerous in the 3rd and the 4th year classes. The Type C were as few as or slightly more than the Type B, however the Type C had a tendency of decrease in aged clams contrary to the Type B. The Type D appeared more frequently than either Type B or Type C, while the Type E appeared most frequently, and were very variable ranging between 0 and 40, although they were counted up to over 20 in many specimens.

The numbers of each types and total of these 3 types (A, B, C) in combination were examined. The maximum number of the Type A in each specimen was 16, and this figure of 16 coincides with the number of days from the second staining to killing, i.e. 16 days. Moreover, all the total numbers in the addition of the Types A and B were just 16, being neither more nor less. On the other hand, the total numbers in the addition to the Types A and C were fluctuated between 11 and 19, and most of them were greater than 16. Total numbers in the addition of all the Types A, B and C amounted to 20 in the maximum, and were either equal or greater than 16.

**DISCUSSION**

For the examination of the formation of growth line during a fixed period, a marking method has been used to indicate the beginning of experiment. However, a notching technique (DAVENPORT, 1938; PANNELLA and MacCLINTOCK, 1968) is not an ideal method for marking, because the shell growth after notching is said to be severely depressed. As another way of marking, vital staining with an alizarin solution was reported by HUDI and HANKS (1968), who immersed *M. mercenaria* shells for 7 days in 5 to 200 ppm alizarin solutions in order to secure long persistence (18 months) in their ecological research.

In the present experiment, clams were put for 30 munites in an alizarin solution with higher concentration than those of HUDI and HANKS, and the result was quite sufficient. alizarin was incorporated into the shell matter during this short period, and the staining resulted in a slight deformation for only 1 to 3 days. Further experiment for a reduction of immersing
time is required to decrease the ill effect of staining, together with a control experiment of the transplantation of non-stained clams.

The marking methods such as staining and notching enabled us to compare the count of growth lines between the marked line and the shell margin directly with the number of days of emplanting. DAVENTPORT (1938) found 4 new lamellae in each specimen of Pecten irradians left 4 days in a tidal box after notching. In M. mercenaria collected 368 days after notching, growth increments between 360 and 370 in number were counted (PANNELLA and MacCLINTOCK, 1968).

In this investigation, the frequencies of 5 types of growth lines (Type A-E) were examined in each of the 38 specimens, during 16 days from the second staining to killing (see Table 3). As mentioned already, the numbers of the Type A, which is conspicuous at the surface of maximum growth (≥3µ), and well discernible in the "inner zone" of outer layer (≥1µ), were 16 in the 2/3 of the specimens, and, in the remaining 1/3, they were 15 in the greater half and ranged from 14 to 11 in the rest. On the other hand, the numbers of the Type B which is well discernible in the "inner zone" (≥1µ) as the Type A, but less conspicuous at the surface of maximum growth (1~3µ) were absent in the greater half of the specimens, in which the numbers of the Type A were consistently 16. Only a few numbers of the Type B appeared in the rest of the specimens, in which the numbers of the Type A were always less than 16, and yet, the total number of the Types A and B were invariably 16. Therefore, the total number of the Types A and B in every specimen, was exactly 16, coinciding the number of the days of emplanting. So, it would not be unreasonable to consider that a single growth line of either Type A or B is formed in every day.

Although the Type C is less discernible within the "inner zone" as are the Types A and B, it is equally conspicuous at the surface of maximum growth, or even more so than the Type B. So, if we ignore the thickness in the "inner zone", those growth lines which are quite conspicuous at the surface of maximum growth are the Types A and C, instead of the Types A and B. However, the total numbers of the Types A and C in each specimen were rather variable ranging between 11 to 19, and showed no consistency in their appearance, contrary to the totals of the Types A and B. It is interesting to know that PANNELLA and MacCLINTOCK (1968) also noted the mode of appearance of growth lines in the peripheral part of the outer layer. According to them, the daily growth increments were completely reflected outer surface of the shell, and the only major lines were visible in the "homogeneous" layer.

Discriminating 2 different categories of the thickness in the "inner zone" well discernible (≥1µ) and barely discernible (<1µ), frequency histogram of thickness at the surface of maximum growth can be depicted separately (see Fig. 3-C, D). In the histogram for the latter group (Fig. 3-D), the Type C is recognized as the thicker
tail of a unimodal distribution mainly consisted of the Type D. The Type B is also recognizable as the thinner member of a unimodal distribution combining the Types A and B. For these reasons, the Types A and B can be recognized collectively as the growth lines of the primary order, and the Types C, D and E as the secondary one.

