Fine banding in the septa of corals.

I. M. Sandeman

Dept of Biology, Trent University, 1600 West Bank Drive, Peterborough, Ont. Canada K9J 7B8

Abstract. Fine banding is apparent in the crystalline areas of thin ground and polished sections of coral septa when viewed with phase contrast optics. In some septa (e.g. *Meandrina meandrites*) the banding is robust and a series of laminations representing several months of growth can be followed. Repeated staining with alizarin of small colonies of *Agaricia* confirmed that the bands are diurnal and indicated that the darker bands (optically more dense) are formed during the day and the lighter bands at night. Ground and stained sections of *A. agaricites* mounted in epoxy resin showed, particularly in the calicoblastic layer areas with high calcification, many bunches of spherical bodies (Golgi apparatus?). These are probably involved in the secretion of the skeletal matrix. Evidence is presented that the Ca^{2+}ATPase pump may be light sensitive and leakage of Ca^{2+} due to lipid peroxidation of the plasma membranes during the day accounts for the higher calcification rates in light. A model of calcification is presented suggesting a mechanism by which the diurnal banding is produced.

Key words: Coral calcification, light, diurnal banding, Ca^{2+}ATPase pump

Introduction

Fine banding in coral skeletons has been studied with a variety of techniques (Risk and Pearce 1992; Perrin 2003; Gill et al 2006). Daily or circadian banding has been reported in the epitheca of corals but is of limited use in environmental studies (Risk and Pearce 1992). Some microbands appear to be the result of secondary skeletonization processes or later infilling of pore spaces between the theca. (Gill et al 2006). Septa, however, have received little attention. They can be easily removed from live corals and, if fine banding is present and can be confirmed as diurnal, septa have the potential to provide a record of recent environmental events on a daily time scale. In addition to providing growth rate information there is the potential of providing chemical proxies which can be related to environmental variables in the form of trace elements and stable isotopes using laser ablation techniques. The aim in this study was to determine whether coral septa have fine diurnal bands and explore how light may be involved in their formation.

Materials and methods

All sea water used for experiments was passed through a column of 4-8 mesh activated charcoal (Sigma) and millepore-filtered (0.45μ). Small pieces of live coral were collected in the back reef at Discovery Bay, Jamaica and were held in the sea water table at the Discovery Bay Marine Laboratory of the University of the West Indies. Small platelike colonies of *Agaricia agaricites* with a thin outer edge proved to be a particularly useful source of material as the structure was simple, consisting of parallel septa, without polyps. Pieces could be easily trimmed to a desired shape with small scissors.

For alizarin staining small colonies of Agaricia were collected from the back reef and at 0900 hrs placed in a large clear plastic bag with 20mg/l of alizarin red. The colonies were removed at 1800 hrs to the water table where they were held in running sea water. Four days later they were again placed in sea water with stain, at midday, and returned to the water table at 1800 hrs. Forty eight hours later the corals were water-piked clean and dried.

Small pieces of *Agaricia* were fixed in modified Karnovsky solution (2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M Sorenson buffer), dehydrated, embedded in LR White (hard) acrylic resin (Sigma) and cured at 60ºC under nitrogen. Slices were cut with a diamond saw. The slices, mounted on glass slides with thermoplastic cement, were ground with a series of microabrasive sheets to a thickness of less than 0.05 mm. The sections were then ground and polished on one side, heated, detached and turned over. After re-attachment they were ground and polished on the second side and stained with toluidine blue. After washing in distilled water and drying the sections each received a drop of immersion oil and a cover glass.

Septa were carefully removed, oriented and mounted on glass slides with thermoplastic cement (Crystal Bond). For cross sections larger septa were first sectioned with a diamond saw, ground with a series of microabrasive sheets, polished on one side,

detached on a hot plate, turned over, reattached and ground and polished on the other side. Progress was monitored regularly under a microscope (Wild M20 provided with phase contrast optics) and turned over several times during grinding to ensure that the final result was a section through the centre of a trabecula. Photographs were taken with an Epson PC800 digital camera.

