The role of microbes in accretion, lamination and early lithification of modern marine stromatolites

R. P. Reid[*], P. T. Visscher[†], A. W. Decho[‡], J. F. Stolz[§], B. M. Bebout[‖], C. Dupraz[*], I. G. Macintyre[¶], H. W. Paerl[#], J. L. Pinckney[**], L. Prufert-Bebout[‖], T. F. Steppe[#] & D. J. DesMarais[‖]

[*] *MGG-RSMAS, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149, USA*
[†] *Department of Marine Sciences, University of Connecticut, Groton, Connecticut 06340, USA*
[‡] *School of Public Health, University of South Carolina, Columbia, South Carolina 29208, USA*
[§] *Department of Biological Sciences, Duquesne University, Pittsburgh, Pennsylvania 15282, USA*
[‖] *NASA Ames Research Centre, Moffett Field, California 94035, USA*
[¶] *National Museum of Natural History, Smithsonian Institution, Washington DC 20560, USA*
[#] *Institute of Marine Sciences, University of North Carolina, Morehead City, North Carolina 28557, USA*
[**] *Department of Oceanography, Texas A&M University, College Station, Texas 77843, USA*

For three billion years, before the Cambrian diversification of life, laminated carbonate build-ups called stromatolites were widespread in shallow marine seas[1,2]. These ancient structures are generally thought to be microbial in origin and potentially preserve evidence of the Earth's earliest biosphere[1–3]. Despite their evolutionary significance, little is known about stromatolite formation, especially the relative roles of microbial and environmental factors in stromatolite accretion[1,3]. Here we show that growth of modern marine stromatolites represents a dynamic balance between sedimentation and intermittent lithification of cyanobacterial mats. Periods of rapid sediment accretion, during which stromatolite surfaces are dominated by pioneer communities of gliding filamentous cyanobacteria, alternate with hiatal intervals. These discontinuities in sedimentation are characterized by development of surface films of exopolymer and subsequent heterotrophic bacterial decomposition, forming thin crusts of microcrystalline carbonate. During prolonged hiatal periods, climax communities develop, which include endolithic coccoid cyanobacteria. These coccoids modify the sediment, forming thicker lithified laminae. Preservation of lithified layers at depth creates millimetre-scale lamination. This simple model of modern marine stromatolite growth may be applicable to ancient stromatolites.

The only known examples of stromatolites presently forming in open marine environments of normal seawater salinity are on the margins of Exuma Sound, Bahamas[4–6]. Our study focused on well-laminated build-ups at Highborne Cay (76° 49′ W, 24° 43′ N) as potential analogues of ancient stromatolites extending back to the Precambrian. Highborne Cay stromatolites form in the back reef zone of an algal-ridge fringing reef complex that extends 2.5 km along the eastern shore of the island, facing Exuma Sound[7]. Surface waters have a salinity of 36–37 parts per thousand and are saturated with respect to both aragonite and calcite. Stromatolites form as intertidal and subtidal build-ups, shoreward of the algal ridge. Results reported here pertain to the subtidal stromatolites, which grow in depths of less than 1 m at mean low tide and form ridges and columnar heads up to half a metre high (Fig. 1).

Surfaces of Highborne Cay stromatolites are covered with cyanobacterial mats. Examination of these mats using a variety of integrated geological and microbiological techniques reveals variations in microbial community structure and composition. Extensive field sampling over a two-year period revealed three mat types, representing a continuum of growth stages with minimal seasonal variability (Fig. 2).

Type 1: About 70% of all mats examined consist of a sparse population of the filamentous cyanobacterium *Schizothrix* sp.[8]. *Schizothrix* filaments are generally vertically orientated and are entwined around carbonate sand grains (Fig. 2a and b).

Type 2: Approximately 15% of mats show development of calcified biofilms, which appear as thin crusts of microcrystalline carbonate (micrite) at the uppermost surface of the mat (Fig. 2c and d). These films are about 20–60 μm thick; they drape over and bridge interstitial spaces between sand grains. Silt-sized carbonate particles, such as tunicate spicules, are commonly embedded in the films. Cyanobacterial filaments are present, but are not abundant in the biofilms, which are comprised mainly of copious amounts of amorphous exopolymer, metabolically diverse heterotrophic microorganisms[9–11] and aragonite needles. Needle-shaped aragonite crystals, approximately 1 μm in length, form spherical aggregates 2–5 μm in diameter and are embedded in the exopolymer matrix (Fig. 2e). Bacteria are abundant and are commonly observed at the edges of the aragonite spherules (Fig. 3). A sparse to moderately dense population of *Schizothrix* underlies the exopolymer biofilm (Fig. 2c).

