

## Growth and Calcification of the Cyanobacterium *Homoeothrix crustacea*

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The annual radial growth rate of *Homoeothrix crustacea* colonies measured *in situ* was  $1.25 \pm 0.3$  mm (mean  $\pm$  95% confidence limits), with maximal growth in early summer and minimal growth in winter. Two types of calcification were observed. In winter, the filaments were encrusted with small anhedra calcite crystals which occupied about 15% of the water-saturated volume of the colony. In summer, a dense (39–56 vol. %) subsurface layer of calcite was deposited, consisting of larger crystals enclosing filaments of *Homoeothrix*, whilst the upper 60–100  $\mu$ m was essentially free of encrustation. The streamwaters in which the *Homoeothrix* was growing were permanently supersaturated with respect to calcite. The results are compared with the growth rates and mineralization processes found in other cyanobacteria.

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### INTRODUCTION

A small number of cyanobacteria are remarkable for their facility to bind or precipitate calcium carbonate on to their sheaths, thereby forming laminate incrustations which are readily preserved as fossils of considerable geological importance (Pentecost & Riding, 1986). Today, calcified cyanobacteria are frequently found in freshwater environments supersaturated with respect to calcite. Precisely how calcium carbonate is precipitated onto the sheaths of these microbes is uncertain but several proposals have been made (Pentecost, 1988*b*). Few cyanobacteria lend themselves to detailed studies of growth and calcification *in vivo* over prolonged periods. They include *Rivularia haematites*, the 'semiglobosa' form of *Schizothrix calcicola*, and *Homoeothrix crustacea*. The growth of *Rivularia* has already been investigated (Pentecost, 1987) but there is little information on other species. *Homoeothrix* often forms hemispherical stromatolitic cushions up to 5 mm in diameter, and previous studies suggest that *Homoeothrix* calcification differs from that of other cyanobacteria (Pentecost, 1988*a*). *H. crustacea* is widely distributed in calcareous waters and the growth dynamics of this species have not been investigated. In this paper, measurements of growth and calcification rates of a *Homoeothrix* species are presented for the first time, together with information on streamwater geochemistry.

### METHODS

*Growth measurements.* Observations were made in Waterfall Beck near Arncliffe, North Yorkshire, UK, at a site 150 m from its confluence with Cowside Beck (National Grid reference 34/908698), altitude 320 m.

Isolated hemispherical colonies of *H. crustacea* Woronichin were measured moist under a binocular microscope fitted with an eyepiece graticule as previously described (Pentecost, 1987). Initial studies showed that growth rate was independent of colony diameter in the size range 1–2.5 mm ( $P < 0.05$ ). A minimum of 30 colonies in this size range were measured at regular intervals over a period of one year during 1987.

*Calcification.* Colonies were air-dried, impregnated with Araldite resin, and 15  $\mu$ m sections prepared for petrological examination. Other colonies were decalcified using 5% (w/v) EDTA pH 8, hand-sectioned and examined by light microscopy. For electron microscopy, colonies were dehydrated in ethanol series, critical-point dried with CO<sub>2</sub>, coated with Au/Pd and examined in a Philips 501B scanning electron microscope.

To obtain the CaCO<sub>3</sub> content, colonies were carefully removed, dried at 105 °C to constant weight, weighed on a five-figure balance then transferred to a small flask from which most of the air was removed with a hand-operated vacuum pump. The colonies were soaked in the flask with air-free, CaCO<sub>3</sub>-saturated water for 15 min, rapidly blotted and reweighed. Colonies were then decalcified with 0.1 M-HCl, washed twice, dried at 105 °C and weighed. Finally, the residue was ignited in a crucible at 500 °C and reweighed, allowing the CaCO<sub>3</sub> content, organic matter and residue to be determined as a fraction of the wet weight. The experimental error for the CaCO<sub>3</sub> content was ± 0.9%. Some colonies were dissected to determine the variation of calcification over time.

*Water chemistry.* Duplicate water samples were collected at the sites and water and air temperature recorded with a thermistor. Samples were returned to a nearby laboratory and analysed for calcium by complexometric titration, and for total CO<sub>2</sub> using Gran plots (Mackereth *et al.*, 1978). pH was determined with a calibrated Corning 120 glass electrode at the temperature of collection and the calcite saturation obtained from the analytical concentrations of calcium, total CO<sub>2</sub> and pH using a BASIC computer program incorporating the most recently available equilibrium constants for the freshwater carbonate system. Details of the methodology are given by Truesdell & Jones (1978). The saturometric method of Stenner (1969) was also used for comparison.