The acetoxymethyl (AM) ester of the cell permeant calcium indicator calcium orange (Molecular Probes Inc.) is permeable to living cells. On entry it is hydrolysed to release the fluorescent calcium indicator in a charged form that is less permeant to cell membranes. 50 μg of calcium orange was dissolved in 0.1ml of a 20% solution of Pluronic® F-127 in dimethyl sulfoxide (DMSO). This was added to 20 ml of sea water to give a 4 μM working solution. Small pieces from the edge of thin plates of *Agaricia agaricites* were incubated for 30 min, rinsed in sea water, trimmed and placed with 0.6 ml sea water in a sample vial in the fluorometer (Sandeman 2006). The sample vial was a 9x30 mm Kimble glass vial with a neoprene 0-ring round the top and the bottom was shielded with a disc of black electrical tape. Excitation at 532 nm was provided by a green laser diode module (E1894, Egismos Technology Corporation). The beam was spread by a double concave lens in its path. Fluorescence (peak at 575-580 nm) collected by the ellipsoidal mirror is passed through a broadband interference filter (560-640 nm, Edmund Optics, A46-159) to the 2.0x2.0 cm blue enhanced silicon photodiode, PerkinsElmer Optoelectronics, at the second conjugate focus of the mirror. In the lamp housing three miniature blue light emitting diodes (LEDs, Maplin Electronics, N31AT) arranged round the excitation beam provided continuous illumination to the sample. The LEDs were independently switched and each provided approximately 100 μmol.m-2.s-1 at the bottom of the sample vial as measured with a LI-COR Quantum Radiometer (Model LI-250). The supply voltages for the laser diode and LEDs were regulated. For fluorescence readings the laser was switched on for as short a time as possible. Fluorescence usually decreased briefly after being switched on but quickly stabilized. Preliminary experiments indicated that photo-bleaching did not occur and fluorescence from coral or algal pigments did not interfere with the signal from the intracellular calcium. There was only slight leakage of the calcium probe.

Results

Of the species investigated *Meandrina meandrites* had robust easily seen banding (Fig. 1a-b). Each septum consistes of a palisade of parallel trabeculae with

sclerodermites, or whorls of fibres, radiating out from growth centres. In longitudinal section the banding is continuous across two or more trabeculae (Fig. 2b). In vertical sections (Fig. 2a) the series of bands
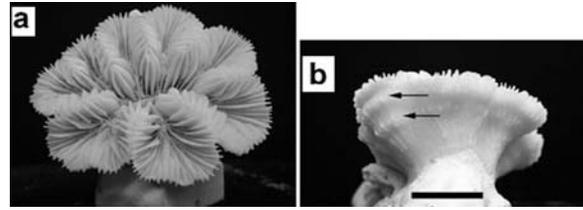

Figure 1a-b: Skeleton of colony of *Meandrina meandrites*, scale bar 1 cm. a septa. b epitheca with summer growth ridges(arrows).

can be followed for a considerable distance and the variation in the separation of the bands is considerable (4-15μ).
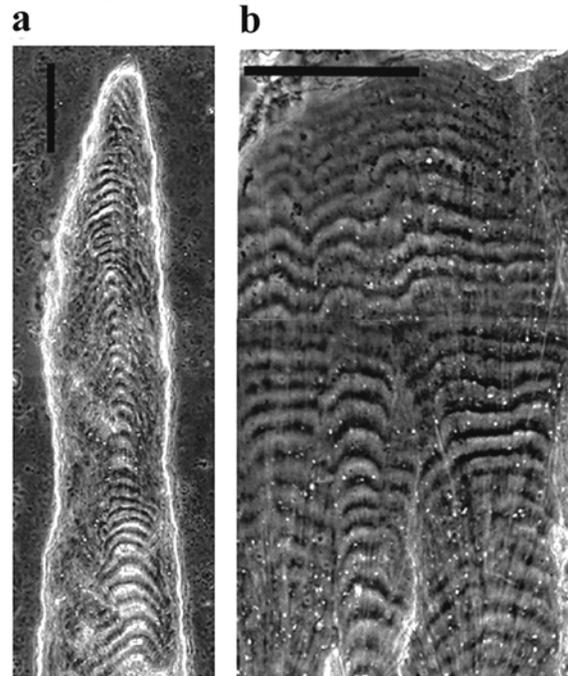

