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Suppression of skeletal growth in scleractinian corals by decreasing ambient carbonate-ion concentration: a cross-family comparison

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Biogenic calcification is influenced by the concentration of available carbonate ions. The recent confirmation of this for hermatypic corals has raised concern over the future of coral reefs because $[CO_3^{2-}]$ is a decreasing function of increasing pCO_2 in the atmosphere. As one of the overriding features of coral reefs is their diversity, understanding the degree of variability between species in their ability to cope with a change in $[CO_3^{2-}]$ is a priority. We cultured four phylogenetically and physiologically different species of hermatypic coral (*Acropora verweyi*, *Galaxea fascicularis*, *Pavona cactus* and *Turbinaria reniformis*) under 'normal' (280 μmol kg^{-1}) and 'low' (140 μmol kg^{-1}) carbonate-ion concentrations. The effect on skeletogenesis was investigated quantitatively (by calcification rate) and qualitatively (by microstructural appearance of growing crystalline fibres using scanning electron microscopy (SEM)). The 'low carbonate' treatment resulted in a significant suppression of calcification rate and a tendency for weaker crystallization at the distal tips of fibres. However, while the calcification rate was affected uniformly across species (13–18% reduction), the magnitude of the microstructural response was highly species specific: crystallization was most markedly affected in *A. verweyi* and least in *T. reniformis*. These results are discussed in relation to past records and future predictions of carbonate variability in the oceans.

Keywords: calcification; carbonate-ion concentration; carbon dioxide; hermatypic corals; fibre microstructure

1. INTRODUCTION

The rate of growth of corals is a major component of their fitness: larger coral colonies have a higher reproductive output and a greater competitive advantage (Wood 1999). Coral growth is intimately linked to calcification and is determined by a number of factors, including light, temperature, feeding and, the concern of this paper, the saturation state of the sea water. The calcium-carbonate saturation state (Ω) is the ratio of the ion concentration product ($[Ca^{2+}] \times [CO_3^{2-}]$) to the solubility product for the mineral deposited. Chemical precipitation is proportional to Ω: the greater the concentration of ions, the greater is the formation of mineral. Studies on hermatypic corals (Gattuso *et al.* 1999; Marubini & Atkinson 1999; Schneider & Erez 2000; Marubini *et al.* 2001), coccolithophores (Riebesell *et al.* 2000), coralline algae (Borowitzka 1981; Agegian 1985) and tropical reef communities (Langdon *et al.* 2000; Leclercq *et al.* 2000) have also shown that biogenic calcification is influenced by Ω. Since $[Ca^{2+}]$ is extremely conservative in sea water, it is $[CO_3^{2-}]$ that determines Ω. CO_3^{2-} is just one of the species of dissolved inorganic carbon present in sea water; the others are bicarbonate (HCO_3^-) and dissolved carbon dioxide (CO_2). Their relative proportions are dependent upon pH. Over the range of natural seawater pH (e.g. pH_{SWS} of 7.7–8.3), HCO_3^- makes up the bulk (*ca.* 85%) and remains relatively unaltered, while a decrease in pH results in a decrease in CO_3^{2-} and a concomitant increase in CO_2. Thus, a central tenet of this paper is that $[CO_3^{2-}]$ is directly proportional to Ω and pH and inversely proportional to CO_2. Although we chose to describe changes in dissolved inorganic carbon chemistry by focusing on $[CO_3^{2-}]$ for consistency with previous work (Marubini *et al.* 2001) and because of its role in inorganic precipitation, it is not clear whether coral calcification is ultimately responding to $[CO_3^{2-}]$ or to another covarying parameter (i.e. pH or CO_2).

