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Long-term effects of induced mineral accretion on growth, survival and corallite properties of *Porites cylindrica* Dana

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Abstract

The mineral accretion technology involves the accumulation of dissolved mineral ions within the vicinity of underwater electrodes and their deposition via electrochemical processes onto the positive electrode forming a natural substrate. Branches of *Porites cylindrica* were reared under the presence of mineral accretion (treated nubbins), without mineral accretion (untreated), and under undisturbed (control) conditions for 6 months (mineral accretion phase). The electricity for the treated nubbins was cut off after the sixth month, and all remaining nubbins were allowed to grow for another 6 months (post mineral accretion phase). The longitudinal growth rate of the treated nubbins was relatively high during the mineral accretion phase then dropped during the post mineral accretion phase. Statistical analysis revealed longitudinal growth to be significant over time, but the significant differences between treatments lay only between the first until the second bi-monthly period (from January to May 2000) and the rest of the observation period (June to January 2001). This indicates that growth enhancement occurred only during the early stages of the mineral accretion phase. There were also significant differences in girth growth between phases and between treatments as well as a significant phase by treatment interaction again indicating that significant differences between treatments occurred only during a certain phase. In terms of overall survival, the treated nubbins fared better than the untreated nubbins. The corals in all treatments showed the same trend in corallite size and density after 1 year. Corallite sizes increased from the tip towards the base of the transplant. The reverse trend occurred with corallite density which decreased from the tip towards the base. Phenotypic alteration of the corallites (decrease in size and increase in density from the middle region of the nubbin towards the base) had occurred in the treated nubbins while they were exposed

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to mineral accretion. This effect disappeared, however, after 6 months. It appears, therefore, that enhancement of growth took place only during active mineral accretion, though there were positive, longer-term effects on survival.

Keywords: $CaCO_3$; Electrochemical deposition; Growth; Mineral accretion technique; *Porites cylindrica*

1. Introduction

Scleractinian corals are major components of a typical coral reef ecosystem. Corals deposit calcium carbonate into their exoskeleton through the process of calcification and skeletogenesis. Both processes are important for coral growth and increasing the density of the skeleton. The calcification process involves the reaction of Ca^{2+} and HCO_3^- ions to form a $CaCO_3$ skeleton in corals (Goreau, 1959; Goreau and Goreau, 1959). The Ca^{2+} ion may follow two possible pathways during calcification: the paracellular pathway which involves passive diffusion of ions through the intercellular spaces and the active transcellular route which is an energy-requiring process (Chalker, 1976; Barnes and Chalker, 1990; Bénazet-Tambutté et al., 1996b; Tambutté et al., 1996; Al-Horani et al., 2003a). Recent studies show that the uptake of small ions (such as Na^+, Cl^-, Ca^{2+}) for cnidarians first takes the paracellular pathway where they freely diffuse from the external environment towards the gastrovascular compartment (coelenteron) (Tambutté et al., 1995, 1996; Bénazet-Tambutté et al., 1996a,b) and are then taken to the site of calcification (calicoblastic epithelium in corals) through the transcellular pathway (Tambutté et al., 1996; Furla et al., 2000) via the intracellular carriers, the Ca^{2+}-binding proteins (Simkiss, 1976; Bronner, 1990). The paracellular pathway is driven by the presence of a concentration gradient of Ca^{2+} between the external epithelium and the coelenteron or by active pumping of the said compartment of fresh seawater through the mouth. Once inside the coelenteron, the Ca^{2+} ions are then actively taken in via an energy-requiring process to the calicoblastic epithelium where they meet with CO_3^{2-} and achieve a supersaturated concentration (Al-Horani et al., 2003a). Skeleton formation is governed by the physical process of precipitation/crystallization assisted by the formation of an organic matrix (Barnes, 1970; Jell, 1974; Johnston, 1980; Gladfelter, 1982, 1983; Cuif and Dauphin, 1998; Cuif and Perrin, 1999; Cuif et al., 1999; Cuif and Sorauf, 2001). The major sources of Ca^{2+} and dissolved inorganic carbon (DIC) for calcification are the external seawater medium (for both) and metabolic CO_2 for DIC (Pearse, 1970; Goreau, 1977; Gattuso et al., 1999; Furla et al., 2000; Al-Horani et al., 2003b).