Stream discharge and current speed were estimated over an even cross-section of the beck using an Ott flow-rate meter; a small correction for turbulent flow was included (Morisawa, 1968).

Mean weekly air temperatures were obtained from Malham Tarn meteorological station, situated 3 km SW at 380 m altitude. A small correction was made for altitude using an adiabatic lapse rate of 8 °C km<sup>-1</sup>, enabling water temperatures to be estimated at the site. Since *in situ* air and water temperature were significantly correlated ( $r = +0.882$ ,  $P < 0.01$ ), stream temperature could be predicted throughout the year using a linear regression equation (Pentecost, 1987), viz.  $Y = 0.628 X + 3.057$ , where  $Y$  is streamwater temperature and  $X$  is corrected air temperature (°C).

## RESULTS AND DISCUSSION

Species of *Homoeothrix* have been reported from many countries and they are widely distributed in calcareous waters (Starmach, 1966a; Kawecka, 1971; Marker, 1976; Sinclair & Whitton, 1977; Pentecost, 1988a). At least four freshwater species have been observed encrusted with calcium carbonate: *H. gracilis* (Hansg.) Komárek & Kovačik, *H. borneti* (Sauv.) Babilie, *H. poljanskii* Muzafurov and *H. crustacea*. These species, along with several uncalcified forms, have been described by Geitler (1932), Starmach (1966b), Komárek & Kann (1973) and Komárek & Kovačik (1987), although the characters used to delimit the species (sheath colour, trichome dimensions) are open to doubt considering their known variability in other cyanobacteria. None of these species exactly matched the material studied here, which had exceptionally narrow trichomes, but the organism most closely resembled descriptions of *H. crustacea*. Another cyanobacterium, *Schizothrix calcicola* f. *semiglobosa*, can be confused with *Homoeothrix*; this organism was present in the stream but was normally confined to deposits of tufa. The trichomes of the Waterfall Beck *Homoeothrix* measured 0.4–1.2 µm in diameter and were found normally in the apical portions of the sheaths. The sheaths were 3–6 µm in diameter, hyaline or pigmented and often fibrous and distally frayed. They exhibited strong form-birefringence under the polarizing microscope. The organism formed hemispherical colonies up to 4 mm wide which sometimes coalesced to form a continuous crust. Within colonies, most of the trichomes were found at or near the surface, in a layer approximately 200 µm thick. During winter, the colony surface was colonized by diatoms which became intermixed with the trichomes. This layer was superficial and did not add significantly to the colony diameter. In older colonies, the innermost areas became colonized by two other cyanobacteria, *Phormidium* cf. *incrustatum* and *Schizothrix calcicola*.

Colonies were firmly attached to rocks and stones in the stream and were never observed exposed to the air. They occurred most abundantly in fully exposed areas subject to flow rates exceeding 0.5 m s<sup>-1</sup>. *Homoeothrix* was only present in parts of the stream where there was calcite precipitation, and it was sometimes associated with *Phormidium incrustatum* and the green alga *Gongrosira incrustans*. A tube-dwelling caddis, *Tinodes*, was frequently observed among the colonies and sometimes destroyed their inner parts (Fig. 1).

Radial growth rates of colonies were variable, even for those colonizing the same stone. The mean growth rate peaked during early summer when water temperature and light intensity were increasing (Fig. 2). The confidence limits attached to this mean overlap with those obtained in

