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Growth and Shell Formation of the Surf Clam, *Spisula sachalinensis* (Schrenck)

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Abstract

For the age determination of the surf clam, *Spisula sachalinensis*, the alternate formation of the prismatic and the nacreous layers in the shell was available, because it confirms the fact that the alternation of each layer is annually cyclic. The formation of the layers is probably influenced by sexual maturity and spawning. The prismatic layer is representative of the growth of the shell, which is rapidly accomplished during several months throughout sexual maturity and spawning. Therefore the sexual maturity and spawning exert a serious influence on growth too. Accordingly the difference in growth pattern between the immature and mature individuals seems to be displayed by this phenomenon. The growth of the shell fits the Gompertz equation.

There are many studies on the growth of bivalves[1], and especially several of them are aimed at the individual growth using annual rings formed on the shell surface as an indicator of age determination, e.g. in *Spisula sachalinensis*[2]. But many subjects remain unknown in regard to the age determination, especially the mechanisms of shell formation by the deposition of calcified tissues[3][4]. Then, in the present paper, an attempt was made to measure the growth of *Spisula sachalinensis* with some considerations of environmental and physiological conditions and of the mechanisms of the shell formation by the deposition of calcified tissues.

The present auther is indebted to the Kamiiso Fishery Co-operation for sampling the material specimens.

Materials and Methods

The specimens used in this study were collected from fourteen stations on the shore of Kamiiso along Hakodate Bay (Fig. 1). Although various methods have, heretofore, been used for the age determination of bivalves, in the present case the rings found on the cutting surface of the shell was available. At first, the shell was cut by a precision cutter from the umbo to the shell margin vertically to the shell surface in an arbitrary direction. The individual variations referring to the

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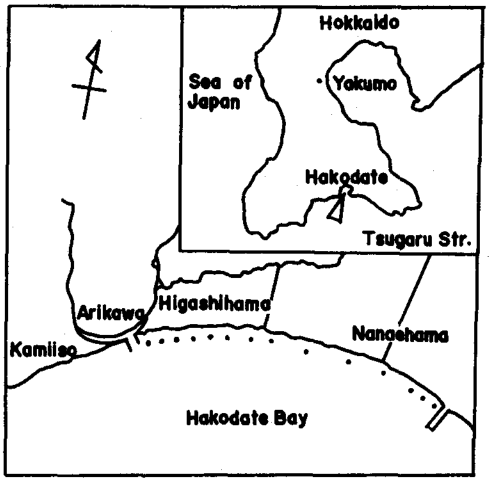

Fig. 1. Map showing the sampling stations expressed by dots.

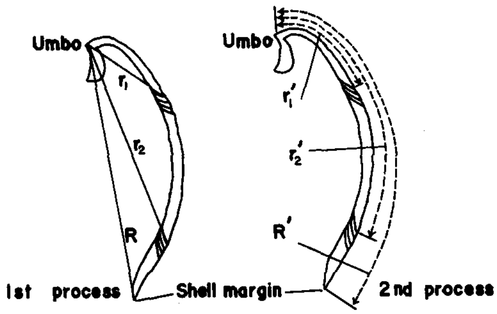

Fig. 2. Methods of measurement of annual rings on a sectioned surface of the shell.

cut direction were negligibly small, because the shell margin can be regarded approximately as the circumference of a circle having the umbo as the centre within a small range of fluctuation of the cutting line as radius. As to the procedure of measuring each ring of the shell two methods were applied; the first process is the measurement of a linear distance (r_i R) from the centre which was fixed on the original umbo to the starting point of each successive ring formation, viz. the translucent nacreous layer, and the second process is the measurement of the curvilinear length along the shell surface (r_i' R') from the centre to the starting point of each ring formation (Fig. 2). For the measurements the posterior part of the left shell was used and the measurements were taken up to 1/20 millimeters with a caliper. In the second process, the length from the centre to each ring was measured by stretching a thin narrow paper ribon closely turned along the shell surface from the umbo to the beginning point of each ring formation, on which point the ribbon was marked. In the present paper, the clams in the period from hatched larvae to the beginning of the first ring formation are called one-year shells, and those until the beginning of the second ring are called two-year shells, and so on. For the detection of the crystal of the shell material, several specimens were crushed into fine particles and tested by the Geigerflex (Rigaku Denki Co. Ltd.; condition (N_i filtered) voltage 35 KV., current 15 mA.). In order to clarify the organic matrix structure of the shell, several sectioned specimens were obserbed microscopically after they were decalcified by E.D.T.A. and stained by hematoxylin and eosin.

