CALCIFICATION RATE OF *CERATOPORELLA NICHOLSONI* (PORIFERA : SCLEROSPONGIAE) : AN *IN SITU* STUDY WITH CALCEIN

TAUX DE CALCIFICATION DE *CERATOPORELLA NICHOLSONI* (PORIFERA : SCLEROSPONGIAE) : MESURES *IN SITU* A LA CALCEINE

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ABSTRACT

The calcareous skeletons of sclerosponges play a secondary but not insignificant role as framework builders on modern coral reefs. They contribute to and strengthen the walls of caves, tunnels and crevices in reefs, are important as framework constructors on the Jamaican deep fore-reef and fill the interstices of the reef even at relatively shallow depths. These facts have led us to investigate the growth rate of the skeleton of sclerosponges.

In the work reported here, calcein, a fluorochrome stain taken up by calcifying tissues, has been employed to mark newly deposited aragonite in the skeleton of Ceratoporella nicholsoni (Hickson). Experiments were performed in situ in a reef tunnel at a depth of 28 m off Pear Tree Bottom, Jamaica. A plastic bag containing a solution of the dye in sea water was tied around each specimen and removed 24 hours later. Identical treatments were repeated on the same specimens after times ranging from one week to six months.

Ground sections perpendicular to the surface of harvested samples of the dried skeleton were examined and photographed by fluorescence microscopy. The distance measured between successive fluorescent lines revealed a growth rate of less than 0.2 mm per year.

Growth lines apparent to the naked eye in sections of C. nicholsoni do not represent annual accretions of aragonite. It is suggested that caution is needed in the interpretation of growth lines in ancient sponges with massive calcareous skeletons.

RESUME

Le squelette calcaire des sclérosponges joue un rôle secondaire mais non négligeable dans l'édification des récifs coralliens actuels. Ces organismes contribuent à l'édification et au renforcement des parois de grottes, de tunnels et de cavités des récifs. Ils constituent d'importants bâtisseurs de la pente externe du front récifal de la côte nord de la Jamaïque, et comblent des cavités, même à des profondeurs relativement faibles. Ceci nous a amenés à étudier la vitesse de croissance du squelette de sclérosponges.

Au cours de cette étude, l'aragonite néoformée du squelette de Ceratoporella nicholsoni (Hickson) a été marquée à la calcéine, un colorant fluorochrome qui s'incorpore aux tissus en cours de calcification. Les expériences ont été réalisées in situ dans un tunnel sous-récifal, à 28 m de profondeur au large de Pear Tree bottom, en Jamaïque. Des sclérosponges ont été isolées du milieu ambiant par des sacs en plastique noués à leur base, et mises en présence de calcéine dissoute dans l'eau de mer pendant 24 h. Des traitements identiques ont été répétés sur les mêmes spécimens après une période de temps variant d'une semaine à six mois. Des échantillons du squelette ont été récoltés, et des lames minces perpendiculaires à la surface ont été réalisées par polissage. Les bandes d'incorporation de la calcéine ont été photographiées en microscopie à fluorescence. La mesure des intervalles entre les bandes fluorescentes a permis d'établir que la croissance annuelle de ces organismes est inférieure à 0,2 mm.

Les lignes de croissance qui apparaissent à l'oeil nu dans des coupes du squelette de C. nicholsoni ne correspondent pas au dépot annuel d'aragonite. La prudence est suggérée quant à l'interprétation des lignes de croissance d'éponges fossiles à squelette calcaire massif.

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INTRODUCTION

Ceratoporellid sclerosponges secrete a massive basal skeleton of calcium carbonate laid down in the form of aragonite. These sponges co-occur with hermatypic scleractinian corals over much of the range of the latter but are not readily apparent because they dwell in shaded habitats created by the living corals. They are found deep in the rubble of branching corals (Scoffin and Hendry, 1984), in tunnels formed by the roofing over of channels between reef buttresses (Hartman and Goreau, 1970) and in other spaces left behind as the coral framework of the reef grows upward (Dustan and Sacco, 1983). In these light-poor habitats sclerosponges contribute to the reef framework by filling in spaces and thus strengthening the framework. On the reefs that lie off Discovery Bay, ceratoporellid sclerosponges begin to appear at the surface of the reef near the lowest reaches of the fore-reef slope at 50 m and increase in abundance on the deep fore-reef escarpment as hermatypic corals decrease in number and disappear at about 100 m. Ceratoporella nicholsoni (Hickson), the most abundant sclerosponge on North Jamaican reefs, reaches its greatest density between 92 and 100 m and then decreases somewhat to the base of the deep fore-reef, continuing sparsely on the island slope down to depths of about 185 m (Hartman, 1973). On the deep fore-reef escarpment sclerosponges are the major contributors to the calcareous framework (Lang, Hartman and Land, 1975).