It was already mentioned that marking experiments, made by some authors (DAVENPORT, 1938; PANNELLA and MACCLINTOCK, 1968), also showed daily formation of a single growth line. Another favorite method for the counting of growth lines is the utilization of annual bands. Although this method cannot be so accurate as the marking methods from the nature of the band itself, the daily formation has also been suggested. The number of growth lines between 2 annual bands attained 300 or more in a clam species, Chione, (BERRY and BARKER, 1968), and also 300–420 with a mean of 358 in an intertidal bivalve, Chione stitchburyi, (COUTTS, 1970). Another data supporting the daily formation was obtained in successively collected specimens of a cockle, Cardium edule, (HOUSE and FARROW, 1968).

The daily formation of growth lines has been discussed also from a viewpoint of the diurnal periodicity of shell formation in relation to the daily rhythm of physiological activities. UNO (1962) reported that growth lines in a turban snail, Turbo cornutus, consisted of daily line—A and finer, hourly line—B, implying the daily formation of a single A-line. In a pearl oyster, Pinctada martensii, 2 different observations has been reported: a single layer of about 0.5 µ thick per day (UCHIDA and UEDA, 1947) in one case, and 2 layers of about 0.3 µ per day (OMORI, 1947) in the other. Studying on the diurnal rhythm of physiological activities, MORI (1948) supported the former opinion of daily formation of a single layer, and suggested that the recognition of 2 layers per day were due to the counting of 2 different zones within a daily increment as 2 individual layers. Such a diurnal periodicity were also reported in the formation of hard tissues in mammals (OKADA, 1938) and that of otolith in fishes (PANNELLA,1972), and NIVILLE (1967) reviewed on the daily layers in animals and plants.

The so-called “missing growth lines” were noted by CLARK II (1968), basing on his result that counts of growth lines during 51 days, ranging from 51 to 34 with an average of 44.2 on 12 juvenile scallops, Pecten diegensis. It means that only 2 individuals formed definitely a single line per day, but the others failed to do so on several days. These missing lines were assumed to be due to discontinuous growth accompanied by very close spacing of the growth lines. However, is not there any possibility of overlooking less conspicuous daily lines such as the Type B in between such closely spaced lines?

Besides the growth lines of daily periodicity, it has been reported that some of the growth lines were formed by the influence of the tide. In a giant clam, Tridacna squamosa, 2 types of daily increments were recognized (PANNELLA and MACCLINTOCK, 1968)—a simple increment consisting of a growth line and a relative-
ly homogeneous layer, and a complex increment where the homogeneous layer has another finer growth line, consequently consisting of 2 growth lines and 2 layers. The 2 kinds of increments form marked patterns with a 14-day periodicity. A similar complex increment is also observed in a cockle, *Clinocardium nuttalli*, (Evans, 1972). These 2 growth lines in a complex increment were said to correspond with 2 exposures in a day during neap tides, and growth lines are considered to be tidal in nature, produced due to the exposure at low tide. The clam examined in this study produced regularly a growth line of the primary order per day, even when they are not exposed during the neap tide. In other subtidal bivalves, daily formation of growth lines is also observed (House and Fallow, 1968). Therefore at least some of growth lines are produced in relation to the diurnal periodicity of the animal, and in addition to this, some others are formed occasionally by the tidal influence. The thinner lines in the complex increments, which appear near the middle of growth increments, would be recognized as those similar to some of growth lines of the second order in the present material: e. g. lines of the Type C in the 13th and 14th increments illustrated in Pl. 2.

Besides these, Pannella and MacClintock (1968) also observed “small bandings” within the daily increments, and explained by temporary closing during periods of maximum activity of shell formation, distinguished from the major growth lines formed during periods of minimum activity. These “small bandings” seem to correspond to the Type D and/or E which were found frequently at the surface of maximum growth and vanished in the “inner zone” of outer layer.

Uno (1962) has observed about 8 to 14 of B-lines in a daily growth increment of *T. cornutus*, and the B-lines are thought to be formed hourly, due to a rhythmic deposition of shell matter. The number of lighter “zonules” found in the dark ground between growth lines in the present species are around 10 per growth increment (Pl. 1-A) in a rough estimation, agreeing with that of the hourly B-line. Numerous thin “subdaily layers” within the daily increment of *M. mercenaria* during the season of rapid growth in summer observed by Pannella and MacClintock (1968: 71, 64 explanation of pl. 1, fig. 2) are also somewhat similar to the zonules of the present species. It might be able to assume that the “zonule” is formed in accordance with cyclic shell deposition. They would be considered to be more or less different in nature from the growth lines of both primary and secondary orders.

**SUMMARY**

To investigate the periodical formation of growth lines, the vital staining and planting of the clam, *Meretrix lusoria*, was carried out on a tidal flat in the Ariake Sea in western Kyushu, on July 9, 1971. The marked shells were collected 16 days after the staining, on July 25.

In spite of a short period of immersion, a well marked, red striation along a
growth line indicated the initial date of emplantation properly. No striking inhibition by the staining was recognized, except a slight deformation of growth lines for only 1 to 3 days just after the staining.