The concentration gradient is known to drive the influx of ions into the coelenteron (Bénazet-Tambutté et al., 1996a,b; Al-Horani et al., 2003a,b). The calcification process, therefore, is greatly affected by the availability of mineral ions in the surrounding seawater, and the pH in the calicoblastic epithelium and of the surrounding water. Increasing the concentration of Ca^{2+} and HCO^{3-} in the external seawater medium increases calcification (Gattuso et al., 1998) and eventually skeletal growth (Marubini and Thake, 1999) in corals.

A technology developed by Wolfe H. Hilbertz in 1974 is capable of accumulating and concentrating dissolved mineral ions in the seawater near the electrodes and electrochemically forming a natural substrate (Hilbertz, 1975, 1976, 1992; Hilbertz and Goreau, 1996) made up of varying proportions of aragonite and brucite (Meyer and Schuhmacher, 1993). This involves passing a low voltage and direct current through two electrodes (cathode and anode separated) to induce electrolysis of seawater where the ions move towards the electrodes by virtue of their ionic charge. Reduction and oxidation reactions take place simultaneously when electricity is applied (Hilbertz, 1992). At the cathode, water is split to form hydrogen gas and two molecules of hydroxide anion. Precipitation of calcium and magnesium occurs with Ca^{2+} combining with dissolved HCO_3^- to form $CaCO_3$, and Mg^+ with the two molecules of OH^- to form $Mg(OH)_2$. The pH environment around the cathode is basic. Simultaneous with this is the oxidation process at the anode creating an acidic environment due to the formation of H^+ and gaseous O_2 from H_2O. The dissolved chlorine ions are also presumably oxidized to form Cl_2 gas.

This method enables an increase in the concentration of mineral ions like Ca^{2+}, Mg^{2+}, CO_3^{2-}, OH^- and HCO_3^-. Transplanting corals onto the cathode exposes them to mineral ion enrichment. This electrochemical attraction of mineral ions in the seawater simulates the experimental addition of Ca^{2+}, HCO_3^- and CO_3^{2-} ions that might have a positive effect on calcification and growth.

Corals exposed to electrodeposition of mineral ions showed an increase in percentage longitudinal and girth growth (Sabater and Yap, 2002). Corallite properties such as size and number per unit area were altered. This process also enhanced the survival of coral transplants (van Treeck and Schuhmacher, 1997; Sabater and Yap, 2002) through firm attachment brought about by the accretion of minerals at the attachment area. Enhanced mineral accretion favored the recruitment of calcareous organisms and selectively eliminated fleshy algae either by burial under the depositing material or by the presence of a high pH environment (Schuhmacher and Schillak, 1994). In another study, the high pH environment enhanced biomineralization of *Millepora dichotoma* Forskål attached to an aluminum plate (Vago et al., 2001). In contrast, lowering of pH caused a decrease in calcification of *Porites compressa* (Marubini and Atkinson, 1999).

The continuous accretion, however, causes a negative feedback (inhibition) in the interaction of the two electrodes, because the cathode is insulated through time with the accreted materials. The effect of mineral ion enrichment could therefore diminish through time. The objectives of this study were to: (1) determine if the enhancement of growth, survival and alteration of the corallite properties would persist after the termination of the mineral accretion; and (2) evaluate the effects of mineral accretion on coral growth and survival on the long term (at least 1 year).