The effect of a change in seawater $[CO_3^{2-}]$ on calcification has very important evolutionary and ecological consequences because carbonates vary over time and space. Since their first appearance 230 Myr ago, scleractinian corals have faced many changes in seawater carbonate chemistry (Buddemeier & Fautin 1996) as geochemical processes have influenced seawater alkalinity and atmospheric CO_2 (Berner 1990; Pearson & Palmer 2000). Owing to the gaseous equilibrium between air and water, any increase in atmospheric pCO_2 is matched by a similar increase in seawater pCO_2, with a concomitant decrease in pH and $[CO_3^{2-}]$. For this reason, the increased rate of CO_2 emissions to the atmosphere by anthropogenic activities has raised concerns about the future of coral reefs (Smith & Buddemeier 1992; Kleypas *et al.* 1999, 2001). By the year 2100, $[CO_3^{2-}]$ is predicted to fall to 180 μmol kg^{-1} from the present concentration of 270 μmol kg^{-1} (Gattuso *et al.* 1999). Natural variability in $[CO_3^{2-}]$ occurs geographically (mostly as a function of the effect of temperature on the solubility of CO_2); $[CO_3^{2-}]$ decreases three-fold from the tropics to the poles, and within the tropical belt it varies from 270 μmol kg^{-1} in the Red Sea to *ca.* 210 μmol kg^{-1} in areas of upwelling

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Table 1. Description of the average carbonate system in each tank for both treatments.

	'normal carbonate'			'low carbonate'		
variable	tank 1	tank 2	tank 3	tank 1	tank 2	tank 3
pH_{SWS}	8.06	8.06	8.06	7.76	7.76	7.75
total Alkalinity ($\mu eq\ kg^{-1}$)	2632	2638	2635	2416	2410	2403
CO_3^{2-} ($\mu mol\ kg^{-1}$)	280	283	285	150	149	145
HCO_3^- ($\mu mol\ kg^{-1}$)	1952	1953	1945	2049	2044	2047
CO_2 ($\mu mol\ kg^{-1}$)	11.2	11.1	10.9	23.0	23.0	23.7
pCO_2 (μatm)	416	412	407	859	857	882
$\Omega_{aragonite}$	4.37	4.41	4.43	2.33	2.32	2.26

(e.g. the Galapagos Islands) (J. A. Kleypas, personal communication). Short-term variability on a diurnal scale is also possible because local pCO_2 is raised at night by community respiration and lowered during the day by photosynthesis (Broecker & Takahashi 1966; Smith & Pesret 1974). Therefore, it might be expected that corals growing in more, or less, favourable areas of the world in terms of $[CO_3^{2-}]$ have adapted their growth responses to take advantage of, or mediate, their surrounding conditions.

Despite conclusive experimental evidence for the effect of $[CO_3^{2-}]$ on coral calcification, it is not known whether all corals of the large geographically, ecologically and physiologically diverse order Scleractinia are affected equally. In a prediction of how reef calcification might change during this century, Kleypas *et al.* (1999) warned of the potential consequences at the community level if the response to lowered $[CO_3^{2-}]$ was species specific. Since calcification in hermatypic corals is a biogenic activity strictly controlled via an organic matrix (Johnston 1980), it is strongly affected by the energetic status of the coral: it is boosted both by the photosynthetic activity of the endosymbionts and by the feeding regime of the host (Wellington 1982; Grottoli 2002). Thus, in the same way that light and food availability have a species-dependent effect on calcification, it is possible that control of calcification by $[CO_3^{2-}]$ is also species specific. Comparison of recent studies (Schneider & Erez 2000; Marubini *et al.* 2001) suggests a possible species-specific response. However, since the experimental designs differed markedly between these studies, it is virtually impossible to conclude whether variability is caused by variability between species or between experimental techniques. Furthermore, information on a greater variety of corals is necessary. The three coral species investigated to date represent only two of the 25 extant families, and all possess a characteristic branching growth form.

We propose that coping mechanisms or adaptive responses to calcification vary between species, and we set out to determine the growth responses of four ecologically and phylogenetically diverse species of hermatypic corals to two levels of $[CO_3^{2-}]$. In addition, we elucidate further the mechanism of growth reduction by examining the microstructure of the growing distal skeletal fibres using scanning electron microscopy (SEM). We ask new questions about the evolutionary responses of these corals to variations in $[CO_3^{2-}]$ over geological time and how global climate change might influence coral reefs over the next century.