Figure 2a-b: Ground and polished thin sections of septa from *Meandrina meandrites* viewed with phase contrast optics, Scale Bar 100μm. a Vertical section, b Longitudinal section.

A similar arrangement of the trabeculae and banding was seen in several other coral species investigated: Dendrogyra cylindricus, *Dichocoena stokesii* and *Eusmilia fastigiata*. In *Montastrea annularis* (Fig. 3a) the trabeculae and pattern of banding were only visible in patches and sometimes obscured by whorls of radiating fibres or sclerodermites. In *Siderastrea siderea* central strips of crystalline banding were surrounded by amorphous material (Fig. 3b) In some species, especially those with thicker septa, eg *Dendrogyra cylindricus*, the trabeculae are not parallel and have a three dimensional   fanlike arrangement

which makes the banding more difficult to follow. Some of the faster growing shallow water corals examined, *Acropora cervicornis, Siderastrea radians* and *Porites porites* appeared to have few crystalline areas with visible banding and most of the skeleton appeared to be deposited in an amorphous form.
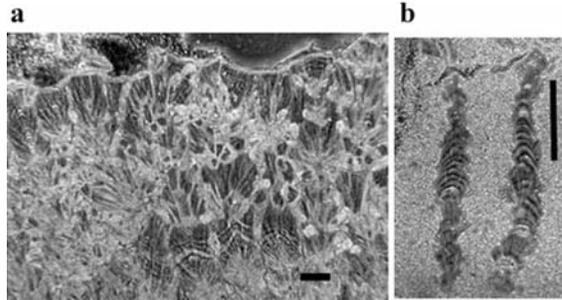

Figure 3a-b: Ground and polished thin sections of septa viewed with phase contrast optics, Scale bar 50μ. a *Montastrea annularis*. b *Siderastrea siderea*.

The epitheca (Fig. 1b) of the colony of *Meandrina meandrites* from which the septa shown in Fig. 2 were taken had annual protruding ridges 4 mm apart. This translates into an average daily growth rate of 11μ which fits well with the separation of the laminations of the septa. Sections from the small colony of *Agaricia agaricites* which had been stained twice with alizarin four days apart (Fig. 4) confirmed that the laminations are diurnal and indicated that calcium was laid down during the day in the darker bands.
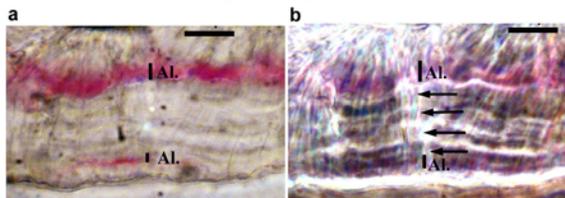

Fig. 4: Ground and polished sections of a septum of *Agaricia agaricites* after double staining. Scale bar 10μ. Al indicates stained layers, arrows dark bands. a standard optics. b phase contrast.

The two layers lining the skeleton of *Agaricia agaricites* can be distinguished in section (Fig.5). Zooxanthellae were not seen in the inner endodermal layer and both layers contain many clumps of vesicles that stain with toluidine blue indicating that they probably contain protein. These clumps of vesicles were profuse where calcification, as judged by the take-up of alizarin by the skeleton, was strongest, particularly on the septa. The stained vesicles were not seen in other tissues and it seems likely from their position and the quantity of material they contain that the vesicles could be the source of the organic matrix.