Type 3: The remaining 15% of mats are characterized by an abundant population of the coccoid cyanobacterium *Solentia* sp. and randomly-orientated *Schizothrix* filaments below a calcified biofilm (Fig. 2f and g). *Solentia* is an endolith, which bores into carbonate sand grains. These bored grains appear grey when viewed in plane polarized light in a petrographic microscope (Fig. 2f), contrasting with the golden-brown colour of unbored grains (Figs 2a and c). The microbored grains are often fused at point contacts and appear 'welded' together (Fig. 2f and g).

The variations in surface mats described above represent changes in microbial community structure and activity in response to intermittent sedimentation. Type 1 mats, characterized by a sparse population of *Schizothrix* filaments, resemble pioneer communities[12], which dominate during periods of sediment accretion. Formation of these mats during intervals of rapid sedimentation is documented by field observations showing that accretion rates of one grain-layer per day produce mats with Type 1 fabrics. The activities of *Schizothrix*, in particular, photosynthetic production of exopolymer, are crucial in the accretion process. Flume studies show that sand grains, which settle from suspension when flow rate is low, adhere to mucous-like exopolymer (B.M.B., unpublished video recordings). These 'trapped' grains are subsequently bound by filaments and exopolymer as *Schizothrix* moves upward to the sediment surface. Populations of diatoms and other eukaryotes are minor to absent in these accreting mats[8,13,14] indicating that, contrary to previous reports[15,16], eukaryotic organisms are not required for the trapping and binding of coarse-grained sediment. Aragonite precipitation is inhibited during this stage through

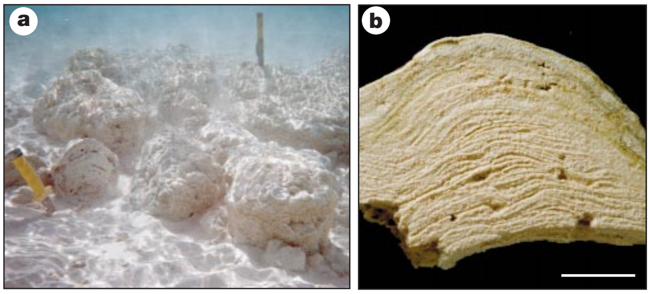

Figure 1 Shallow subtidal stromatolites, Highborne Cay, Bahamas. **a**, Extensive columnar build-ups. **b**, Vertical section showing lamination; scale bar, 2 cm.

calcium ion binding by exopolymer and low-molecular-weight organic acids excreted by *Schizothrix*[11].

Type 2 mats represent a more mature[12,17] surface community characterized by development of a continuous surface film of exopolymer. This mat type develops during quiescent periods when sedimentation ceases and mats begin to lithify. Formation during calm periods is indicated by carbonate silt, such as tunicate spicules, which is commonly entrapped in the surface films but is characteristically lacking in Type 1 mats. Mesocosm manipulations suggest that continuous surface biofilms form in a matter of days. These surface films support heterotrophic activity of both aerobic and anaerobic bacteria[9,11], which metabolize the low-molecular-weight organic compounds and the labile fraction of the amorphous exopolymer[10,11]. Sulphate reduction takes place despite the presence of oxygen at the surface and sulphate-reducing bacteria account for a significant fraction (30–40%) of the organic carbon consumption by the community[9,10]. This bacterial activity promotes aragonite precipitation as evidenced by microscale observations that high rates of sulphate reduction coincide with micritic crusts[18]. In addition, microautoradiography of radiolabelled organic matter shows a close association between bacteria and aragonite needles (H.W.P., unpublished data). The net result of these processes is calcification of the biofilm and formation of a thin micritic crust. When additional carbonate sand is accreted onto the stromatolite, this surface-coating film persists into the subsurface as a nearly continuous thin sheet of micritic cement.