2. MATERIAL AND METHODS

(a) *Coral preparation*

Four species of hermatypic coral were chosen from four different suborders: *Acropora verweyi* (Archaeocoeniina), *Galaxea fascicularis* (Meandrina), *Pavona cactus* (Fungiina) and *Turbinaria reniformis* (Dendrophyllina). Colonies were taken from culture reserves in the Oceanographic Museum of Monaco. For each species one mother colony was used, except for *A. verweyi* in which three colonies were selected to investigate interclonal variability. Coral nubbins (Davies 1995) were obtained for each species. Branches (2–3 cm tall) of *A. verweyi* were cut with bone cutters. The cut surface was ground flat on a grindstone, washed in running sea water and glued with viscous cyanoacrylate glue ('Superglue') to acrylic tiles (30 mm × 30 mm × 3 mm). Corallites of *G. fascicularis* were detached with pliers at the base of the coenosteum; a single corallite was glued on an acrylic tile with a small amount of underwater epoxy (Devcon11). For *P. cactus* and *T. reniformis*, which have a foliose structure, small parts (2–3 cm^2) were detached from the mother colony and attached to acrylic tiles with underwater epoxy. All samples were returned to the culture rooms and allowed to recover and grow for two months before starting the experiment. In all cases, damaged tissue completely regenerated within two weeks.

(b) *Experimental set-up and conditions*

Six 20 l glass aquaria were provided with light from overhead metal halide lamps (Osram H-QI 400 W) at an intensity of $300 \pm 30\ \mu mol\ m^{-2}\ s^{-1}$ for 12 h per day. Each tank was provided with a temperature sensor and a 500 W heater (Rena) connected to an independent temperature controller (ElliWell PC 902/T), and the temperature was maintained at 26.5 ± 0.2 °C. A small aquarium pump (Rena C20) ensured continuous water motion in each tank. Sea water flowed into the tanks at $3\ l\ min^{-1}$ from the flow-through system that pumps Mediterranean water from a depth of 50 m. Each tank contained between three and five nubbins per colony. Nubbins were initially grown for 8 days under standard conditions (with no chemical alteration to the incoming sea water) to acclimate them to the light, water motion and tanks of the experimental set-up. Thereafter, three tanks were chosen for the 'normal carbonate' treatment (pH_{SWS} of 8.1) and three for the 'low carbonate' treatment (pH_{SWS} of 7.7). Solutions of NaOH (*ca.* 0.1 N) and HCl (*ca.* 0.1 N) were delivered by peristaltic pump at a rate that matched the inflowing rate of fresh sea water into each tank. We measured pH daily on the seawater scale, and every 3 days we determined total alkalinity (TA) potentiometrically using an automated titrator (Mettler DL70), as described in Gattuso *et al.* (1993). Components of the carbonate system (pCO_2, HCO_3^-, CO_3^{2-}) were calculated from TA and pH using the apparent dissociation

constants of Mehrbach *et al.* (1973) for carbonic acid. Ca^{2+} was determined from salinity and temperature. The aragonite saturation state was calculated according to Mucci (1983). Nubbins were maintained in these experimental conditions for 8 days.

(c) *Measurements*

At the start and end of the experiment, nubbins were weighed in water using a Heraus precision balance (0.1 mg) fitted with a density-determination kit. Buoyant weights were transformed into dry weights after determining the skeletal density for each species as described in Davies (1989). The calcification rate was calculated as the change in weight over time.

Because, in corals, growth rate is a function of colony size, it is essential to standardize to some measure of size. We chose protein content as our standardizing unit. Thus, at the end of the experiment, coral tissue was removed by soaking each nubbin in a solution of NaOH (1 N) at 90 °C for 30 min. The protein content of the homogenized slurry was measured, using the method of Lowry *et al.* (1951), in an autoanalyser (Alliance Instruments) using bovine gamma globuline as a standard.