Fluorescence levels from pieces of *Agaricia* loaded with calcium orange leveled off within 10-15 minutes (Fig.6). The variation in fluorescence levels between different pieces of *Agaricia* was high and depended on the extent to which the fluorescent probe

was taken up by the coral pieces. When illuminated continuously with blue light from one or more LEDs the fluorescence level dropped quickly and adjusted to a new level. When the LEDs were switched off fluorescence returned slowly to close to its original level.
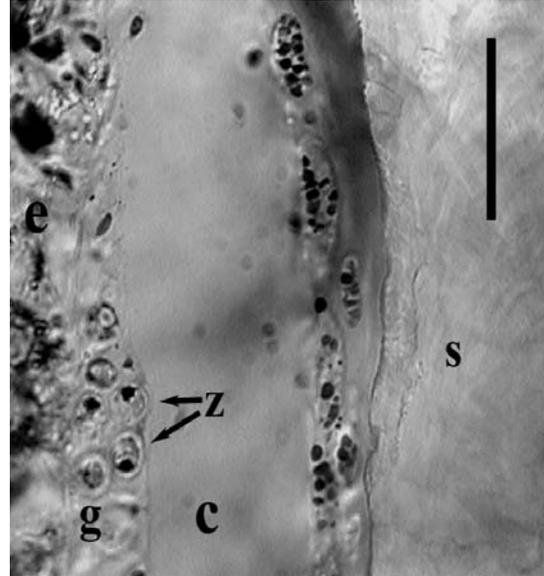

Figure 5: Section of *Agaricia agaricites* showing the calicoblastic endodermal and ectodermal layers, detached from the septal wall, with cells with stained secretory vesicles. c coelenteron, e epidermis, g gastrodermis, s septum, z zooxanthellae, Scale bar 50 μm.

Fig. 6 shows a typical result obtained using the same piece of *Agaricia* exposed to three different light levels with a dark period. As can be seen the change in fluorescence was proportional to the light intensity. With two LEDs switched on ($200\mu mols.m^{-2}.s^{-1}$) the fluorescence dropped to a new mean level 33.5 % lower than the original level (n=7).
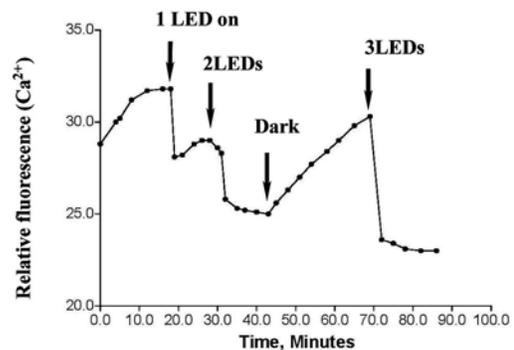

Figure 6: Relative fluorescence of *Agaricia agaricites* loaded with the intracellular fluorescent probe calcium orange. The relative change of fluorescence with time is shown for changes in light level (each blue LED emits 100 μmol.m-2.s-1).

Discussion

Comparison of the average separation of the bands of *Meandrina meandrites* (Fig. 2) with the annual ridges on the epitheca (Fig. 1b) and the sequential alizarin

staining of *Agaricia agaricites* (Fig. 4) indicated that the banding is diurnal. From the septa of the corals examined it is clear that a few species have the potential to provide a useful daily record of growth over periods of several months. These are generally species in with large, thin septa with robust banding in which the trabeculae are parallel and growth takes place in a single plane, eg *Meandrina meandrites*, *Fungia* sp. and *Eusmilia fastigiata*. In species such as *Dendrogyra cylindricus* with thick septa, many trabeculae grow at an angle to the main axis and these thicken the septae. However, this makes it difficult to find and follow the main growth axis. In other species the banding is either too patchy to follow or there are deposits of amorphous material with little or no banding visible. Variation in the separation of the bands in the septae of *Meandrina* was from 4-15 µ and bands can be counted up to the surface so that the exact date of variations can be pinpointed accurately.

