Mollusk Shell: Daily Growth Lines

Abstract. *Ridges forming the concentric sculpture on the shells of laboratory-grown specimens of* Pecten diegensis *Dall show daily periodicity. Missing growth lines account for all scatter in the data, so that the maximum, not the average, line count is most representative. The variation in spacing between growth lines can be correlated among specimens.*

Daily growth lines have potential value in geochronometry. Wells (*1*) noted that geophysical theory suggests that the earth's rotation is slowing, so that in the geologic past, a year contained a greater number of shorter days than in the present. He reasoned that a fossil with both daily and yearly growth lines could be used to check this theory, and once the relation was well established any such fossil would be a true geologic clock, capable of indicating not just its relative but its absolute age. He tested this concept with some Devonian corals, and counted about 400 growth lines per annual increment, with extremes of 385 and 410. These results agree with the geophysical estimates (*2*).

This approach has been applied to other cycles and other invertebrates. Scrutton (*3*) found bands, apparently representing lunar months, of from 27 to 35 presumably daily ridges on Devonian corals. His average of 30.59 days in a Devonian lunation is compatible with geophysical theory (*4*). Barker (*5*) and Pannella and MacClintock (*6*) have worked with bivalves which appear to show daily and tidal periodicities. Their evidence indicates that the tidal cycle, and therefore the lunar month, was longer during the Pennsylvanian period than now.

The daily growth line has not been well studied, and experimental evidence is limited. Wells based his interpretation on the indirect evidence that in some recent corals there are about 360 growth lines in a distance equivalent to an average year's growth. Pannella and MacClintock found between 360 and 370 growth increments on shell formed over a year's time on marked intertidal bivalves. Davenport (*7*) found four new increments on scallops left 4 days in a tide box; he also counted 48 to 56 increments on shells believed to be about a month and a half old. This evidence indicates that these lines are daily growth increments, and strongly supports the circumstantial evidence of the fossil growth lines. However, both groups of evidence also indicate that these growth lines are neither exclusively nor infallibly a record of a day's passage, for in virtually every case there are specimens showing both more and fewer growth lines than days. Moreover, in some of the fossil studies it is not obvious that the factors responsible for fewer lines than days are related to the factors responsible for more lines than days; consequently they do not necessarily occur with equal frequency. In such cases the average growth-line count might be of little significance.

I now report documentation of daily growth lines in a different bivalve. In this study consideration of missing growth lines is the most important factor in the interpretation of the data. Moreover, I show how such missing lines may be compensated for in certain cases.

Twelve juvenile specimens of *Pecten diegensis* Dall were kept in running seawater at the Kerckhoff Marine Laboratory, Corona del Mar, California, from 12 May to 2 July 1967 (*8*).

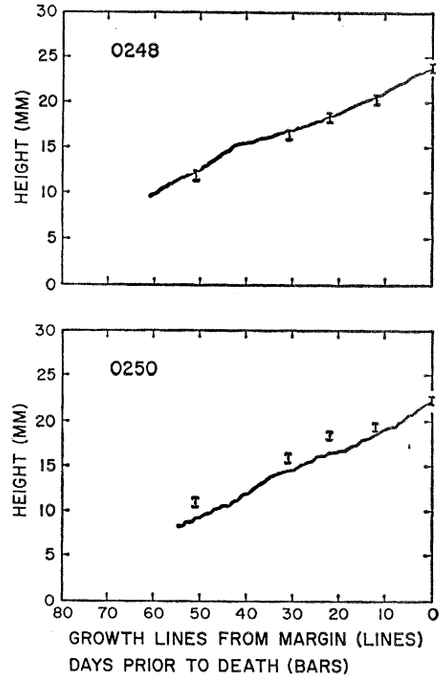

Fig. 1. Cumulative growth relative to growth lines (continuous line) compared with cumulative growth relative to calendar days (bars) for two specimens. Right border (margin of shell) is used as base point for comparison.

The height of the shell (perpendicular distance from hinge line to margin) of each specimen was measured five times during the experiment (*9*). Inasmuch as each growth line represents the position of the shell margin at the time of formation of that growth line, the perpendicular distance from the hinge line to any growth line represents the height of the shell at the time that growth line was formed (*10*). Thus, if the height of the shell 10 days before the death of the animal is the same as the height of the tenth growth line from the margin, then for that period of time it is likely that one growth line formed each day.