The microstructure of the freshly deposited skeleton was examined using SEM. After washing the bare skeletons several times in distilled water, they were dried at room temperature. Four nubbins of each species were randomly selected from each treatment and were coated with gold–palladium for 3D-observations with SEM. Observations (more than 50 per nubbin) focused on the distal tips of crystal fibres across septa.

(d) *Data presentation and statistical analyses*

The response of each coral species to the treatment was analysed separately because variations were found to be heterogeneous between species and no data transformation proved useful. We used a nested analysis of covariance (ANCOVA), testing for the effect of treatment and for the effect of tank nested within treatment. The dependent variable was the rate of calcification during the experimental period, expressed as mg $CaCO_3\ d^{-1}$. The covariate was the protein content of each nubbin. This was statistically better than running an ANOVA on growth data standardized according to protein content because such an adjustment assumes that the slope of the regression of calcification rate on protein equals exactly 1, which was not the case in this study. By adjusting the growth rate for protein content with the ANCOVA we increased the precision of the analysis (Sokal & Rohlf 1995). Within the species *A. verweyi*, all three colonies were tested simultaneously because assumptions of homogeneity of variances and normality of residuals were met; thus, the effect of colonies and the interaction term of treatment and colonies were also included in the analysis using two-way nested ANCOVA. All mean calcification-rate data presented in § 3 are adjusted by the covariate 'total protein'. Standard errors for adjusted means were calculated as described in Sokal & Rohlf (1995). All analyses were carried out using STATISTICA v. 5.5.

3. RESULTS

The concentration of carbonate ions in the 'normal carbonate' treatment was 283 μmol kg^{-1}, twice as much as in the 'low carbonate' treatment. Corresponding values for Ω_{arag} were 4.4 and 2.3, respectively, while CO_2 was 11 μmol kg^{-1} in the 'normal carbonate' treatment and 24 μmol kg^{-1} in the 'low carbonate' treatment. For each treatment, the three independent tanks were maintained with the same chemistry (table 1). There was no significant effect of tank in any analysis of calcification. The experimental design, therefore, avoided pseudoreplication (Hurlbert 1984).