Results

Relation between the shell weight and dimensions

The relations between the body weight (W), the shell length (L), the shell breadth (B), and the shell height (H) were determined, thus fitting the formula $y=a\,x^b$ or $\log y=\log a+b\log x$, where y is W in decigram or L and B in mm, x is L or H in mm, and a and b are constants. The total number of individuals for calculation were 410. The curves of these relations prove to fit the observed values very well, however some of them break into two parts for shells under two years and shells over three years (Table 1, Fig. 3). That is, in the W-L relation, the slopes of the straight line in logarithm "b" are almost the same as 3 throughout the growth, but discontinuity occurs during the period between the first and the second ring formation; in the W-B relation, a marked break of line occurs, at which point the "b" value is about 3.0 in the shells under two years, and lower to about 2.5 in those above three years; and in the B-L relation the slope is hightened markedly from the shells under two years to those above three years. These facts prove that the shell breadth increases abruptly from two-year shells to three-year shells. The L-H relation is invariable throughout the whole course of growth, and shows isauxetic relative growth. The W-R' relation is expressed by a straight line throughout the period of growth, whereas the existence of discontinuity is unknown because of the lack of data for smaller shells. As mentioned later, R corresponds to H, but R' expresses a substantial shell height.

Age determination and growth

The shell, viz. the calcified tissue, of the surf calm can be obviously discriminated into two layers, that is, one is a white opaque layer and the other is a gray translucent layer. According to the general designation, the former is named "prismatic layer" and the latter "nacreous layer". The rings of the shell constructed by the gray translucent layer were taken into account as age indicator and for the measurement of growth, as mentioned above in the methodological description. In regard to the mean of each ring radius (r_i) measured by the first and second processes, the growth equations were tested for fitness. Consequently the growth equation of von Bertalanffy did not show so good fitness, but the Gomperz equation.[5)]

$$y_t = y_\infty e^{-ae-bx}$$

showed a good fitness, where y_t represents the length of the shell at any time t, y_∞ is the theoretical maximum length, and a and b are constants. This formula can be transformed into

$$Y_t = Y_\infty\left(1-\frac{a}{Y_\infty}e^{-bx}\right)$$

Table 1. *Relationship between shell length (L), body weight (W), shell breadth in brackets represent 95% confidence interval.*

Relation		Observed numbers	log a	b
$W=aL^b$	Under 2 years	100	−2.883	3.08 (2.94–3.22)
	Over 3 years	115	−2.616	3.01 (2.90–3.12)
$W=aB^b$	Under 2 years	100	−1.777	3.03 (2.89–3.17)
	Over 3 years	115	−0.960	2.49 (2.47–2.51)
$B=aL^b$	Under 2 years	100	−0.314	0.98 (0.92–1.04)
	Over 3 years	115	−0.598	1.17 (1.13–1.22)
$L=aH^b$		105	+0.099	1.00 (0.98–1.03)
$W=aR'^b$		111	−3.944	3.10 (2.96–3.24)