Longer hiatal periods allow formation of Type 3 mats, which are more fully developed than Type 2 mats and include an abundant population of the coccoid cyanobacterium *Solentia* sp. These *Solentia*-rich mats represent the 'climax' community of the stromatolite system (Fig. 2). Excretion products of *Solentia* and *Schizothrix* support high rates of bacterial respiration[10,12]. Microscopy and culture experiments have revealed an unusual process of boring and infilling associated with *Solentia*[19,20]. Boreholes are filled in with aragonite as *Solentia* advances[19,20]. Moreover, as *Solentia* crosses between grains at point contacts, infilling of the microbored tunnels obliterates grain boundaries and grains become fused together (Fig. 2g). Observations of organic matter in some

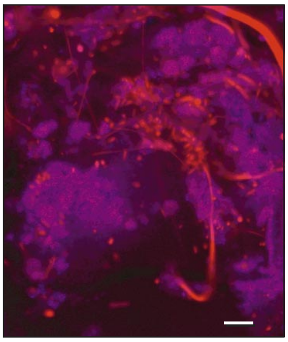

Figure 3 Scanning laser confocal microscope image of a surface biofilm. Bacteria (red fluorescence) are abundant and show an intimate association with carbonate precipitates (blue autofluorescence). Large, uncalcified filament in upper left is *Schizothrix* sp. Sample is stained with propidium iodide; scale bar, 5 µm.