2. Materials and methods

2.1. Study site

This study was done at Quezon Island (16°13′30″N, 120°02′43″E) in the Hundred Islands National Park, northern Philippines (Fig. 1). The park is composed of numerous

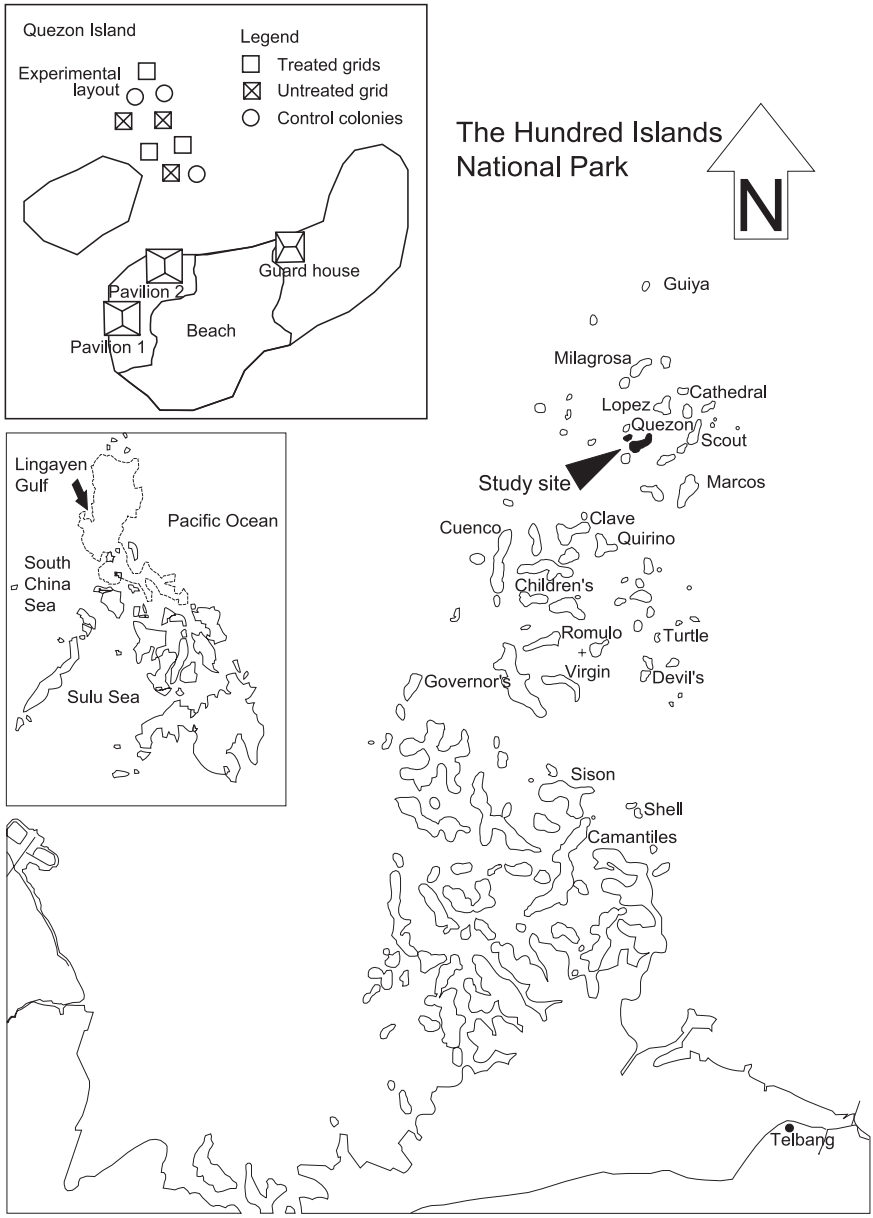

Fig. 1. Location of the Quezon Island study site (16°13′ 30″N 120°02′ 43″E) at the Hundred Islands National Park in the Lingayen Gulf (middle-left inset), northern Philippines. The experimental grids were located at a depth of 4.3–7.6 m. The lay-out of the experimental grids is shown in the upper left inset where the replicates of one treatment are interspersed with the other treatments to minimize the confounding effect of location and depth for a particular treatment.

limestone islands distributed northward from the mainland. The reef is characterized by a narrow flat which slopes to a sandy bottom. The experiment was conducted at a depth ranging from 4.3 to 7.6 m on the reef slope. The substrate was composed of patches of sand, silt and consolidated rubble interspersed with colonies of *Porites cylindrica* and *Acropora echinata* Dana. The experimental set-ups were placed in areas with bare sand patches. The study was conducted from December 1999 until January 2001.