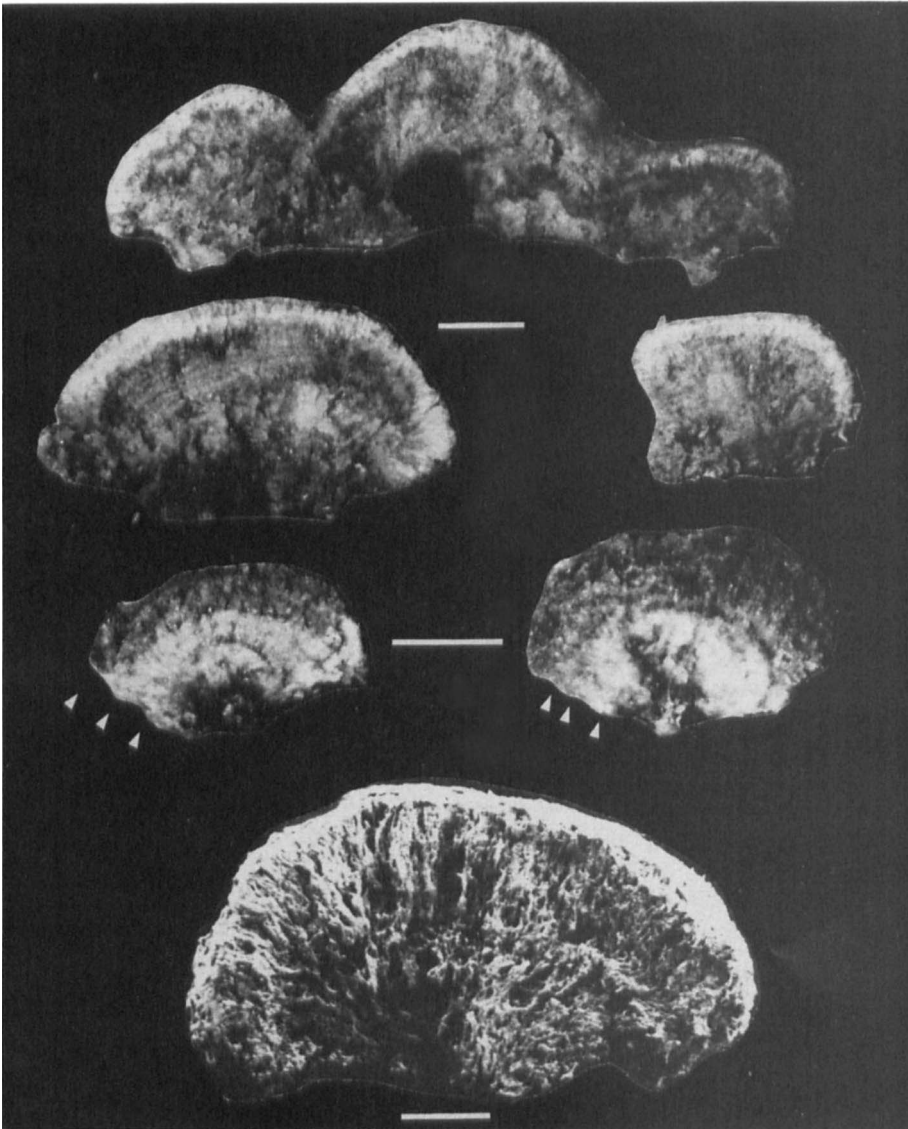


Fig. 1. Sections through colonies of *Homoeothrix* from Waterfall Beck. Top row: three coalesced colonies, the centre colony bored by *Tinodes*, 14 August 87. Note surface calcification. Second row: two further colonies collected on 14 August 87 showing surface calcified layer 0.2–0.4 mm thick and part of a lower pale zone formed in the previous summer. The winter layers have a series of narrow calcified bands. Bar, 10 mm. Third row: measured colonies removed on 21 December 87, showing a dark surface layer and a lower pale summer zone. Arrows mark colony size; from top to bottom the dates are 17 October, 14 August and 19 June 1987. Bar, 10 mm. Fourth row: scanning electron micrograph of a section through a colony collected on 19 June 87, showing the radiating filamentous structure. Critical-point-dried material. Bar, 5 mm.

midsummer and autumn but the radial growth rate was significantly lower during winter. The mean radial growth rate ranged from 2.02 to 4.9  $\mu\text{m d}^{-1}$ , giving an annual radial growth of 1.25 mm (Table 1). Despite the high summer growth, the correlation obtained between growth rates and stream water temperature was not very significant ( $r = +0.578$ ,  $P < 0.25$ ).

This low correlation contrasted with the significant results found in an associated cyanobacterium, *Rivularia haematites* (Pentecost, 1987). The annual radial growth of

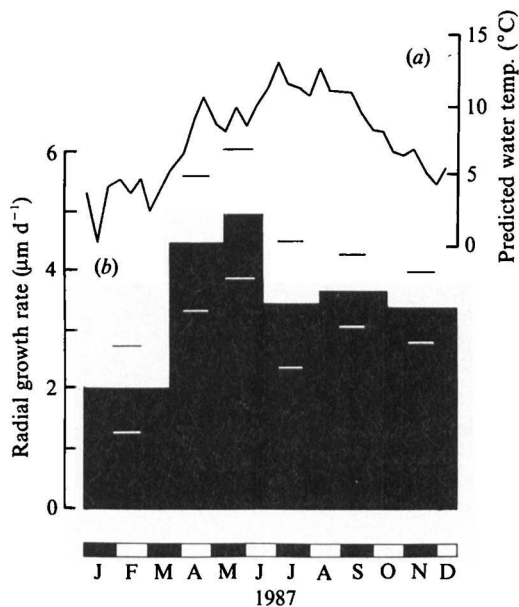


Fig. 2. (a) Predicted 10 d mean water temperature of Waterfall Beck, 1987. (b) Radial growth rate of *Homoeothrix* over a one-year period; 95% confidence limits to the mean are indicated by horizontal lines.