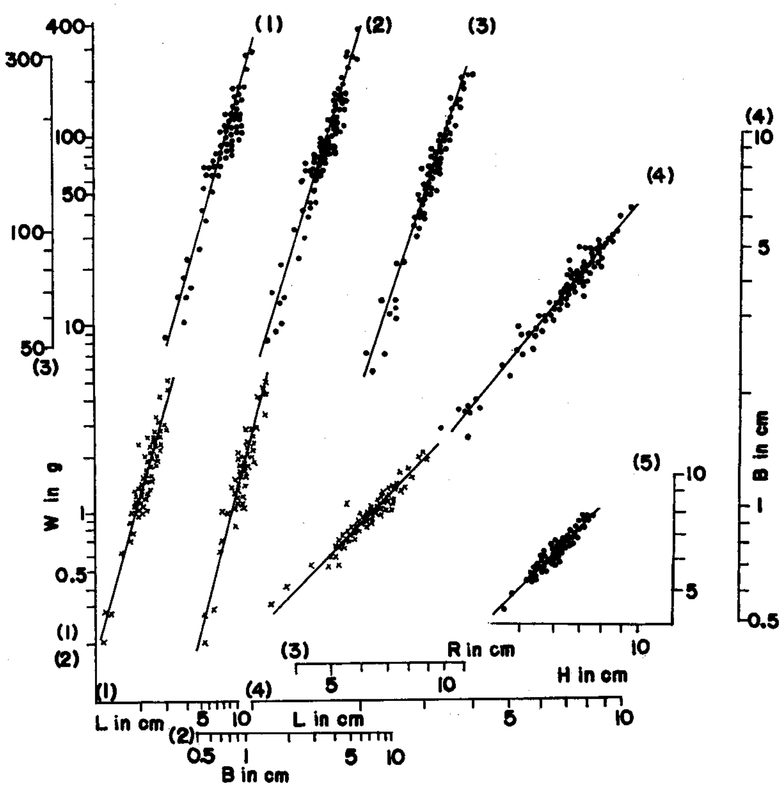

Fig. 3. Relations between body weight and shell dimentions. (1) W/L relation, (2) W/B relation, (3) W/R' relation (4) B/L relation, (5) L/H relation. ● :Individuals over 3 years old, ×: Individuals under 2 years old.

(B), shell height (H), and the length R′ in Fig. 2. a and b are constants. Figures

Correlation coefficient	Adjusted mean	Comparison between b	Comparison between adjusted men
0.991	2.453	F=1.23 df:1, 211 P>0.25	F=48.75 df:1, 212 P<0.005
0.998	2.124		
0.994	2.108	F=550.8 df:1, 211 P<0.005	F=62.33 df:1, 212 P<0.005
0.999	2.132		
0.999	1.293	F=10.68 df:1, 211 P<0.005	F=0.17 df:1, 212 P>0.10
0.999	1.293		
0.999			
0.999			

where $Y_t = \log y_t$, and $Y_\infty = \log y_\infty$. The results of the calculation by this formula are as follows (Fig. 4):

For the values by the first process

$$Y_t = 0.882(1 - 2.103e^{-0.902x}) \tag{1}$$

or

$$y_t = 7.62e^{-4.456e^{-0,902x}} \tag{2}$$

and by the second process

$$Y_t' = 1.040(1 - 1.860e^{-0.865x}) \tag{3}$$

or

$$y_t' = 10.97e^{-4.456e^{-0.865x}} \tag{4}$$

As the relation between the shell length (L) and the shell height (H) is

$$L = 1.26\ H^{1.003} \tag{5}$$

(Table 1) and the value of y_t in the equation (2) is regarded as the same as the shell height at time t, the theoretical shell length can be evaluated by means of the substitution of y_t for H in the equation (5). In the same way, as the relation between the length along the shell surface from the umbo to the shell margin, y_t', and the body weight W was shown in

$$W = 0.0011 y_t'^{3.10} \tag{6}$$

(Table 1) and the correlation coefficient between $\log y_t'$ and $\log W$ was as high as 0.999, the theoretical body weight at time t can be evaluated by inserting the

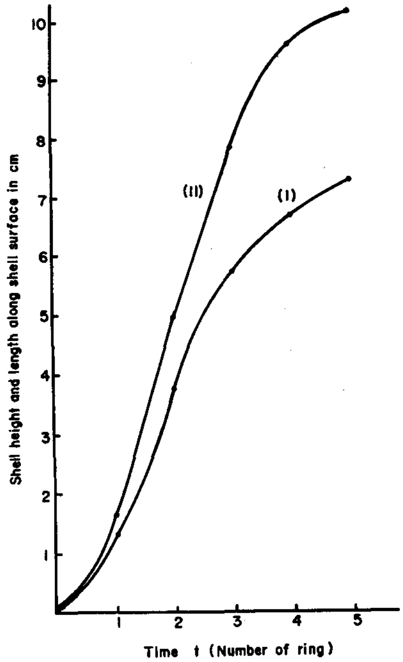

Fig. 4. Growth curves of the shell height by the 1st process (I) and the length along the shell surface by the 2nd process (II).

value of y_t' of the equation (4) into the equation (6). Thus, the theoretical shell length and body weight were calculated as shown in Table 2.