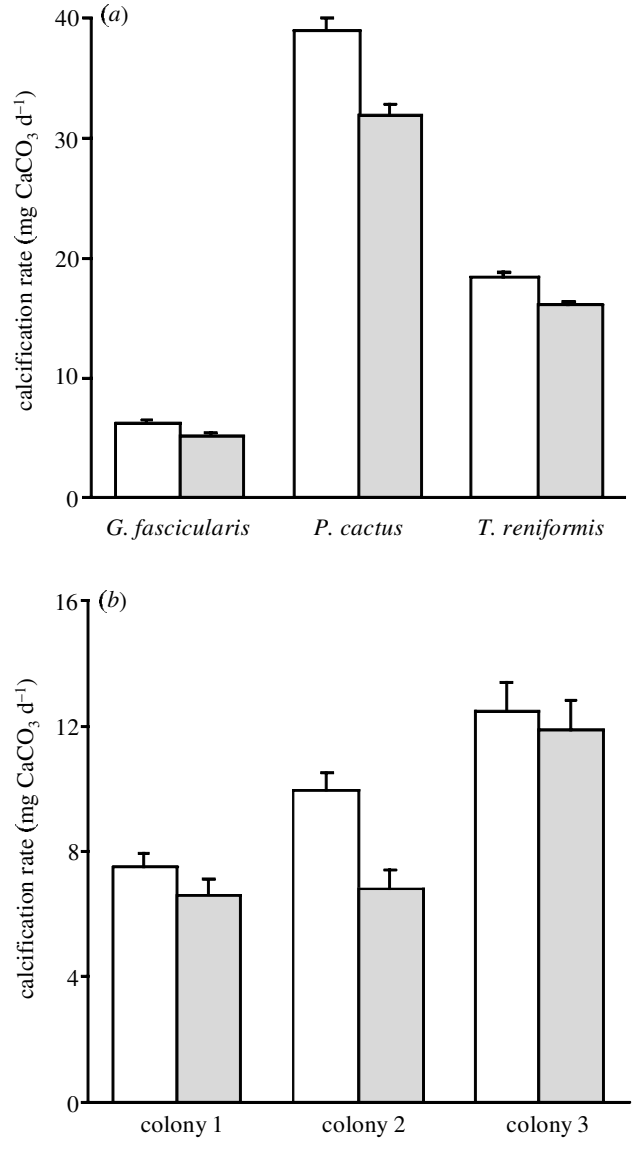

Figure 1. Daily calcification rates (mg $CaCO_3\ d^{-1}$) for (*a*) nubbins of *G. fascicularis*, *P. cactus* and *T. reniformis*, and (*b*) nubbins from three colonies of *A. verweyi*. Corals were cultured for 8 days under 'normal' (open bars) and 'low carbonate' (grey bars) conditions. Results are given as adjusted means (per protein content) and $s.e._{adj}$.

Calcification rate was found to depend on $[CO_3^{2-}]$ in all species: higher growth rates were found in the 'normal carbonate' tanks (figure 1). *Galaxea fascicularis* deposited, on average, 6.2 mg $CaCO_3\ d^{-1}$ in the 'normal carbonate' treatment and only 5.2 mg $CaCO_3\ d^{-1}$ in the 'low carbonate' treatment: a 16% reduction. The effect of treatment was highly significant (ANCOVA $F_{1,25} = 9.47$, $p < 0.01$). *Pavona cactus*, the fastest growing coral, deposited 39.0 and 31.9 mg $CaCO_3\ d^{-1}$ in the 'normal' and 'low carbonate' treatments, respectively: an 18% reduction in calcification in the 'low carbonate' treatment, which was highly significant (ANCOVA $F_{1,18} = 19.33$, $p < 0.001$). *Turbinaria reniformis* grew by 18.4 and 16.1 mg $CaCO_3\ d^{-1}$ in the 'normal' and 'low carbonate' treatments, respectively: a 13% reduction in the 'low carbonate' treatment which was also highly significant (ANCOVA $F_{1,12} = 16.81$, p