Table 1. Water chemistry (a), *Homoeothrix* growth rate (b) and calcification (c): means and ranges

	Mean	Range	Date/period	No. of measurements
(a) Water temp. (°C)	7.55	0.0–16.4	1987	354
Discharge* (l s <sup>-1</sup> )	51	5–91		7
pH*	8.29	7.95–8.47		14
Total CO <sub>2</sub> * (mM)	2.75	2.13–3.59		14
Calcium* (mM)	1.43	1.16–1.78		14
(b) <i>Homoeothrix</i> radial growth rate ± confidence limits (µm d <sup>-1</sup> )	3.42 ± 0.82			7
(mm year <sup>-1</sup> )	1.25 ± 0.30			7
Colony diameter (mm)	2.17	0.49–4.25	Aug. '87	50
(c) CaCO <sub>3</sub> content of water-saturated colonies (vol. %)	25.6	22.5–27.9	Aug. '87	10
(wt %)	48.0	43.6–51.2	Aug. '87	10
Organic matter (wt %)	8.2	6.1–9.7	Aug. '87	10
Inorganic residue (wt %)	0.73	0.57–1.10	Aug. '87	10

\* Time-weighted means.

*Homoeothrix* is marginally higher than *Rivularia* in this stream and is substantially higher than that for *Calothrix* measured at a nearby site (Pentecost, 1988a). These rates of growth indicate that, on average, only one or two new cells are produced per trichome per day. This might result from the low mean water temperature (7 °C). Nutrient depletion is not suspected, as bryophyte growth rates in the stream were substantial.

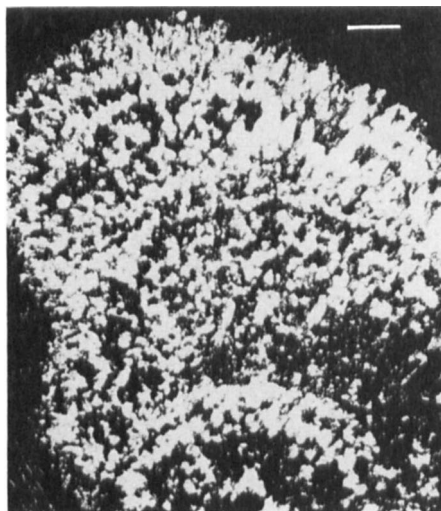


Fig. 3. Vertical section through a colony edge, viewed in cross-polarized light showing bands of microspar and micrite. Note the periodic banding in the outer calcified zone, which is 120–180  $\mu\text{m}$  thick, and a conspicuous double band below, probably deposited during spring. Sampled 14 August 87. Bar, 50  $\mu\text{m}$ .

Since the colonies attained a mean diameter of 2.2 mm (maximum about 4 mm) (Table 1), few could have survived for more than a year. This must be a consequence of the unstable habitat, characterized by high flow rates and compounded by the destructive activities of *Tinodes*.

The colony surface changed markedly as the season progressed. With the increase in growth during late spring, the originally farinose carbonate incrustation was replaced by a thin uncalcified network of *Homoeothrix* filaments. This consisted of a series of reticulate ridges 30–60  $\mu\text{m}$  high and 10–50  $\mu\text{m}$  wide at their apices. During September the farinose surface reappeared, with irregular crystals of calcite deposited upon and among the filaments.

All colonies examined were internally heavily encrusted with calcite although the degree of encrustation varied with the season. During May–August a pale, heavily calcified layer 0.2–0.5 mm in thickness was evident close to the colony surface (Fig. 1, rows 1–2). More detailed examination showed that this layer often consisted of a series of dense calcified bands (Fig. 3). The deposit in this layer consisted of microspar with irregular crystal faces and a tendency for the crystals to be elongated parallel to the *Homoeothrix* filaments. Many crystals were 50–100  $\mu\text{m}$  in length, often enclosing large numbers of filaments. Bands of smaller crystals, 5–20  $\mu\text{m}$  in diameter, were also occasional in this zone (Fig. 3). At the colony surface, calcification was much less in evidence and the surface 60–100  $\mu\text{m}$  was almost free of encrustation, with occasional anhedral crystals present in the hollows between the network ridges and a thin veneer along the ridges themselves. This dense summer layer could be discerned in material collected later in the year (Fig. 1, 3rd row) and contained 39–56 vol.% calcite. The calcification corresponded to areas with low sheath pigmentation.