Ring formation

From the diffraction pattern by X-rays, it was shown that both the prismatic and nacreous layers are formed by aragonite (Fig. 5). Kawahara and Maita[6] have also shown the same result of analysis. The direction of cleavage, which is observed when the shell is cut away into a thin layer, coincides with the orientation of the organic matrix or crystal microscopically. A decalcified preparation treated by E.D.T.A. was stained by the hematoxylin-eosin method for the microscopic observation. The portion which seemed to be a prismatic layer was dyed by hematoxylin, and the portion which seemed to be a nacreous layer was dyed by eosin separately. Bevelander and Benzer[7] have already shown the same result. And on the sectioned surface of the shell, only the prismatic layer is dyed clearly by the alizanin red dry solution (Fig. 6). The same fact has been shown by Hayashi[8]. From these facts, the difference between the prismatic and the nacreous layers is not due to the characteristics of the crystal itself, but it seems to follow the difference of the orientation of the crystal or the difference of the organic compounds in the organic matrix wrapping the crystals. Each layer of the surf clam exhibits an alternative cycle unlike the pearl oyster (Fig. 6). In the surf clam, observing

Table 2. *The theoretical shell length and body weight with 95% confidence interval at the time of the annual ring formation.*

Age	1	2	3	4	5
Mean H (cm)	1.32±0.05	3.74±0.07	5.72±0.04	6.69±0.03	7.33±0.28
Theoretical H (cm)	1.34	3.78	5.72	6.65	7.27
Theoretical L (cm)	1.69	4.78	7.23	8.57	9.21
Mean y' (cm)	1.65±0.07	4.95±0.11	7.81±0.12	9.41±0.03	1.001±0.03
Theoretical y' (cm)	1.68	4.97	7.85	9.58	10.35
Theoretical W (g)	0.7	20.7	85.3	155.3	200.9

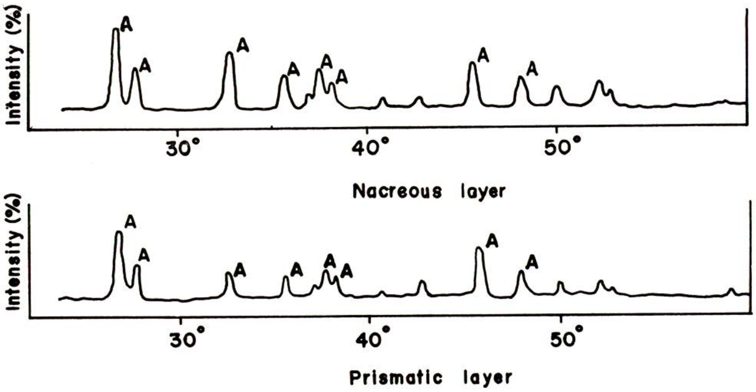

Fig. 5. X ray diffraction pattern of each layer of the shell. Mark A indicates a peak of reflex angle of aragonite.