In the present work we have studied the calcification rate of Ceratoporella nicholsoni in shaded habitats of Jamaican reefs to compare the rate with that of scleractinian corals, the major framework builders of Recent reefs.

MATERIALS AND METHODS

Specimens of Ceratoporella nicholsoni, 10 to 18 cm in diameter (Fig. 1 and 2), were labeled in situ with the fluorescent dyes, tetracycline or calcein, in a reef tunnel at a depth of 28 m at Pear Tree Bottom, 5 km east of Discovery Bay, Jamaica. Each specimen was enclosed within a plastic bag (with a volume of 4 l) secured around its base with nylon cord or a rubber band. Sufficient fluorescent dye, dissolved in sea water, was injected into each plastic bag to make a final concentration of 25 or 100 mg/l in the case of calcein (Fluka 21030) or 10 or 100 mg/l in the case of tetracycline hydrochloride (Fluka 87130). The bags of stain were removed from the sponges after 12 or 24 hours. The sponges were stained three times: on July 9, 1984; six days later; and on February 15, 1985, 220 days later.

Skeletal samples, with attached tissue, about 1 cm^3 in volume, were removed with hammer and cold chisel from the periphery of the sponge specimen on July 18, 1984, and on February 25, 1985 and were fixed in 50% ethyl alcohol. A sample of one specimen was taken 46 days after the initial staining. Following dehydration in a graded series of alcohols, the samples were embedded in Spurr's medium (Spurr, 1969). Sections, cut perpendicular to the surface and ground to a thickness of 10-15 µm were observed and photographed by fluorescence microscopy (Reichert Univar microscope). Growth of the calcareous skeleton was established by measuring the linear extension in micrometers between two calcein stained lines along growth axes at two sites: (1) the infilling aragonite at the bottom of the lumen of the calicles (Figures 3 to 5) and (2) the apical edges of the wall separating two calicles (Figure 3, 4 and 6).

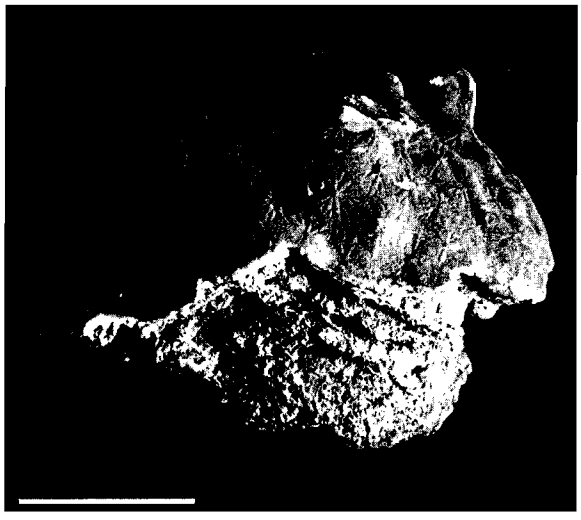

Fig.1.
Lateral view of a dried specimen of Ceratoporella nicholsoni, collected aboard PC-8 submersible off Discovery Bay, Jamaica, at a depth of 117 m. Scale = 5 cm.