The height of individual growth lines was determined from measurements on enlarged photographs of the specimens (*11*). In Fig. 1 the cumulative growth derived from these measurements, plotted against growth line number, is compared with cumulative growth derived from the measurements made during the growth of the animals, plotted against calendar days. Six of the 12 specimens, like 0248, have formed growth lines in numbers compatible with daily formation. The other six, like 0250, have formed too few growth lines for this interpretation. This ambiguity might be resolved if the growth lines were assumed to be daily in both cases, but were too few in number in some specimens due to discontinuous growth.

Many bivalves stop growing at times of environmental or biological stress (*12*). Such a pause usually leaves a distinct line of disturbance on the shell, and is often accompanied by very close spacing of the growth lines. All of the specimens in my experiment showed zones of closely spaced growth lines, and many appeared to have one or more disturbance lines. The interval between growth lines was measured (*13*) and plotted against growth-line number to form a growth-rate curve (Fig. 2) (*14*). The first three curves are quite similar, and if horizontal offset is discounted, all six curves show features in common. The correction for this offset would be in the direction of more growth lines; the specimens requiring this offset in growth-rate curve also have too few growth lines by their cumulative growth curves (0250, Fig. 1). This correlation strongly suggests that some growth lines are missing.

To test the validity of this interpretation, the horizontal axis (in units of growth lines) of both the cumulative

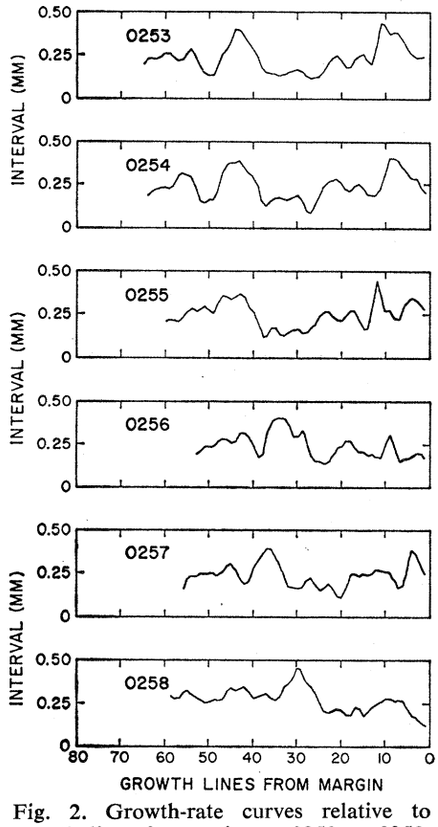

Fig. 2. Growth-rate curves relative to growth lines for specimens 0253 to 0258. The curves have been smoothed by the weighted running mean.

growth curve and the growth-rate curve was divided into segments at points corresponding to disturbance lines on the specimen. If each segment represents a portion of the shell where a growth line formed each day, then segments of the cumulative growth

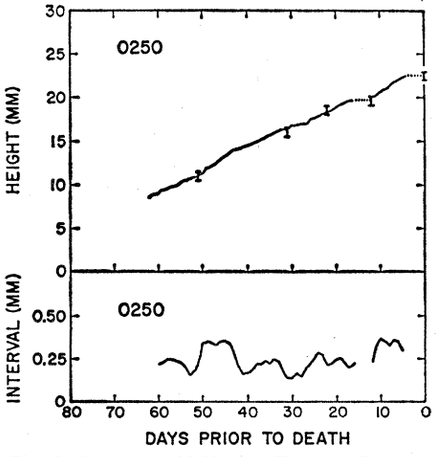

Fig. 3. Specimen 0250 (see Fig. 1). Growth data relative to growth lines have been offset along the horizontal axis in two segments to fit the growth data relative to days, demonstrating two locations of missing growth lines (dotted lines). Comparison data for lower curve not shown (see Figs. 2 and 4).

curve should fit the measurements at the same offset that corresponding segments of the growth-rate curve most closely match the growth-rate curves of specimens lacking no growth lines. This offset should represent the number of missing growth lines. This technique was applied (Fig. 3) and confirmed offsets for the six specimens with too few growth lines. Moreover, the growth curves of four of the remaining six specimens showed improved relations through the introduction of minor offsets, and thereby demonstrated the sensitivity of this method. Figure 4 shows the corrected growth-rate curves for the six specimens shown in Fig. 2. The horizontal lines above the curves indicate the location and extent of missing growth lines.