Cohen and McConnaughey (2003) suggested that the role of the vesicles in the calicoblastic epithelium was the transport of seawater into the calcifying space. The clumps of vesicles seen in the two layers lining the skeleton in this study look like Golgi bodies which are often associated with the secretion of glycoproteins. The vesicles take up stain and are therefore unlikely to contain seawater and are interpreted here as the probable source of the organic matrix. The volume occupied by the vesicles (Fig. 5) appears to be enough to provide the 3-4 µ layer that would be laid down daily. No zooxanthellae were seen in the inner 'endodermal' layer and the two layers appeared very similar and vesicles were present equally in both layers, indicating that both layers may be involved in skeletogenesis. Physical extension of the coral takes place at night (Vago et al 1997) and this is presumably when the organic matrix is secreted. The role of the matrix is reviewed by Cohen and McConnaughy (2003). The matrix has been shown by Tambutté *et al* (2007) to consist of structural proteins which also play a catalytic role similar to that of carbonic anhydrase. It has also been shown to have calcium binding properties (Isa and Okazaki 1987; Constantz and Weiner 1988; Puverel et al. 2005). The dynamics of the synthesis and deposition of the organic matrix is not well known (Allemand et al, 1998) but its production may be the determining factor for calcium deposition rather than Ca^{2+} (Wainwright, 1963; Allemand et al 1998). The observation that the concentration of vesicles is highest in the areas of the coral where growth is highest may confirm this view.

Corals, like other organisms, maintain low intracellular Ca^{2+} levels (Barnes and Chalker 1990; Al-Horani et al, 2003) and failure of the Ca^{2+}ATPase pump at high temperatures was involved in 'dark' bleaching (Sandeman 2006). 'Solar' bleaching in-

volved build-up of H_2O_2 produced in photosynthesis. H_2O_2 at lower levels may also play a role in the regulation of algal density (Sandeman 2006). It is also evident, from the stable isotope fractionation of calcium (Gussone et al 2005; Bohm et al 2006), that the Ca^{2+}ATPase pump is involved in transporting Ca^{2+} into the extracellular calcifying fluid (ECF) where it is deposited. Many mechanisms have been invoked to explain the significantly higher calcification rates of corals in light than in the dark, these were reviewed by Gattuso et al (1999). More recently Al-Horani et al (2003), using Ca^{2+} microsensors found that the Ca^{2+} concentration under the calicoblastic layer of *Galaxea fascicularis* increased in the light and when the light was switched off the Ca^{2+} decreased. This was interpreted as calcium transport to the skeleton site being triggered by light with the additional ATP needed being supply from photosynthetically driven respiration. Cohen and McConnaughy (2003) proposed a model of calcification based on this light-sensitive action of the Ca^{2+}ATPase pump. The experiments undertaken in this study with *Agaricia agaricites* loaded with the fluorescent intracellular calcium probe indicated that the intracellular Ca^{2+} concentration decreases rapidly by 33% in light (200 µmol photons m^2. s^{-1}). The decrease was more or less proportional to the light intensity. In the dark the Ca^{2+} concentration returned slowly to its previous level. These results are interpreted as confirmation that the Ca^{2+}ATPase pump is light sensitive. However increased activity of the Ca^{2+}ATPase pump in the light does not explain how Ca^{2+}, which because of its charge is unable to move passively through the plasma membrane, gets into the calicoblastic layer in the first place from the large pool (Goreau 1959) in the coelenteron. Mechanisms involving special channels (Zoccola et al 1999; Clode and Marshall 2002) or special cells (Tambutté et al 1996) or transport of seawater by pericellular pathways or vesicles (see Cohen and McConnaughey 2003) have been suggested. Sandeman (2008) showed that lipid peroxidation in *Agaricia agaricites* in light (800µ mol photons) increased to a maximum in about four hours then reduced back to its original level. Lipid peroxidation, produced as a result of build up of H_2O_2 is known to make membranes leaky to Ca^{2+} (Halliwell and Gutteridge 1999) and it was suggested that the leakage provides a route for additional Ca^{2+} and other ions to enter cells of the calicoblastic layer. In that study the build-up of lipid-peroxidation parallels, almost exactly, the way that calcification rates of *Galaxea fascicularis* in light (200 µmol photons) measured by al-Horani (2007) increased to a maximum in six hours then decreased again. The difference in timing can be attributed to difference in light levels. Lipid peroxidation levels at the beginning of experi-