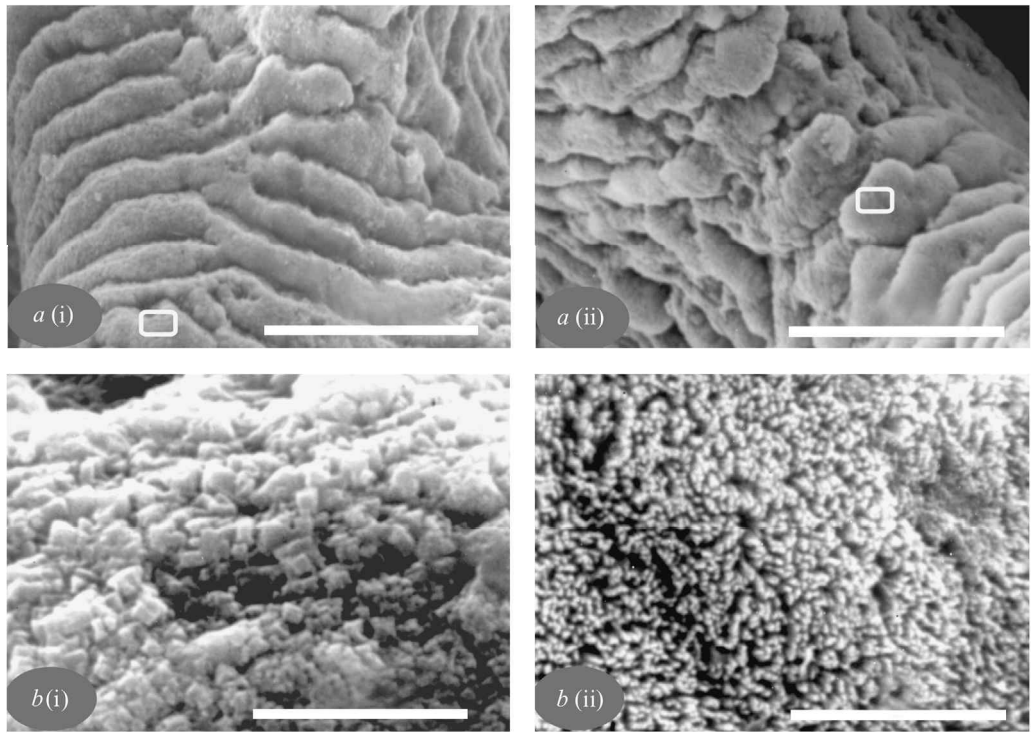

Figure 2. Scanning electron micrographs of the growing edges of septa of *A. verweyi* grown in (*a*(i),*b*(i)) 'normal' and (*a*(ii),*b*(ii)) 'low carbonate' conditions. At the lower magnification (*a*(i,ii); scale bars, 50 μm) the large and flattened skeletal units cannot be differentiated, but at the higher magnification (*b*(i,ii); scale bars, 5 μm) the crystallization pattern of fibres reveals a marked difference between the treatments.

< 0.002) (figure 1*a*). *Acropora verweyi* was also affected by the treatment: all three colonies grew less in the 'low carbonate' treatment (figure 1*b*) and the difference was highly significant (ANCOVA $F_{1,36} = 9.87$, $p < 0.005$). Growth rate was significantly different between colonies (ANCOVA $F_{2,36} = 14.94$, $p < 0.0001$), but there was no interaction between treatment and colony (ANCOVA $F_{2,36} = 1.03$, $p > 0.3$). Therefore, it was possible to pool all colonies to compare *A. verweyi* with the other species: on average, 10.1 mg $CaCO_3$ d^{-1} was deposited in the 'normal carbonate' treatment but only 8.3 mg $CaCO_3$ d^{-1} in the 'low carbonate' treatment: an 18% reduction. The four species of coral differed in the ratio of protein to skeleton: it was greatest in *G. fascicularis* with 25.9 mg g^{-1}; it was 16.2 mg g^{-1} in *T. reniformis*; 12.4 mg g^{-1} in *P. cactus* and only 6.4 mg g^{-1} in *A. verweyi*.

At a gross morphological level, SEM revealed no obvious differences in the size or shape of skeletal fibres between treatments. For example, the large and flattened skeletal units of *A. verweyi* at low magnification were similar in both treatments (figure 2*a*(i,ii)). However, treatment effects were revealed in all species at a much higher magnification by observing the crystallization patterns of fibres. Differences were found in the size of the microcrystalline units and in their arrangement. The magnitude of the difference was species specific: *A. verweyi* fibres were the most reactive, *T. reniformis* the least and *G. fascicularis* and *P. cactus* were intermediate. Fibres of *A. verweyi* in 'normal' conditions were built of densely packed microcrystals (figure 2*b*(i)), but in the 'low carbonate' treatment the fibre-growing units were elongated and much narrower (figure 2*b*(ii)). Clearly, mineral density was lower in these fibres. In *G. fascicularis* the distal end, of fibres in nubbins from the 'normal carbonate' treatment were characteristically rough, because of very well-developed microcrystals (figure 3*a*(i)), but appeared softer in the 'low carbonate' specimens (figure 3*a*(ii)). In *P. cactus* a decrease in the size of microcrystals was frequently observed in the 'low carbonate' treatment (figure 3*b*(i,ii)), a feature common also to *T. reniformis* (figure 3*c*(i,ii)), albeit only rarely encountered.

4. DISCUSSION

Skeletogenesis in scleractinian corals is, essentially, the process of incorporating calcium and carbonate ions from sea water into a crystal structure strictly controlled by an organic matrix (Johnston 1980). We measured both its quantitative (the amount of calcium carbonate deposited) and qualitative (the appearance of growing fibres) response to a change in carbonate-ion concentration. With regard to the quantitative growth response, our data show that the rate of calcification in the hermatypic corals *G. fascicularis, P. cactus, T. reniformis* and *A. verweyi* is a function of $[CO_3^{2-}]$ of sea water and, thus, of Ω_{arag}; the lower the Ω_{arag}, the lower the rate of calcification. This supplements and complements recent work carried out on hermatypic corals (Gattuso *et al.* 1998; Marubini & Atkinson 1999; Schneider & Erez 2000; Marubini *et al.* 2001). The magnitude of this response is remarkably constant between species: it only varies from 13% to 18% in the four species tested. We used corals with four-fold differences in the ratio of tissue (measured as protein content) to skeleton and three-fold differences in their calcification rates. None the less, the change in seawater $[CO_3^{2-}]$ affected all coral species equally. In addition, we can calculate the percentage reduction in calcification over the same