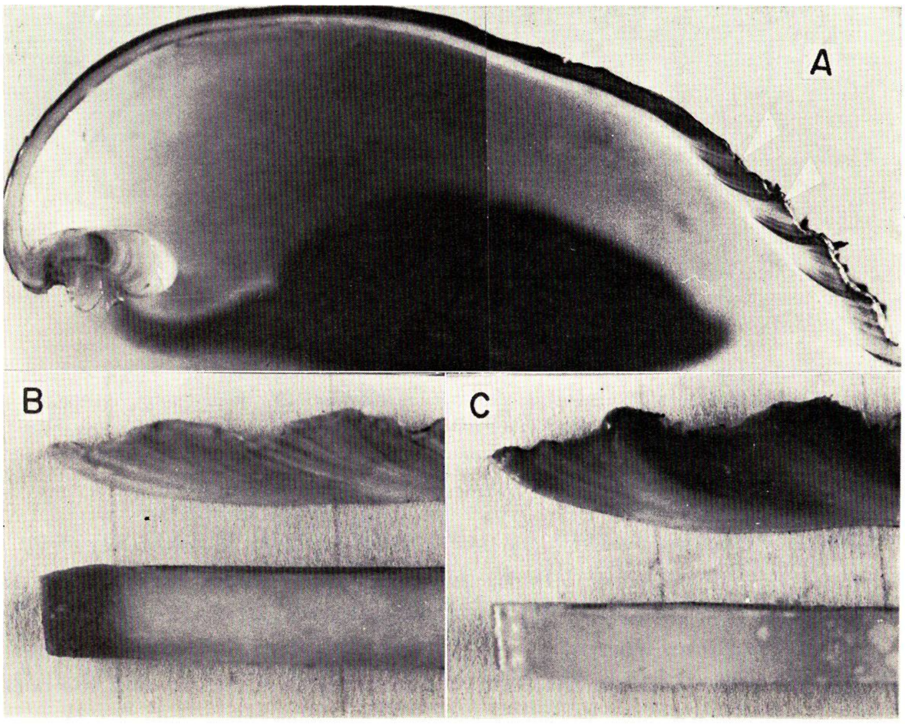

Fig. 6. A. Photographs showing the beginning point of annual ring formation (arrow) and construction of annual translucent (nacreous) and opaque (prismatic) layers, dying with alizanin red dry solution, by which only the prismatic layer is dyed on the sectioned surface of the shell.

Fig. 6. B. Sectioned surface view of the shell margin (up), and view from inside of the valve (down), in May.

Fig. 6. C. The same as Fig. 6b in September.

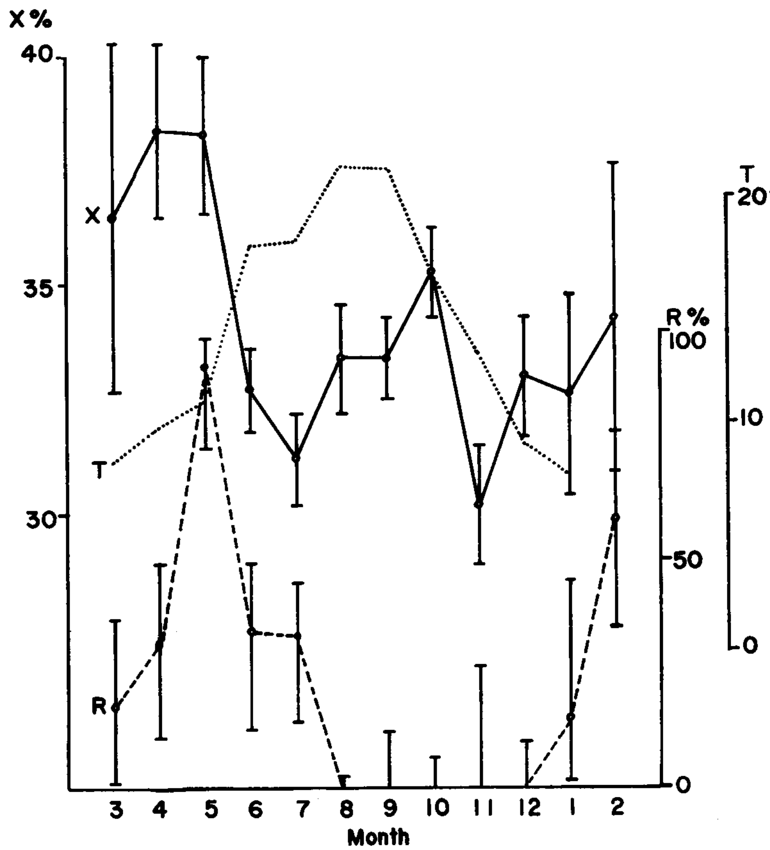

Fig. 7. Monthly change in prismatic layer formation, percentage of flesh weight, percentage of dried flesh weight, and temperature of sea water. X: Flesh weight exclusive of shell/total weight, R: Individuals forming prismatic layer at the shell margin/total individuals, T: Mean surface temperature of sea water. Vertical lines represent 95% confidence intervals.

the pattern of alternative layer on the cutting surface, it looks like that the translucent or nacreous layer wraps up the opaque or prismatic layer at the time of ring formation. It is due to the complete periodical alternation of each layer at the shell margin, whereupon we suggest the term of "wrapping up" to represent this fact, therefore the term "ring" means a condition that the nacreous layer is wrapping up the prismatic layer in the umbo-side.