ments (Sandeman 2008), before exposure to light, and at the end of experiments were about 50% of the highest levels in light. This indicates that there may be a constant level of lipid peroxidation even in the dark and continuous leakage of Ca^{2+} and other ions into the tissues accompanied by a low level of Ca^{2+}ATPase pump activity. In light the activity of the Ca^{2+} pump increases and this may explain the higher calcification rate in light for ahermatypic corals. Zooxanthellate corals would experience even higher rates of calcification during the day as a result H_2O_2 build-up, increased lipid-peroxidation and leakage of more Ca^{2+} into the calicoblastic layer. The results and elements of the above discussion are combined into a model, following Adkins et al (2003), presented in Figure. 7.
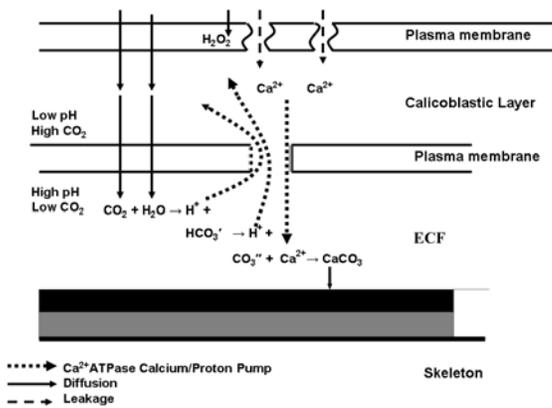

Figure 7: A calcification model for diurnal banding. Low levels of intracellular Ca^{2+} are maintained by the Ca^{2+}ATPase/proton pump which is light sensitive and maintains a lower level of Ca^{2+} in light. H_2O_2 produced by zooxanthellae builds up during the day, causes lipid peroxidation of the plasma membrane, makes it leaky to Ca^{2+} and provides a route for more Ca^{2+} (and other ions) to enter the calicoblastic layer. Secretion by the calicoblastic layers of a new layer of organic matrix takes place during the night. It has Ca^{2+} binding and enzymatic properties which enhance adhesion and the formation and deposition of $CaCO_3$. Ca^{2+} is actively transported into the ECF by the Ca^{2+}ATPase pump and protons are transported in the opposite direction. The removal of protons enhances the formation and deposition of $CaCO_3$ and the pH changes enhance the diffusion of CO_2 into the ECF. There is greater deposition on the new layer of the organic matrix during the day. This results in alternating low and high density laminations.

References

Adkins JF, Boyle EA, Curry WB, Lutringer A 2003 Stable isotopes in deep-sea corals and a new mechanism for "vital effects" Geochim Cosmochim Acta 67:1129-1143

Al-Horani FA, Al-Moghrabi SM, De Beer D 2004 The mechanism of calcification and its relation to photosynthesis and respiration in the scleratinian coral Galaxea fascicularis. Marine Biol 142:419-426

Al-Horani FA, Tambutté É, Allemand D 2007 Dark calcification and the daily rhythm of calcification in the scleratinian coral, *Galaxea fascicularis.* Coral Reefs 26:531-53