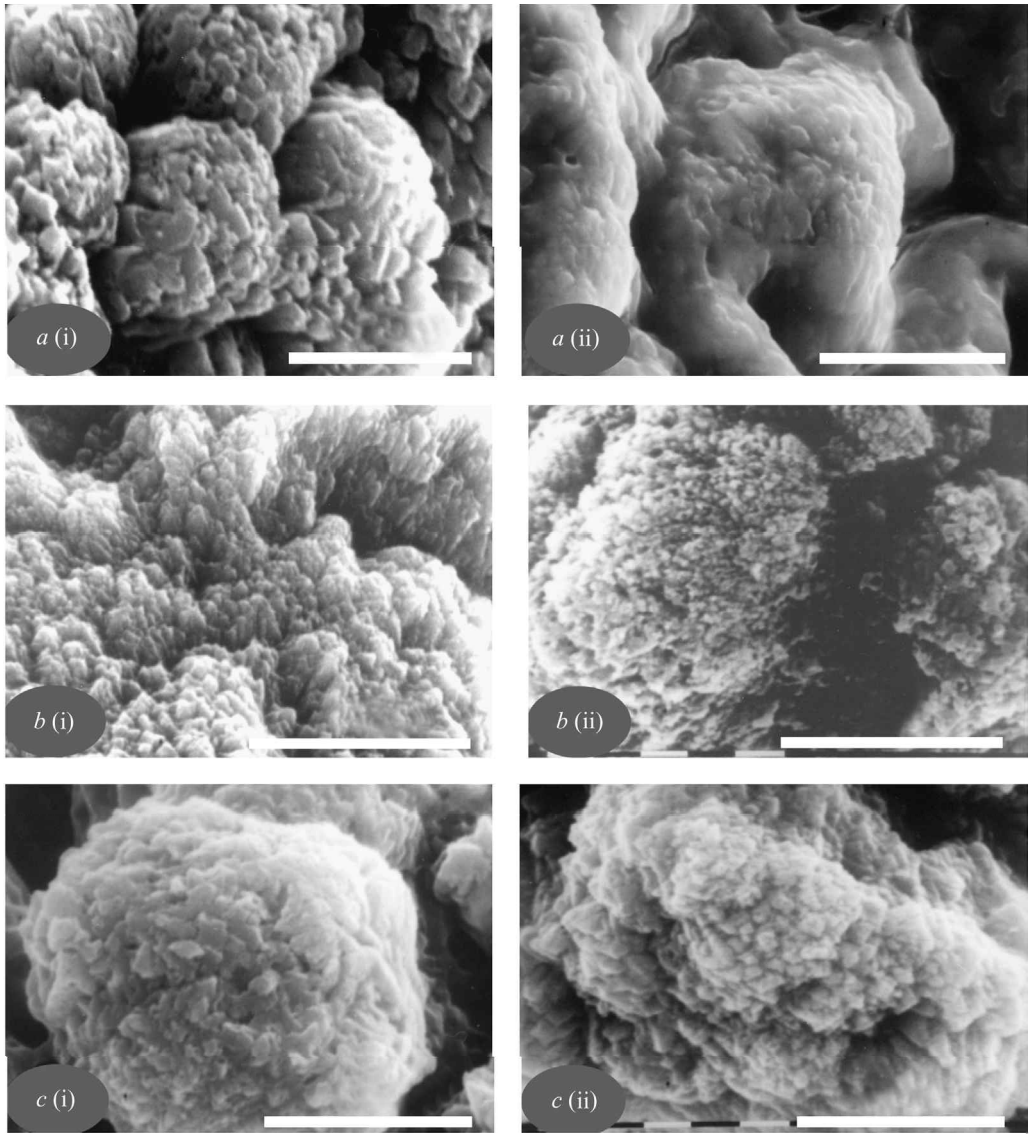

Figure 3. Scanning electron micrographs of the growing edges of septa of *G. fascicularis* (*a*(i,ii)), *P. cactus* (*b*(i,ii)) and *T. reniformis* (*c*(i,ii)) grown in 'normal' (*a*(i), *b*(i), *c*(i)) and 'low carbonate' (*a*(ii), *b*(ii), *c*(ii)) conditions. Scale bars, 5 μm.

range of $[CO_3^{2-}]$ values for previously published data, provided that the carbonate system had been controlled, experimentally manipulated and measured in a comparable manner. Thus recalculated, the calcification rate was reduced by 19% in *Porites compressa* (Marubini *et al.* 2001) and by 15% in *Stylophora pistillata* (F. Marubini, unpublished data). Therefore, the same response is shared by corals from six common genera, spanning five suborders, with differences in growth form (laminar, branching, massive), habitat preference (in terms of turbidity and wave exposure), reproductive strategy (brooders and broadcast spawners), symbiont associations and physiology (Veron 2000).

In addition to suppressing growth, $[CO_3^{2-}]$ can also affect the skeletal morphology of calcifying organisms. Riebesell *et al.* (2000) demonstrated structural disruption of the skeletal plates of coccolithophores under conditions of low $[CO_3^{2-}]$. We also found an effect of treatment on the morphological appearance of the distal tips of growing fibres. However, contrary to the growth response, microstructural changes in the crystal fibres were species specific, being most evident in *A. verweyi.* The fibre growth process in hermatypic corals is characterized by cyclic organo-mineral depositions (Cuif *et al.* 1999) that involve the organic matrix at every step (Johnston 1980). It is the species-specific character of the organic matrix that produces differences at the species level in the size and shape of growing fibres (Cuif *et al.* 1999). Our observations, therefore, suggest that the organic matrices of different species differ in their reaction to the chemical changes brought about in the mineralizing fluid compartment by a decrease in seawater $[CO_3^{2-}]$. Alternatively, but not mutually exclusively, there may be differences between species in the completion of the crystallization cycle, just as there are differences in the thickness of each cycle (Cuif *et al.