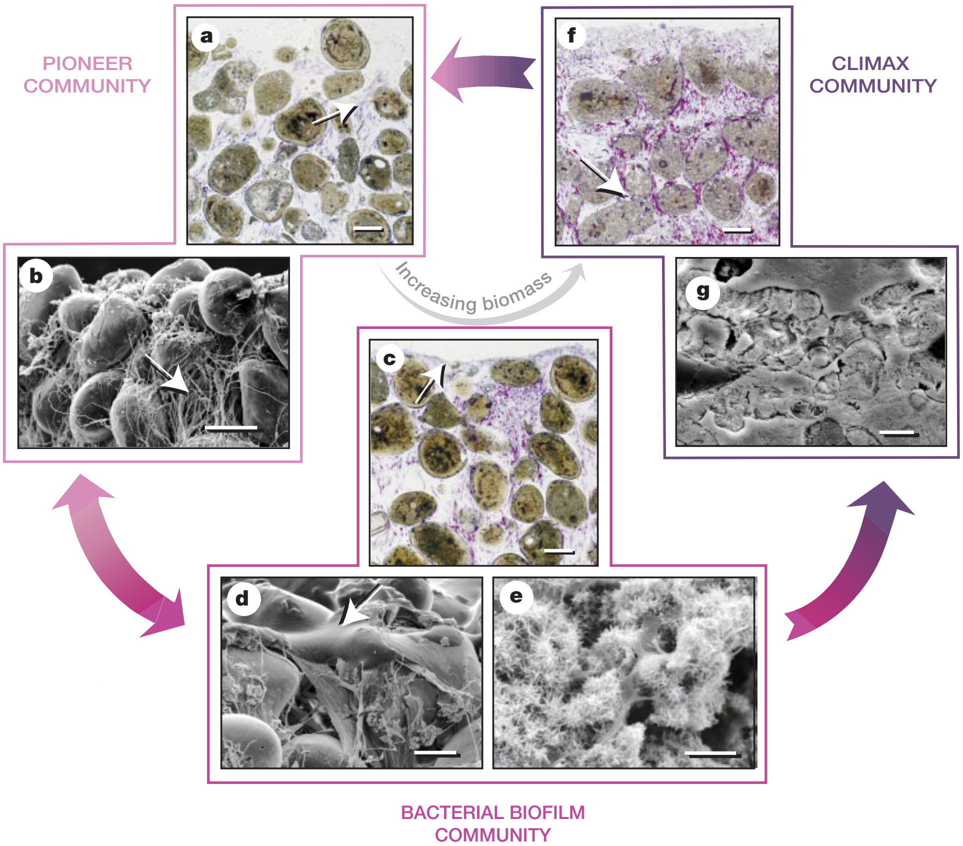

Figure 2 Dominant prokaryotic communities on stromatolite surfaces. Cycling between communities, indicated by large arrows, is a response to intermittent sedimentation (see text). **a**, **b**, Pioneer community: filamentous cyanobacteria (arrows) bind carbonate sand grains. **c–e**, Bacterial biofilm community: a continuous sheet of amorphous exopolymer (arrows, **c**, **d**) with abundant heterotrophic bacteria (Fig. 3) forms uppermost surface; aragonite needles precipitate within this surface film (**e**). **f**, **g**, Climax community: a surface biofilm overlies filamentous cyanobacteria and endolith-infested grains, which appear grey and are fused (arrow, **f**). Precipitation in tunnels that cross between grains leads to welding (**g**). **a**, **c**, **f**, Petrographic thin sections, plane polarized light; cyanobacteria are stained with methylene blue. **b**, **d**, **e**, **g**, Scanning electron microscope images. Scale bars: **a**, **b**, **c**, **f**, 100 µm; **d**, 50 µm; **e**, 5 µm; **g**, 10 µm.

boreholes, together with high sulphate reduction activity in these layers[18] indicates that, as in Type 2 mats, heterotrophic activity may be important in the precipitation process. In contrast to the conventional view that microboring is principally a destructive process[8,21], the microboring and infilling processes associated with *Solentia* activity in these mats is a constructive process. This process fuses grains together to create laterally cohesive carbonate crusts. These crusts persist into the subsurface and provide structural support for the growth and long-term preservation of the stromatolite. Field and laboratory studies show that layers of fused microbored grains are formed in periods of weeks to months[19]. As *Solentia* is a photosynthetic microorganism, such prolonged periods of microboring activity can only be sustained when this population remains at the surface during long hiatal periods. Even longer hiatal periods result in a community succession to eukaryotic algal communities, which do not form laminated structures[8,14].

Laminations in the fossilized part of the stromatolites represent a chronology of former surface mats (Fig. 4). Stromatolite laminae are most easily observed on water-washed, cut surfaces where lithified layers stand out in relief (Fig. 4a). Although lamination is readily apparent in hand samples, it has a subtle expression in petrographic thin sections. Detailed microstructural analyses show, however, that the lithified layers have two distinct petrographic appearances (Fig. 4b). These laminae correspond to (1) thin crusts of micrite, 10–60 μm thick (blue lines in Fig. 4b and c), and (2) layers of fused, microbored grains infested with *Solentia* sp.; these layers are 1–2 mm thick (orange lines in Fig. 4b and d) and underlie micritic crusts. Light microscopy combined with scanning electron microscopy shows that the thin crusts are identical in thickness, composition and texture to the calcified biofilms described above; they are also similar in thickness to micritic laminae in many ancient stromatolites[3,22]. In addition, microstructural features of the layers of fused, microbored grains are identical to those formed by the climax community described above.

Lithified layers, which represent former surfaces of mats, show a millimetre-scale distribution. This is indicated by petrographic analyses of the upper several centimetres of 37 stromatolites containing 453 micritic crusts and 174 microbored layers. The micritic crusts form at intervals averaging 1–2 mm. Distances between tops of layers of fused, microbored grains show two modes, one at 2–3 mm and a second at 4–5 mm. Typical marine cements, such as acicular fringes of aragonite, are notably absent during these early stages of growth. Thus, the lithified mats provide initial structural support for the development of laminated build-ups with topographic relief.

To our knowledge, this is the first study to define a specific set of mechanisms that link lamination in marine stromatolites to a dynamic balance between sedimentation, a succession of prokaryotic communities and early lithification. Integration of detailed geological and microbiological analyses of stromatolites in a modern marine system has shown that the structure and composition of surface mats alter in response to intermittent sedimentation and that mats lithify during hiatal periods. Lithification depends on two fundamentally important microbial processes: photosynthetic production by cyanobacteria and heterotrophic respiration by bacteria. A laminated microstructure is formed by precipitation of laterally continuous sheets of micrite in surface biofilms, which are formed during frequent discontinuities in sedimentation. In some