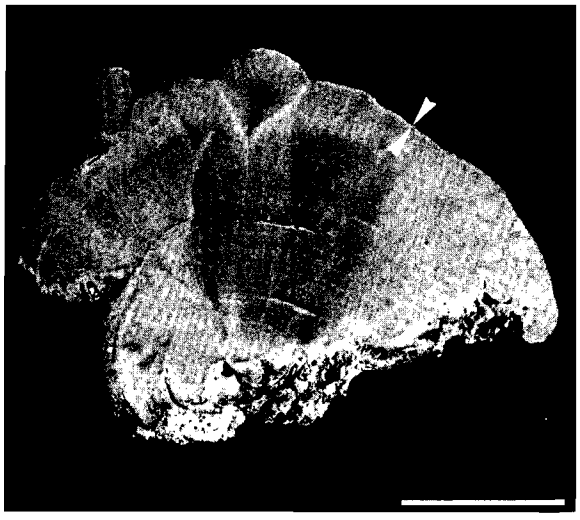

Fig.2.
Section of the same specimen perpendicular to the surface showing the thinness of the living (here dried) superficial tissue (► ◄) compared to the massive skeleton. Note irregularly spaced concentric lines in skeleton. The estimated age of this specimen is 527 ± 50 years. Scale = 5 cm.

RESULTS

No label was detected under fluorescence microscopy in specimens incubated in the presence of tetracycline at either concentration (10 or 100 mg/l) and for either incubation time (12 or 24 hours). The same negative result followed whether the samples were collected 10 days or 220 days after labeling.

In the case of labeling with calcein, distinct fluorescent lines were observed in ground sections with fluorescence microscopy (Figs. 4, 5 and 6) at either final concentration of the dye (25 or 100 mg/l) and after either incubation time (12 or 24 hours). The intensity of the label increased with both concentration and incubation times. The method is sensitive enough to detect growth intervals of six days in the skeleton of Ceratoporella nicholsoni (a distance of ± 3 µm; see Fig.6).

Measurements of the linear extension of the skeleton were based on the longest time intervals (220 days), however, to reduce error.

No adverse effects of either dye on the sclerosponges were noted at the concentrations and times of incubation used.

Table 1 presents mean values of the linear extension of the skeleton of four specimens marked on July 9 and 15, 1984 and again on February 15, 1985, 220 days later. Comparison of the mean values of the linear extension of the skeleton of all four specimens after 220 days shows no significant difference between the measurements made at the outer edge of the calicular walls and in the zone of infilling at the base of the calicles (Mann-Whitney test, $p>0.35$).

TABLE 1

Linear Extension Rate of Skeleton of Ceratoporella nicholsoni

Site of measurement		Outer edge of calicular walls			Infilling zone at base of calicles		
	S	$\bar{X}$	n	SD	$\bar{X}$	n	SD
Linear extension in 220 days (µm)	A	132.21	11	17.13	124.52	9	16.58
	B	104.27	13	19.50	122.57	8	22.21
	C	169.74	15	14.95	88.58	8	18.29
	D	79.10	15	23.91	67.39	14	15.04
Mean linear extension rate		111.05 ± 11.71 µm/220 days.					

S = specimen; $\bar{X}$ = mean linear extension; n = number of measurements; SD = standard deviation

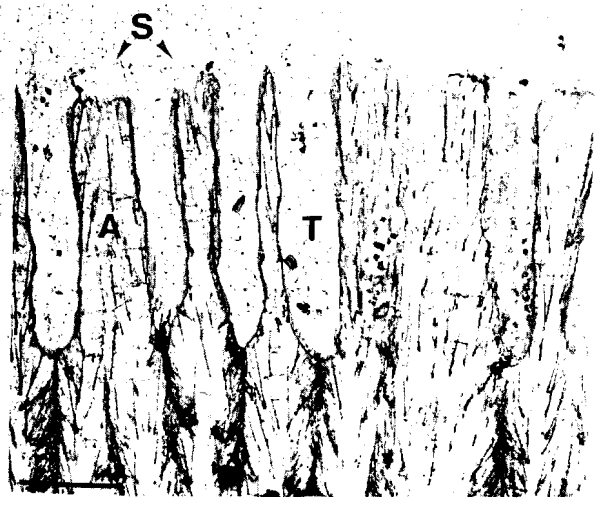

Fig.3.
Ground section of a sample taken at the surface of C. nicholsoni, viewed by phase contrast microscopy and showing the skeleton. A. Aragonitic skeleton. S. Surface of the living tissue. T. Living tissue. Scale = 250 µm.