The specimens over 3 years old having the ability of spawning were sampled from March 1970 to February 1971 (about 30 individuals every month), on which the pattern of alternation of each layer was inquired on the cutting surface of the shell. From these observations, a percentage rate of individuals forming the prismatic layer at the shell margin of the total individuals, and the mean rate of

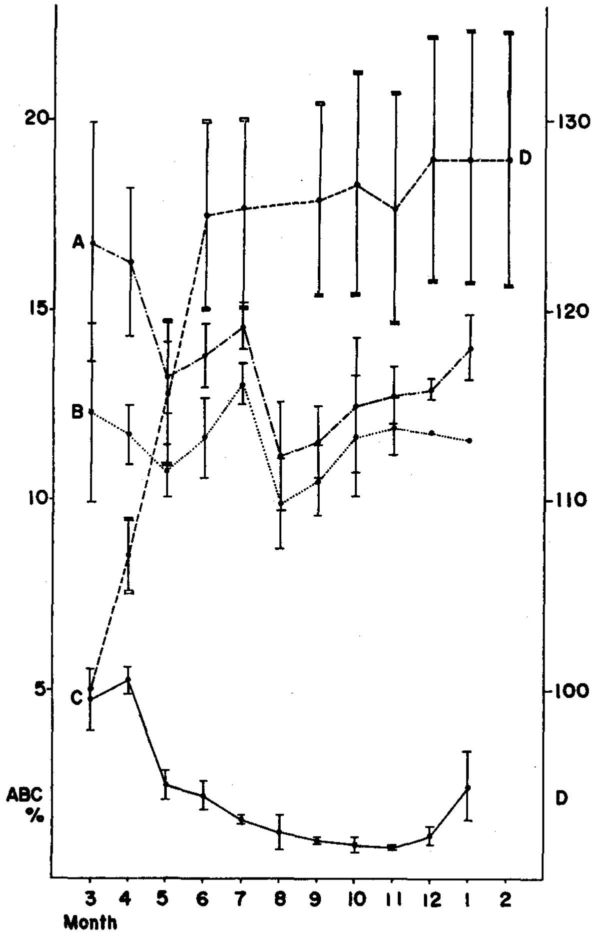

Fig. 8. Growth pattern represented by monthly changes in percentages of flesh and visceral organs to valves in dry weight.
A: Dry total flesh weight/dry valves weight.
B: Dry flesh weight exclusive of visceral organs/dry valves weight C: Dry visceral organs weight/dry valves weight. D: Monthly mean shell lengths/mean shell length in March (%) in 2-year shell.
Vertical lines represent 95% confidence intervals.

flesh weight exclusive of shell to the total body weight were calculated every month (Fig. 7). Significant differences of the mean values were observed between March, April or May, and June, more markedly especially between May and June. This marked decrease from May to June may be due to the spending of reproductive materials, which occurs during the period of abrupt increase of the surface temperature of sea water. It may coincide with the spawning period of the surf clam, as observed by Kinoshita,[9] which occurs from May to June in Kamiiso. There is

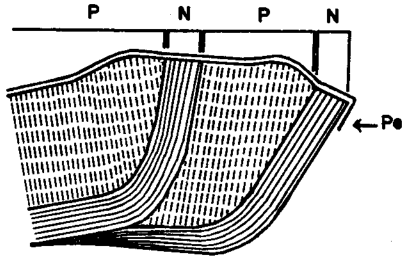

Fig. 9. Assumed pattern of orientation of each layer on the sectioned shell surface. N: Nacreous layer, P: Prismatic layer, Pe: Periostracum.

also a difference between October and Nobember, however it is very small and probably an error due to sampling deviations, because of no evidence in another instance (Fig. 8).

The percentage of individuals forming the prismatic layer decreases with the above-mentioned changes, so that the formation of the nacreous layer begins immediately after spawning by June at the shell margin and continues till December-January, from which the formation of the prismatic layer begins to occur at the shell margin and continues till July the following year. The same results were also shown in the 1968-population in which the changes in the length from the last ring to the shell margin were investigated from June to October (unpublished). Takahashi, Takano and Yamamoto[10)11)] investigated the cycle of the seasonal change in ovary and testis histologically using the surf clams collected in Yakumo near Kamiiso, and thereby the cycle coincided completely with the present macroscopical data.

The specimens similar to those in Fig. 7 were sampled from March 1973 to January 1974. Their bodies were dissected and separated into valves, muscles and visceral organs, and then each part was weighed after being dried at 60°C. In the surf calm it is difficult to take out only the reproductive organs from the body, because the intestine penetrates through the reproductive organs coiling upon itself without cavity between both tissues. Thereby the weight of total visceral organs was substituted for the weight of the reproductive materials. The change in each part was considered by the ratio that the weight of each part was divided by the weight of the values in order to diminish the deviations caused from the shell size (Fig. 8). From these considerations it is clear that the reproductive materials exist during the period from December-January to June-July, which coincides with the period of the prismatic layer being formed (Figs. 7 and 8). The muscular part seems to be almost constant throughout a year, but a significant change is found in July-August immediately after the spawning season.