In the inner part of the outer layer of the shell, a slightly different zone, which is called the "inner zone" in this paper, was observed, where curved growth lines became straight and change their direction more or less parallel to the inner shell surface.

The thicknesses of growth lines were measured on scanning electron photomicrographs under a measuring microscope. The thickness along the surface of maximum growth showed a trimodal distribution, and fell into 3 groups ($\geq 3\mu$, $3 \sim 1\mu$, $<1\mu$), on the other hand, the thickness in the "inner zone" had a bimodal distribution and was divided into 2 groups ($\geq 1\mu$, $<1\mu$). Based on these data, growth lines were classified into 5 types (the Type A: $\geq 3\mu$ at the surface of maximum growth, and $\geq 1\mu$ in the "inner zone"; the Type B: $1 \sim 3\mu$, $\geq 1\mu$; the Type C: $\geq 3\mu$, $<1\mu$; the Type D: $1 \sim 3\mu$, $<1\mu$; and the Type E: $<1\mu$, $<1\mu$).

They can be brought under following 2 categories: the growth line of the primary order (Types A and B) well discernible in the "inner zone" and more or less conspicuous at the surface of maximum growth, and the secondary one (Types C-E) barely visible or invisible in the "inner zone" and fainter at the surface of maximum growth.

The frequencies of 5 types in each specimen from the staining to killing were counted. Both in the specimens where the primary line included only the Type A, the Type B being absent, and in the rest of specimens where the Type A formed the great majority, a few of the Type B being included, the total of the frequency of these primary lines (only A or A+B) in each specimen was always exactly 16, coinciding the number of days of emplanting. The primary and secondary lines are considered as that of daily and subdaily formations. Closer observation revealed that the dark ground between the conspicuous lines was not quite homogeneous, and was composed of an alternation of lighter and darker zonules.

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ハマグリにおける貝殻成長線の日周期的形成について

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貝殻の成長線は、ほぼ日周期的に形成され、また日成長の年周期的変化をともなうことから、先史時代貝塚
産貝類の採集期の推定に有効な形質であるといわれている。このような成長線の周期的な形成をより厳密に調
べるために、ハマグリ（Moretrix lusoria）を用いアリザリンで生体染色し、16日間放置した。染色された貝
殻の縦断面に赤色に染まった成長線が顕微鏡下で明瞭に観察され、実験開始の時日を正確に認めることができた。
貝殻の外層はその内側がやや異なり、成長線が半円の曲線から直線的となるに方向を変わり内殻面と平行に整
列するので、ここでは外層の“内帯”と呼ぶ。走査乾燥写真上で計測した成長線の厚さは、外層の最大成長線
では3ミリ以上・1ミリ以下・1ミリ以下の3グループに分かれ、外層の“内帯”では1ミリ以上・1ミリ以下の2グループに分か
れた。この2か所での計測値の組合合わせから、成長線をA～Eの5型に分類した。さらにこの5型は、“内帯”
で明瞭に検出でき、最大成長面でも顕著な第1次の成長線（A・B型）と、“内帯”では消失もしくはか
らうじて見い、最大成長面でも比較的細線である第2次のもの（C・D・E型）に大別される。5型の各型につき、
染色線から貝殻にたる各亜殻での出現数を計測すると、全殻体の約2/3では第1次線中にはA型のみでB
型を欠き、残りの1/3ではA型が数多く占めるが2・3本のB型が加える。上記のいずれの場合（Aのみか、
A＋B）にも第1次線の合計はかまに16本となり、この数は経過日数と一致した。一方最大成長面で比較的顯
著な厚さを持つが、外層の“内帯”では消失するC型と前記のA型の各殻体での合計は、11～19本と不形であっ
た。したがって外層の“内帯”で明瞭に検出できる第1次の成長線は日周期的形成され、第2次線はその
間に生ずるものと思われる。このような成長線の識別法をもとにすれば、先史時代のハマグリ採集期の推定も
可能であると考えられる。
Plate 1. Scanning electron photomicrographs of growth lines (KM64D).
A—General view, showing a stained growth line (SL).
B—Details of the growth increment shown in A, showing conspicuous growth lines (C) and fainter growth lines (F).
C—The profile of the location in B (A'-A line) showing the sharp peaks of the conspicuous growth lines.
Plate 2. Five types of growth lines from the second staining to killing (KM12SII), illustrating the Type A (thick solid line with number), the Type B (thick broken line with number), the Type C (thin solid line) the Type D (thin broken line) and the Type E (thin dotted line). Some of growth lines such as the Type A of No. 12 and No. 14 and 2 lines of the Type C in the No. 13 and No. 14 increments are seen to be composed of closely adjoining 2 lines at the surface of maximum growth. But the thinner lines are annexed in a half way to the “inner zone”, so these adjoining lines were not measured or counted separately.