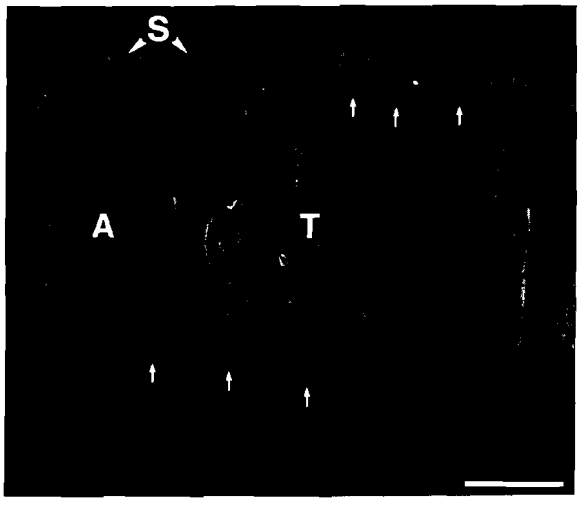

Fig.4.
The same section, viewed by fluorescence microscopy. The living tissue stands out through autofluorescence. Key to letters, same as Fig.3. Scale = 250 µm. Arrows indicate fluorescent lines where calcein has been incorporated into the skeleton.

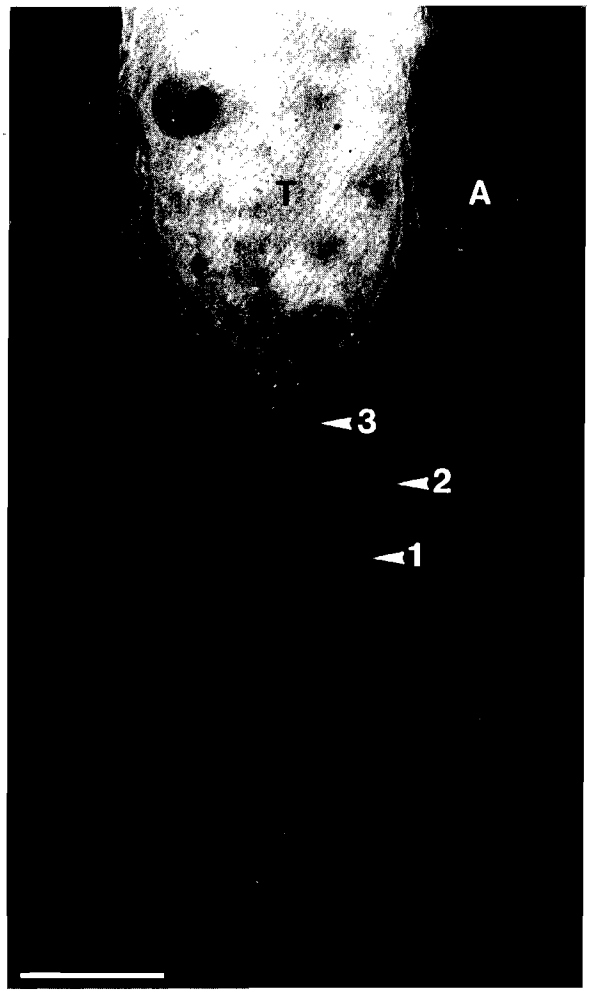

Fig.5.
Zone of infilling at the base of a calicle, viewed by fluorescence microscopy.
A. Aragonitic skeleton. T. Living tissue.
1, 2, 3. Fluorescent lines where calcein has been incorporated into the skeleton. Interval between 1 and 2 = 6 days. Interval between 2 and 3 = 220 days. Scale = 100 μm.