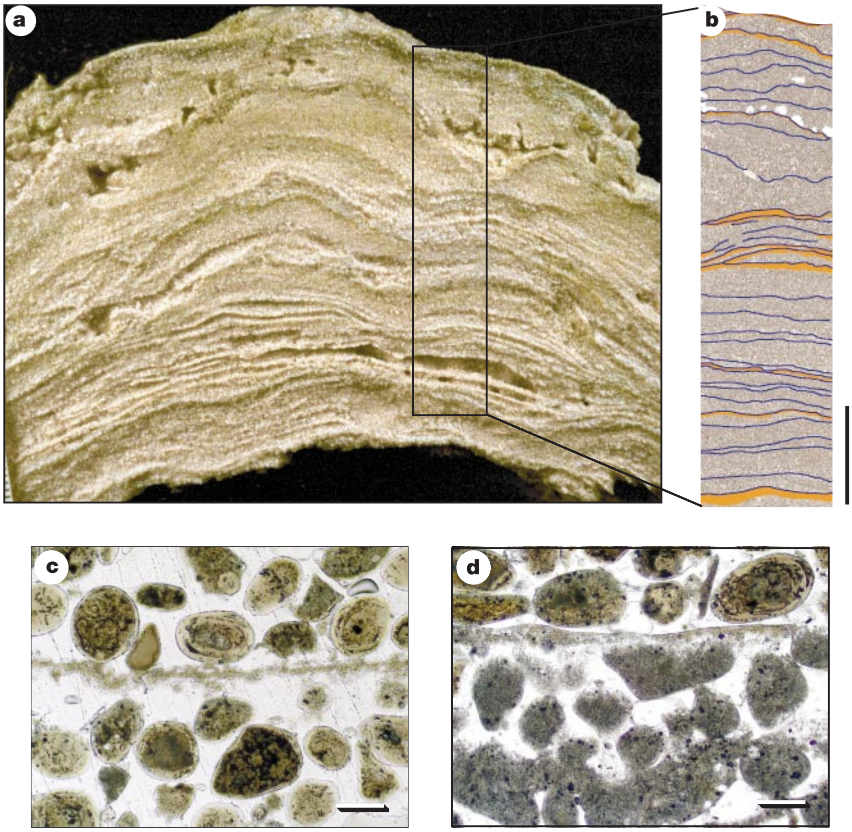

Figure 4 Lamination and microstructure in stromatolite subsurface. Lithified layers representing former surface mats form at 1–2 mm intervals. **a**, Water-washed vertical section showing lithified laminae, which stand out in relief. **b**, Low magnification thin-section photomicrograph of boxed area in **a** showing the distribution of lithified layers. Blue lines represent micritic crusts (**c**); orange lines represent welded, micritized grains (**d**). **c**, Thin-section photomicrograph of a micritic crust; these crusts represent calcified biofilms (Type 2 mats). **d**, Thin-section photomicrograph of a layer of microbored, fused grains, which underlie a micritic crust; these layers represent former climax communities (Type 3 mats). **c**, **d**, Plane polarized light. Scale bars: **b**, 10 mm; **c**, **d**, 100 μm.

cases, thicker layers of fused grains form below these biofilms in response to microboring activities and precipitation, probably resulting from polymer degradation in boreholes.

These findings provide insight into the role of microbes in stromatolite accretion, lamination and lithification. Although most researchers agree that, "microbial mats and their associated sediments must be lithified early in order to be preserved in the record as stromatolites"[1], the proposed mechanisms and precise timing of early lithification have been "vigorously debated"[1]. Historically, early lithification was attributed to abiotic processes of submarine cementation[23,5] or to calcification of cyanobacterial sheaths[24] related to photosynthetic activity. More recently, attention has shifted to heterotrophic bacterial decomposition of cyanobacterial sheaths in subsurface, aphotic zones[25,26]. Although field studies have documented bacterial precipitation of micrite on the sheaths of dead cyanobacteria in the subsurface of laminated microbial mats in tidal flats[25,27], these mats do not form fully lithified laminae and stromatolitic build-ups. We argue that growth of laminated microbial structures with topographic relief, such as those that dominated the fossil record for three billion years, depends on penecontemporaneous lithification of surface mats. This lithification process occurs by decomposition of an amorphous matrix of bacterial exopolymer (not sheath material) in the photic zone across the stromatolite surface. Similar processes of precipitation within the amorphous exopolymer matrix of biofilms, rather than on cyanobacterial sheaths, offer an additional mechanism to account for the paucity of preserved microfossils in Precambrian stromatolites, which is typically ascribed to recrystallization and/or rapid degradation of sheaths[1,26,28]. The potential role that climax microbial communities, functionally equivalent to the endolithic coccoid cyanobacterial communities in modern marine stromatolites, may have played in the growth and lithification of ancient stromatolites remains to be evaluated. □