If the growth lines examined had been counted on fossil shells, the interpretation might have been different. Counts made on the 12 shells range from 34 to 51 lines, with an average of 44.2. A frequency distribution of the data shows a strong skew to the lower values, which might be attributed to the small size of the sample. With no better reason for believing the proper distribution to be other than normal, the average value should probably be accepted as representative. In fact, the distribution is far from normal, since 10 of the 12 specimens have missing lines, and apparently none have extra lines. The true number of days represented here is 51, the maximum value counted.

As mentioned before, Scrutton (*3*) took the average of his growth-line counts to represent the number of days in the Devonian lunar month. The data he presents, unfortunately not in raw form, indicate a frequency distribution with a strong skew to the lower values. Moreover, he mentions that similar bands on modern corals have been observed to have "approximately" 28 increments. This value, presumably an average, is lower than the 29.5 days in a present-day lunation. These factors suggest, but do not prove, that missing growth lines are more important here than extra ones. If reevaluation of Scrutton's data gave a slightly higher value, these figures would still be compatible with geophysical theory (*15*).

Short-term environmental control of growth rates, as implied by the correlation which we found between growth-rate curves, would mean that each specimen in a fossil assemblage could carry an environmental record in its

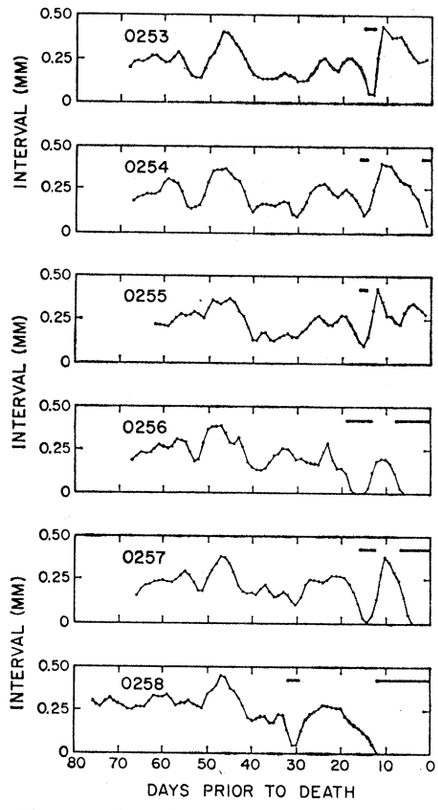

Fig. 4. Growth-rate curves relative to calendar days for six specimens. Horizontal line segments near top of curves indicate extent of missing growth lines. Curves do not always drop to zero at these locations due to the smoothing process (*14*).

growth lines. Even before we learn to interpret the environmental information in such a record, the growth-rate curves of the different specimens could be compared in a search for correlations. When the growth-rate curves correlate in nearly all the specimens and end at the same point, the assemblage was most likely a fossil community which suffered a catastrophic death. In cases when growth-rate curves correlate, but with considerable overlap between specimens, the assemblage was probably a fossil community whose members lived their usual life-spans. Lack of correlation between growth-rate curve probably indicates that the assemblage was not a community. This type of information is of prime importance in paleoecologic investigations (*16*). Of potentially greater significance is the possibility of using the growth-line record to determine absolute environmental conditions in the past. Tree-ring studies based on very similar measurements, have provided information on terrestrial paleoclimates for periods extending far beyond the

lifetime of any single tree (*17*). Although the necessary relations have not yet been established, there is no reason to believe that growth lines on marine invertebrates are less significant.

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8. The specimens were dredged in a depth of about 60 meters off Santa Catalina Island, from R.V. *Velero IV*, station No. 11467, 20 April 1967.
9. Specimen identity was preserved by the use of individual plastic cages.
10. The hinge line does not significantly change position during growth.
11. The techniques used minimized the effects of photographic distortion, and resulting error was considered insignificant beside the errors in the measurements made during the growth period.
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13. Again, this was done on enlarged photographs.
14. The curves have been smoothed by the weighted running mean.
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18. Supported in part by NSF grant GB-6275. Contribution 1530, Division of Geological Sciences, California Institute of Technology.