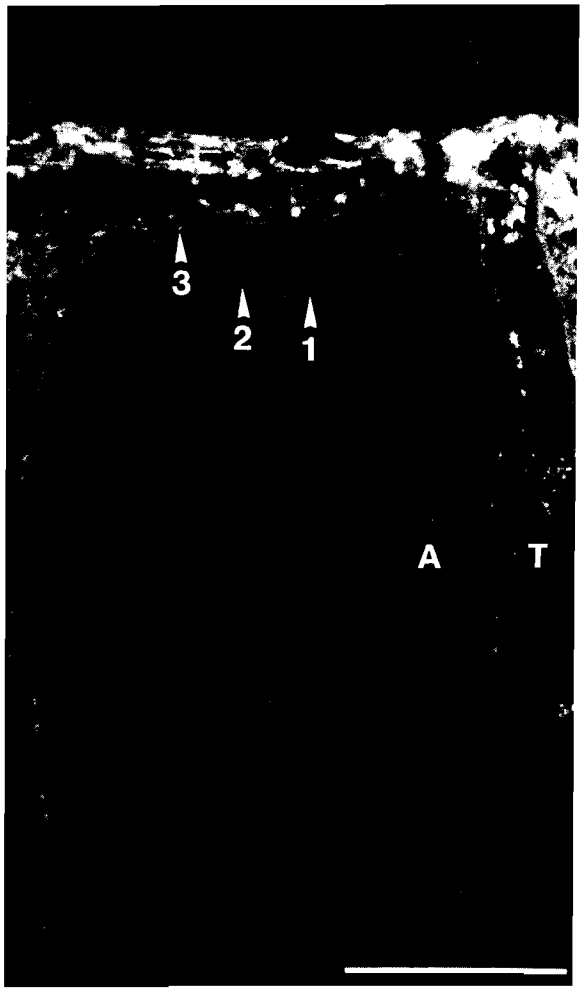

Fig.6.
Outer edge of wall of calicle.
1, 2. Fluorescent lines with 6 day interval.
3. Outer edge of skeleton (unmarked by calcein). Interval between 1 and 2 = 6 days. Interval between 2 and 3 = 46 days. Scale = 100 μm.

DISCUSSION

Tetracycline has been employed successfully in calcification studies of scleractinian corals (Barnes, 1971, 1981), echinoids (Kobayashi and Taki, 1969; Märkel, 1975, 1981; Régis, 1979) and holothuroids (Ebert, 1978). However, it was not incorporated into the calcareous skeleton of Ceratoporella nicholsoni at the concentrations used. In samples incubated in dye twice with a six day interval, no fluorescent band could be detected in the newly deposited skeleton. It is, of course, possible that the bands were obscured by the autofluorescence of the tissue, but Skinner and Nalbandian (1975) note that in human bone the golden ultraviolet-induced fluorescence of the areas labeled by tetracycline is readily distinguished from tissue autofluorescence. Further, if labeling had occurred, one would have expected to detect it in samples collected 220 days after exposure to the fluorochrome, but this was not so. Failure of tetracycline to label the skeleton of Ceratoporella is most likely explained by one of the following: the use of too weak a solution, the slow growth rate of the sclerosponge, the fact that no growth was occurring at the time of staining (see Ebert, 1978), perhaps because of inhibition of mineralization caused by too high a concentration of the dye (Harris, 1960), or fading of the dye during preparation of the specimens. Incubation of the sclerosponge in a solution of tetracycline caused no apparent harm to the organism, although it is not known what effect its antibiotic properties might have had on the intercellular symbiotic bacteria characteristic of this sponge.

Calcein was first synthesized by Diehl and Ellingboe (1956) as an indicator for EDTA titration of calcium in the presence of magnesium. Suzuki and Mathews (1966) first used it as an intravital fluorescent tag in mammalian bones, noting that its green fluorescence contrasts with the yellow fluorescence of tetracycline. Use of both dyes makes it possible to mark layers of bone sequentially. Calcein has since been used

in studies of bone deposition and remodeling (Rahn and Perren, 1970, who used a variant producing a blue fluorescence; Vignery and Baron, 1980). Its use in studies of calcification in marine invertebrates has not been reported previously, to our knowledge.

Extrapolation of the growth rate of C. nicholsoni reported for the experimental period gives a mean annual growth rate of 184.2 ± 19.4 µm for the skeleton. A linear extension of 1 mm will occur in 5 2/5 yrs. Measurements were made in midsummer and midwinter and hence sample the growth rate at both extremes of the sea water temperature range in Jamaica. They do not, however, provide information on possible seasonal variation of calcification rates. The large specimen illustrated in Figures 1 and 2 is 16 cm in diameter and 9.7 cm in height. It is estimated to be 527 ± 50 yrs old according to the growth rate given above.