Methods

This study combined a range of geological, microbial and chemical analyses. An extensive field program was conducted during January and June 1997, and March and August 1998. Physicochemical indices of stromatolite mats were determined *in situ*, primarily with O_2, sulphide, pH needle electrodes (0.8 mm outer diameter)[9], whereas microstructural, chemical and microbial analyses and incubations were done in the laboratory at the field site and in home institutions. Mat communities and microstructural features were identified using a variety of microscope techniques (light, scanning electron, transmission electron, and scanning laser confocal[29]) and microbial populations were enumerated using epifluorescence microscopy counts[9], most-probable number enumerations[9,10] and molecular phylogenetic techniques. Microbial activities were assessed using depth profiles measured with microelectrodes[9] and radioisotope incubations using ^{3}H, ^{14}C and ^{35}S (refs 9, 10 and 30). Heterotrophic activity was also studied with microautoradiography of labelled organic matter uptake[30]. Microscale distribution of sulphate reduction was assessed using Ag foil coated with $^{35}SO_4^{2-}$ (ref. 18). Exopolymer distribution and production were evaluated by physical and chemical extractions and ^{14}C-bicarbonate experiments, respectively[11]. Other methods used are described elsewhere[9,10,11,13,29,30].

Received 17 April; accepted 26 May 2000.

1. Grotzinger, J. P & Knoll, A. H. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annu. Rev. Earth Planet. Sci.* **27,** 313–358 (1999).
2. Walter, M. R. in *Early Life on Earth* (ed. Bengtson, S.) Nobel Symposium Vol. 84, 270–286 (Columbia Univ. Press, New York, 1994).
3. Walter, M. R. in *Earth's Earliest Biosphere* (ed. Schopf, J. W.) 187–213 (Princeton Univ. Press, Princeton, 1983).
4. Dravis, J. J. Hardened subtidal stromatolites, Bahamas. *Science* **219,** 385–386 (1983).
5. Dill, R. F., Shinn, E. A., Jones, A. T., Kelly, K. & Steinen, R. P. Giant subtidal stromatolites forming in normal salinity water. *Nature*, **324,** 55–58 (1986).
6. Reid, R. P., Macintyre, I. G., Steneck, R. S., Browne, K. M. & Miller, T. E. Stromatolites in the Exuma Cays, Bahamas: Uncommonly common. *Facies* **33,** 1–18 (1995).
7. Reid, R. P., Macintyre, I. G. & Steneck, R. S. A microbialite/algal ridge fringing reef complex, Highborne Cay, Bahamas. *Atoll Res. Bull.* **466,** 1–18 (1999).
8. Golubic, S. & Browne, K. M. *Schizothrix gebeleinii* sp. nova builds subtidal stromatolites, Lee Stocking Island. *Algolog. Stud.* **83,** 273–290 (1996).
9. Visscher, P. T. *et al.* Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. *Am. Mineral.* **83,** 1482–1491 (1998).
10. Visscher, P. T., Gritzer, R. F. & Leadbetter, E. R. Low-molecular weight sulfonates: a major substrate for sulfate reducers in marine microbial mats. *Appl. Environ. Microbiol.* **65,** 3272–3278 (1999).
11. Decho, A. W., Visscher, P. T. & Reid, R. P. Cycling and production of natural microbial exopolymers (EPS) within a marine stromatolite. *Aquat. Microb. Ecol.* (submitted).
12. Stal, L. J., van Gemerden, H. & Krumbein, W. E. Structure and development of a benthic microbial mat. *FEMS Microbiol. Ecol.* **31,** 111–125 (1985).
13. Pinckney, J. L. & Reid, R. P. Productivity and community composition of stromatolitic microbial mats in the Exuma Cays, Bahamas. *Facies* **36,** 204–207 (1997).
14. Seeong-Joo, L., Browne, K. M. & Golubic, S. in *Microbial Sediments* (eds Riding, R. E. & Awramik, S. M.) 16–24 (Springer, New York, 2000).
15. Awramik, S. M. & Riding, R. Role of algal eukaryotes in subtidal columnar stromatolite formation. *Proc. Natl Acad. Sci. USA* **85,** 1327–1329 (1988).
16. Riding, R. in *Biostabilization of Sediments* (eds Krumbein, W. E., Paterson, D. M. & Stal, L. J.) Vol. 84, 183–202 (Oldenburg Univ. Press, Oldenburg, Germany, 1994).
17. Van Gemerden, H. Microbial mats: A joint venture. *Mar. Geol.* **113,** 3–25 (1993).
18. Visscher, P. T., Reid, R. P. & Bebout, B. M. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology* (in the press).
19. Macintyre, I. G., Prufert-Bebout, L. & Reid, R. P. The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites. *Sedimentology* (in the press).
20. Reid R. P. & Macintyre, I. G. Microboring versus recrystallization: further insight into the micritization process. *J. Sediment. Res.* **70,** 24–28 (2000).
21. Golubic, S., Seeong-Joo, L. & Browne, K. M. in *Microbial Sediments* (eds Riding, R. E. & Awramik, S. M.) 57–67 (Springer, New York, 2000).
22. Bertrand-Sarfati, J. in *Stromatolites, Developments in Sedimentology* (ed. Walter, M. R.) Vol. 20, 251–259 (Elsevier, New York, 1976).
23. Logan, B. W. *Cryptozoon* and associate stromatolites from the Recent, Shark Bay, Western Australia. *J. Geol.* **69,** 517–533 (1961).
24. Monty, C. L. V. in *Stromatolites, Developments in Sedimentology* (ed. Walter, M. R.) Vol. 20, 193–250 (Elsevier, New York, 1976).
25. Chafetz, H. S. & Buczynski, C. Bacterially induced lithification of microbial mats. *Palaios* **7,** 277–293 (1992).
26. Bartley, J. K. Actualistic taphonomy of cyanobacteria: implications for the Precambrian fossil record. *Palaios* **11,** 571–586 (1996).
27. Krumbein, W. E., Cohen, J. & Shilo, M. Solar Lake (Sinai). 4. Stromatolitic cyanobacterial mats. *Limnol. Oceanogr.* **22,** 635–656 (1977).
28. Walter, M. R., Bauld, J., DesMarais, D. J. & Schopf, J. W. in *The Proterozoic Biosphere* (eds Schopf, J. W. & Klein, C.) 335–338 (Cambridge Univ. Press, Cambridge, 1992).
29. Decho, A. W. & Kawaguchi, T. Confocal imaging of in situ natural microbial communities and their extracellular polymeric secretions (EPS) using nanoplast resin. *BioTechniques* **27,** 1246–1252 (1999).
30. Paerl, H. W., Bebout, B. M., Joye, S. B. & DesMarais, D. J. Microscale characterization of dissolved organic matter production and uptake in marine microbial mat communities. *Limnol. Oceanogr.* **38,** 1150–1159 (1993).