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Interferon Inducers Protect Mice against Plasmodium berghei Malaria

Abstract. *Injection of mice with two interferon inducers, Newcastle disease virus or statolon, 20 hours after inoculation with* Plasmodium berghei *sporozoites, prevented or delayed the development of detectable malarial parasitemia and death.*

To know whether the antimicrobial action of interferon is limited to viruses or extends to other classes of intracellular parasites is important with respect to the mechanism of action of interferon, to the metabolism and mechanism of reproduction of intracellular parasites, and to the range of potential therapeutic or preventive applications of interferon and of inducers of interferon activity. Interferon inhibits the development of psittacosis and trachoma-inclusion conjunctivitis (TRIC) agents (*1*), a group of intracellular parasites distinct from viruses (*2*). The experiments reported here show that two interferon inducers, statolon (*3*) and Newcastle disease virus (NDV), exert a protective effect in vivo on a protozoal infection, *Plasmodium berghei* mouse malaria.

The cyclical propagation of *P. berghei* in rodents and in *Anopheles stephensi* (*4*) and the method of harvesting sporozoites from the mosquitoes' salivary glands (*5*) have been described. In this system, development of parasites previous to their invasion of erythrocytes takes place in liver cells, chiefly during the first 40 to 46 hours after injection of sporozoites, and detectable parasitemia appears usually from the 3rd to 7th day, depending upon the number of sporozoites injected (*6*). Female CF1 mice (4 weeks old, Carworth Farms) were injected intravenously with 5000 sporozoites in 0.2 ml of a solution of 50 percent saline, and 50 percent human serum. Seventeen to 20 hours later, they were injected with the interferon inducers or with control materials. A 4 percent suspension of statolon (*7*) was prepared and diluted in 1 percent sodium bicarbonate just before use. Control materials included 1 percent bicarbonate, the stabilizer (*7*), and the 4 percent statolon suspension after heating at 95°C for 1 hour, since this procedure has been reported to decrease the interferon-inducing activity of statolon (*3*). Statolon and the respective control materials were injected intraperitoneally in 0.2 ml of 1 percent sodium bicarbonate. Allantoic fluid was harvested from NDV-infected chick embryos and titrated in chick embryo cell cultures by the plaque method (*8*). Dilutions of NDV were prepared in physiological saline. Control materials included physiological saline, normal allantoic fluid from 13-day-old chick embryos, and the supernatant of the infected allantoic fluid after centrifugation at 100,000*g* (most but not all infectious virus was removed by centrifugation). Portions (0.2 ml) of NDV or of the respective control materials were injected intravenously.

Three to six mice were bled for determination of serum interferon 8 hours after the injection of NDV (or of respective control fluids) or 16 hours after the injection of statolon (or of respective control fluids). The pooled serums from NDV-injected mice, and those from the respective control mice, were dialyzed at *p*H 2.0 for 3 to 4 days to destroy residual infectious virus. Interferon activity was determined by the ability of twofold dilutions of the tested pooled serums to inhibit vesicular stomatitis virus (VSV) plaque formation in mouse L cells. The reciprocal of the highest dilution of the tested pooled serum causing a reduction of 50 percent or more in the number of control plaques was considered the interferon titer (*9*). Blood smears obtained from the remaining mice (usually 10 per group) on the 4th, 5th, 6th, 7th, and 8th days after the sporozoite injection and twice a week thereafter were stained with Giemsa. The parasitized erythrocytes per 10,000 erythrocytes were counted, and the following end points were determined: (i) the number of mice that showed detectable parasitemia (patency); (ii) the duration of the mean interval between sporozoite injection and first smear with one or more parasites per 10,000 erythrocytes (prepatent period); and (iii) the mean number of parasitized erythrocytes on a given day. Patent animals were observed daily until they died (up to 35 days) and nonpatent ones for 21 days. Deaths occurred only in patent animals, and all patent animals died.

Allantoic fluid from NDV-injected embryos exerted a protective effect up to dilutions of 10^{-2} to 10^{-3} (Table 1). This effect was not associated with the injection of fluid per se (saline control), nor with the constituents of normal allantoic fluid (allantoic fluid control). The protective effect was decreased by the centrifugation of NDV allantoic fluid. Thus, it was associated either with the infectious virus particle or with a particle present only in infected allantoic fluid and sedimenting at 100,000*g*. The dilution end point of the protective effect of NDV was about the same whether it was measured by the number of surviving animals or whether it was measured by the mean prepatent period. The protective effect of statolon (Table 2) was not due to the diluent or to the stabilizer. It was decreased after heating at 95°C, as was the interferon-stimulating effect of statolon (experiment 4).

From the results shown in Tables 1 and 2, it can be concluded that (i) two interferon inducers of different origin and composition, statolon and NDV, exert a protective effect against sporo-