* 1999). Since only the uppermost cycle of calcification is observable by SEM, in species where calcification is completed at each cycle, a decrease in calcification rate will result in an obvious change in the appearance of the distal fibres (e.g. *A. verweyi)*. However, in species where fibre crystallization continues after a new cycle has been deposited, the same decrease in calcification rate will be shared among the different cycles, diffusing the observable effect at the distal tips (e.g. *T. reniformis*).

While the mechanism by which a change in seawater [CO_3^{2-}] affects skeletogenesis in corals remains elusive, we have confirmed that a decrease in [CO_3^{2-}] results in a decrease in the calcification rate. In addition, we have linked the reduction in calcification to a change at the microstructural level. Our results suggest that, on a reef, a short-term decrease in [CO_3^{2-}] will inhibit aragonite deposition by the same magnitude in all coral species. However, if the effect at the microstructural level results in a change in skeletal bulk density, then some species, for example *A. verweyi*, might be more affected than others, especially in terms of their resistance to boring invertebrates and wave damage. Over the long-term and over evolutionary time corals might be able to evolve strategies to cope with conditions of low [CO_3^{2-}]. One potential strategy in extreme low [CO_3^{2-}] conditions might be to avoid the deposition of a skeleton completely (Buddemeier & Fautin 1996; Stanley & Fautin 2001). If the next 100 years are too short a time for physiological modifications to take place in any species, then the predictions outlined above should hold true; however, the precise magnitude of the change in calcification will also depend on how the predicted increase in temperature affects calcification (Lough & Barnes 2000).

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