Acknowledgements

D. A. Dean prepared petrographic thin sections; T. Kawaguchi provided confocal images; N. Pinel analysed layer distribution. Numerous students assisted in field and laboratory studies. Logistical field support was provided by the crew of the RV *Calanus* and Highborne Cay management. This study is a contribution to the Research Initiative on Bahamian Stromatolites Project and International Geological Correlation Project Biosedimentology of Microbial Buildups. Funding for this research was provided by the US National Science Foundation, Ocean Sciences Division and NASA's Exobiology Program.

Correspondence and requests for materials should be addressed to R.P.R.
(e-mail: preid@rsmas.miami.edu).

Ant-like task allocation and recruitment in cooperative robots

Michael J. B. Krieger*†, Jean-Bernard Billeter‡ & Laurent Keller*

* *Institute of Ecology, University of Lausanne, BB, 1015 Lausanne, Switzerland*
‡ *Laboratoire de micro-informatique, Swiss Federal Institute of Technology, 1015 Lausanne, Switzerland*

One of the greatest challenges in robotics is to create machines that are able to interact with unpredictable environments in real time. A possible solution may be to use swarms of robots behaving in a self-organized manner, similar to workers in an ant colony[1–5]. Efficient mechanisms of division of labour, in particular series–parallel operation and transfer of information among group members[6], are key components of the tremendous ecological success of ants[7,8]. Here we show that the general principles regulating division of labour in ant colonies indeed allow the design of

---

† Present address: Department of Entomology and Department of Microbiology, University of Georgia, Athens, Georgia 30602, USA.