Allemand D, Tambutté É, Girard J-P, Jaubert J 1998 Organic matrix synthesis in the scleratinian coral Stylophora pistillata: role in biomineralization and potential target of the organotin tributyltin. J Exp Biol 201:2001-2009

Barnes DJ, Chalker BE 1990 Calcification and photosynthesis in reef-building corals and algae. In Dubinsky Z (ed) Coral reefs. Elsevier, Amsterdam, pp 109-131

Böhm F, Gussone N, Eisenhauer A, Dullo W-C, Reynaud S, Paytan A 2006 Calcium isotope fractionation in modern scleratinian corals. Geochim Cosmochim Acta 70:4452-4462

Chalker BE 1976 Calcium transport during skeletogenesis in hermatypic corals. Comp Biochem Physiol 54:455-459

Clode PL, Marshall AT 2002 Low temperature X-ray microanalysis of calcium in a scleratinian coral: Evidence of active transport mechanism. J Exp Biol 205:3543-3552

Cohen A, McConnaughey TA 2003 Geochemical perspectives on coral mineralization. Rev Min Geochem 54:151-187

Constantz BR, Weiner S 1988 Acidic macromolecules associated with the mineral phase of scleratinian coral skeletons. J Exp Zool 248:253-258

Gattuso J-P, Allemand D, Frankignoulle M 1999 Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. Amer Zool 39:160-183

Gill IP,Dickson JAD, Hubbard, DK 2006 Daily banding in corals: implications for paleoclimatic reconstruction and skeletonization. J. Sed. Res. 76:683-688

Goreau TF 1959 The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biol Bull 116:59-75

Gussone N, Bohm F, Eisenhauer A, Dietzel M, Heuser A, Teichert BMA, Reitner J, Wörheide G, Dullo W.-Chr 2005 Calcium isotope fractionation in calcite and aragonite. Geochim Cosmochim Acta 69:4485-4494

Halliwell, B. & J.M.C. Gutteridge. 1999 Free radicals in biology and medicine. (Third Edition) Oxford University Press, Oxford, England.

Isa Y, Okazaki M 1987 Some observations on the Ca^{2+} -binding phospholipids from scleratinian coral skeletons. Comp Biochem Physiol 87B:507-512.

Perrin, C 2003 Compositional heterogeneity and microstructural diversity of coral skeletons: implications for taxonomy and control on early diagenesis. Coral Reefs 22:109-120

Puverel S, Tambutté E, Pereira-Mouriès L, Zoccola D, Allemand D, Tambutté S 2005 Soluble organic matrix of two scleratinian corals: Partial and comparative analysis. Comp Biochem Physiol 141B:480-487

Risk MJ, Pearce TH 1992. Interference imaging of daily growth bands in massive corals. Nature 358:572-573

Sandeman IM 2006 Fragmentation of the gastrodermis and detachment of zooxanthellae in symbiotic cnidarians: a role for hydrogen peroxide and Ca^{2+} in coral bleaching and algal density control. Rev Biol Trop 54 (Suppl. 3):79-96

Sandeman IM 2008 Light driven lipid peroxidation of coral membranes and a suggested role in calcification. Rev Biol Trop 56 (Suppl. 1):1-9

Tambutté S, Tambutté E, Zoccola D, Camaniti N, Lotto S, Moya A, Allemand D, Adkins J 2007 Characterization and role of carbonic anhydrase in the calcification process of the azooxanthellate coral *Tubastrea aurea.* Mar Biol 151:71-83

Vago, R, Gill E, Collingwood, JC 1997 Laser measurement of coral growth. Nature 386:30-31

Wainwright SA 1963 Skeletal organization in the coral *Pocillopora damicornis.* Quart J Micros Sci 104:164-183

Zoccola D, Tambutté E, Senegar-Balar F, Michiele JF, Faille JP, Jaubert J, Allemand D 1999 Cloning of a calcium channel α1 subunit from the reef-build-ing coral *Stylophora pistillata.* Gene 227:157-167