The very slow growth rate of the calcareous skeleton of Ceratoporella nicholsoni, the most abundant of the Caribbean sclerosponges, is consistent with the hypothesis that the forebears of Recent sclerosponges have been outcompeted as reef builders by the more rapidly calcifying scleractinian corals. While still contributing importantly to the framework of modern reefs, this contribution is now secondary to that of the corals. The open framework of scleractinian reefs, permeated by tunnels, caves and crevices, creates an environment where relict reef-dwellers may persist and still contribute to the reef structure. The significance of the symbiotic relationship between corals and zooxanthellae and its importance in fostering more rapid calcification also creates a depth-limited distribution which allows the slow-growing, zooxanthella-free sclerosponges to reach the peak of their importance on the deep fore-reef environment where hermatypic corals are absent.

Among both hermatypic and ahermatypic scleractinian corals, the rates of skeletal growth greatly exceed those of Ceratoporella. The growth rates of two of the most important scleractinian framework builders on Caribbean reefs as well as a coral that occurs with or without zooxanthellae may be cited as examples. Tunnicliffe (1980) reported a mean linear branch extension of 12.2 cm/yr in Acropora cervicornis (Lam.) at Discovery Bay, Jamaica. In the massive coral Montastrea annularis (Ellis & Sol.) Dustan (1975) reported mean upward extension rates of 1.6 to 6.7 mm/yr depending upon depth at the same location. Dodge (1981) reported mean growth rates of 7.9 to 10.5 mm/yr for M. annularis at Vieques, Puerto Rico. Reed (1981) found that mean annual linear extension of the branches of Oculina varicosa Lesueur was 1.13 cm/yr at 6 m where the coral has dinoflagellate symbionts and was 1.61 cm/yr at 80 m where the coral exists in an aposymbiotic state. The massive shape of the calcareous skeleton of Ceratoporella is similar in form to that of M. annularis, the often mound shaped scleractinian coral cited above. Despite this similarity, the annual extension rate of Ceratoporella is still only about 10 % that of the slowest rates reported for colonies of Montastrea.

It is difficult to know the extent to which the skeletal growth data for Ceratoporella nicholsoni can be used to interpret the growth rates of ancient sponge groups with massive basal calcareous skeletons. Ceratoporella and its related genera have been united in an order Ceratoporellida by Hartman and Goreau (1972) who postulate that the group arose from the Chaetetida (see also West and Clark, 1984).
This group might be expected to be closest to Ceratoporella in growth rate. Lustig (1971) interpreted growth bands in Pennsylvanian Chaetetes spp., each band comprising a light and dark region in the skeleton, as annual accretions and noted that these average 2 mm in thickness. Such a rate of growth compares favorably with the lowest growth rates recorded for Montastrea annularis by Dustan (1975) but is more than 10x faster than the rate for Ceratoporella. Concentric growth bands are evident in calcareous skeletons of Ceratoporella sectioned perpendicular to the surface (Figure 2) but are believed to originate in the organic matrix of the skeleton (Hartman and Goreau, 1970); they seem to mark a greater than annual periodicity. These bands, each comprising a light band of variable width and a narrow dark band, range in total width from 0.4 to 2.4 mm.

In another ancient group of sponges with a massive basal calcareous skeleton, the stromatoporoids, concentric growth bands, called latilaminae (Lecompte, 1956), have been interpreted as evidence of annual environmental cycles (Stearn, 1984). Meyer (1981) has related the latilaminae of several species of Devonian stromatoporoids to the presumed annual growth bands (marked by decreases in dissepiment size) of intergrown specimens of favositids. In this way he has determined the vertical linear growth of these stromatoporoids to vary from 1.3 to 3 mm per year, a rate comparable to that of chaetetids and to the lower rates of the Recent massive scleractinian Montastrea annularis but considerably faster than that of Ceratoporella. The periodicity of the growth bands of past groups of sponges with massive basal calcareous skeletons is still uncertain. We have shown that the annual vertical extension of the calcareous skeleton of the Recent sclerosponge Ceratoporella nicholsoni is much less than the growth bands apparent to the naked eye in sections of the skeleton. This observation should inspire caution in interpreting the periodicity of growth bands in extinct organisms of this nature as representing annual accretions of calcium carbonate.

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