During the winter, the deposits occupied 13–16% of the colony volume, indicating a less intense period of calcification. The crystals were smaller, 5–20  $\mu\text{m}$  in diameter, randomly orientated and were seen enclosing filaments less frequently. Narrow bands of more intense calcification were occasionally observed in the layer deposited during this period (Fig. 1, 2nd row). The average calcium carbonate content of colonies is given in Table 1, together with the organic matter content and the acid-insoluble residue. *Homoeothrix* occupied about 15% of the stream bed and precipitated approximately 100 kg of calcite in Waterfall Beck in the course of a year.

The formation of a dense summer band of calcite suggests a change in conditions within, or just below, the zone of active growth. During this season, deposition appears most intense in a region 70–500  $\mu\text{m}$  below the surface, and in the upper part of this layer, trichomes are abundant within the sheaths. Since photosynthesis causes alkalization of the area adjacent to the trichome, calcification may be subsequently enhanced (Pentecost, 1988*b*) but it is difficult to

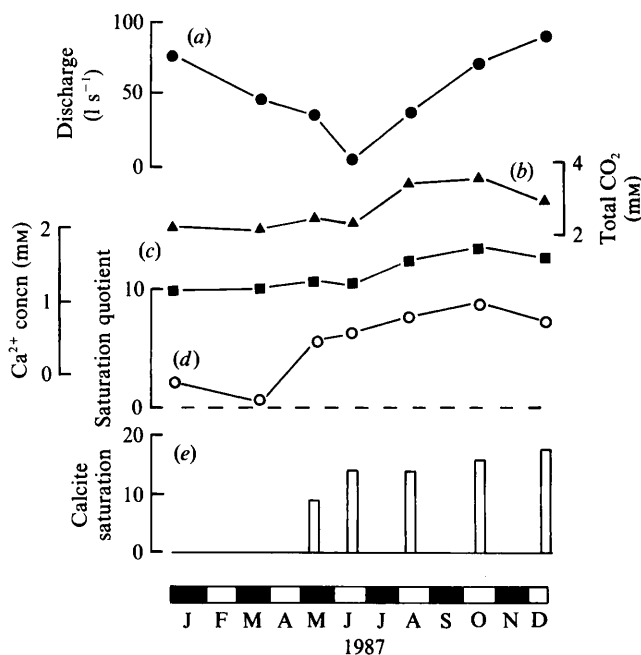


Fig. 4. (a) Waterfall Beck discharge (●). (b) Total CO<sub>2</sub> (▲). (c) Calcium concentration (■). (d) Calcite saturation quotient,  $\Omega$  (○). (e) Calcite saturation expressed as the quantity of CaCO<sub>3</sub> (mg l<sup>-1</sup>) in excess of that in water at equilibrium with pure calcite at streamwater temperature (no data available for the first two samplings).

explain why the surface layer remains poorly mineralized. Because the amount of carbon fixed in these microstromatolites is small in comparison to the amount of calcite present (Table 1) it is doubtful if the amount of calcite formed is stoichiometrically equivalent to the amount of carbon dioxide fixed by photosynthesis. It is more likely that photosynthesis raises the carbonate ion activity sufficiently to nucleate calcite crystals which subsequently grow by the addition of ions diffusing into colonies from the overlying supersaturated water.

Growth and calcification of oncolites colonized by *Calothrix* in a nearby stream was also maximal during summer, though at a much lower rate (Pentecost, 1988a). In *Rivularia*, narrow bands of calcification were noted during summer and attributed in part to photosynthetic activity, whilst during winter, a dense deposit of calcite was formed abiogenically at the colony surface (Pentecost, 1987). Thus these genera, whilst closely related, show a distinctly different rate of growth and pattern of calcification.

It is tempting to attribute the banding patterns of calcification and sheath pigmentation in *Homoeothrix* and *Rivularia* to a common factor. On the basis of the available evidence, abiogenic calcification appears associated with zones of pigmented sheaths, and photosynthetically induced deposits associated with hyaline zones. This may indicate that sheath pigmentation is related to periods of slow growth.

Waterfall Beck is a permanent limestone stream with a pronounced minimum discharge during the summer (Fig. 4). The major ions present in the water were Ca<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup>. Calcium and total CO<sub>2</sub> concentrations varied with the season, reaching a maximum in the autumn (Fig. 4). The pH was close to 8 at all times and the water was always supersaturated with respect to calcite at the growth-measurement site. Values of the saturation quotient,  $\Omega$ , ranged from 1.37 to 9.36, with the highest supersaturation in autumn and the least during winter and spring (Fig. 4). The amount of precipitable calcite ranged from 9 to 18 mg l<sup>-1</sup> and tended to support the results obtained from the equilibrium calculations.

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