The growth process of 12 individuals of 2-year shells is also shown in Fig. 8.

These specimens were reared in running natural sea water over a year period at the Hakodate Fishery Experiment Station. The mean shell length in March being 100, indices of the mean shell length in the successive months were compared with it. Significant changes were found during the period from March to June, which coincides with the period of the prismatic layer formation. That is, it shows that a rapid growth is performed during the spawning season, and no growth occurs during the remaining months. Therefore the prismatic layer is obviously conformed to the growth layer.

From these facts mentioned above, it is clear that the formation of each layer alternates once a year accompanying the reproductive cycle in the mature shells, but it seems that some problems remain unknown in the immature shells, whether there exists a growth of gonads in a cyclic manner or not, and whether the temperature is only a factor on the growth and formation of the shell or not.

Discussion

The age determination of the surf clam by means of the shell cutting has some advantages, e.g. a facility to distinguish the true annual rings from false ones which might occur by some physiological disturbances, and clearness of rings over 3-year-old shells, especially the nacreous layer in order to observe the beginning point of formation. It may be understood that the physiological effects of spawning are more abrupt than those of maturity process of organisms. In the experiments for elucidating the shell formation, the orientation pattern of the calcareous crystal or organic matrix was observed microscopically in many specimens (Fig. 9). According to these observations, the crystals in the prismatic layer line up in a parallel direction to the previously formed part of the nacreous layer, and then the nacreous layer is formed vertically or obliquely to the prismatic layer and further encloses it on the edge of the shell at a definite season. From these facts it may be understood that a physiological condition suddenly changes, and since this change is annually cyclic, its representation on the shell is available for age determination.

Out of the two growth equation mentioned above, the equation (2) give the pattern of the growth of the shell length owing to the relative growth of *L-H*, and the equation (4) gives the pattern of the growth in weight by taking into account the equation (6), where it is due to the shell growth that the majority of this bivalve weight (about 50% in wet weight) is an additional growth of inorganic tissue. There are lots of occasions when the nacreous layer is formed wider than the prismatic layer in older shells. In fact, in the immature shells under 2 years of age the nacreous layer "wrapping up" the prismatic layer is presented as a thin layer. In the surf clam, although the prismatic layer represents the growth layer, the nacreous layer should be called the "thickness layer", for the addition of the nacreous layer on the shell margin contributes to a thickening of the shell width rather than an enlargement of the shell.

There may be some differences between immature and mature individuals in the mechanisms of shell formation. Now, in the surf clam, it should be considered that the alternation of the prismatic layer to the nacreous one is due to the change in the mechanisms of the shell formation followed by physiological changes. Although there is a hypothesis that the different portions of the mantle form the different calcified tissues on the pearl oyster, *Pinctada martensii*,[12][13] on the surf clam the prismatic and nacreous layers are not formed in piles on the outer and inner sides of the shell, but form alternatively and additively on the shell margin once a year. From these facts another hypothesis that periodical changes in the permeability of periostracum make the mechanism of the changes in calcification, namely that they are due to the sensitivity of the biological membrane against the environmental and physiological changes, seems to be more prevalent.[14][15][16] Since the surf clam is not considered to be a special case in the calcification mechanisms, this hypothesis may be more appropriate not only to the surf clam but to other bivalves. There may be a case when the nacreous layer "wrapping up" the prismatic layer is probably thin in other bivalves (e.g. *Phacosoma japonica* (Reeve), *Mactra sinensis carneopicta* Pilsbry, *Crenomytilus grayanus* (Dunker)), but it may not be clear. And apart from the formation of the layers, it is shown that the aragonite crystal pattern will be changed due to physiological conditions, and so on.[17]

In the present studies, the annual periodicity suggests that the physiological rythm which especially occurs in the processes of growth, maturity, and spawning may change the mechanisms of calcification in the shell, deducing from the facts that the period of prismatic layer formation coincides with that of maturity and spawning, after which the period of nacreous layer formation comes. The greatest part of growth is attained in the period of the prismatic layer formation. That the features of growth are different between immature and mature stages being expressed by the discontinuities in the relations of W/L, W/B and B/L, it is suggested that the thickening of the nacreous layer may participate in post-spawning physiological conditions and also in the discontinuity of the relative growth.

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