2.2. Experimental design

The experimental design consisted of three treatments: treated (with mineral accretion), untreated (without mineral accretion) and the control (natural colonies). Each treatment had three replicates resulting in a total of nine experimental grids. The replicate grids of one treatment were interspersed with the other treatments (Fig. 1) to minimize the effect of location.

2.3. Mineral accretion set-up

A mineral accretion set-up had four primary components: an anode, a cathode, a power source and a structural platform (Fig. 2). Three Ringsdorff™ graphite anode bars were placed equidistantly and parallel to each other above a galvanized steel mesh cathode

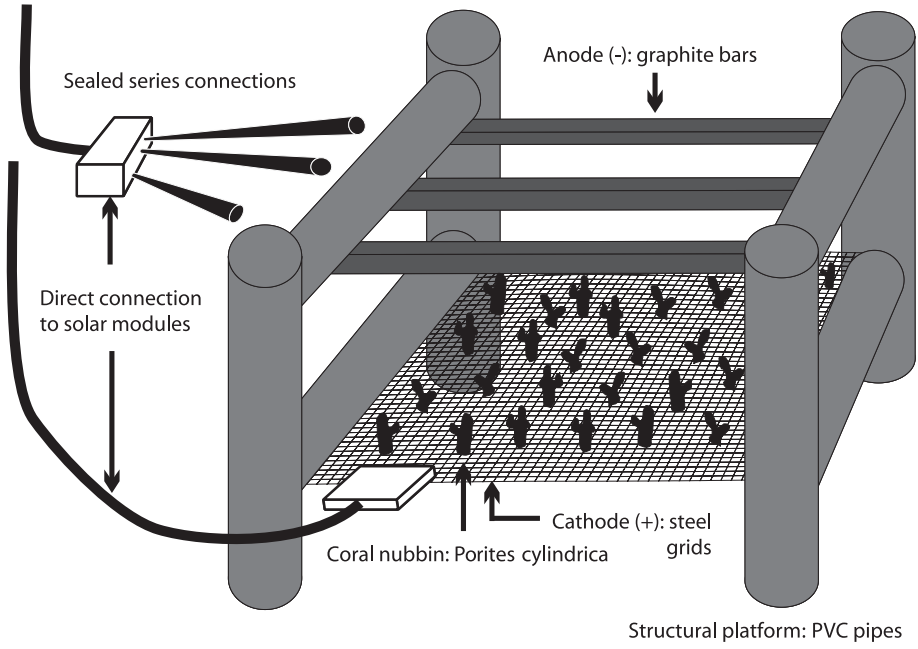

Fig. 2. The mineral accretion unit showing the major components: (1) anodes made up of three graphite bars; (2) a galvanized cathode steel mesh; (3) a structural platform made up of PVC pipes; (4) *P. cylindrica* transplants; and (5) the sealed wire connections.

(area: 1 m^2; mesh size: 10 mm; wire diameter: 0.7 mm). Thick copper cables were used to connect the electrodes to the power source on land. All connections were sealed using silicon sealant. The electrodes were assembled into a single unit (= experimental grid) using a polyvinyl chloride (PVC) pipe framework.

Sixteen AEG™ solar modules served as the power source. The modules produced a maximum voltage and direct current of 18 V and 4.18 A, respectively. The daily amperage followed a sinusoidal function where the level depended on the angle of the sun having its peak (4.18 A) at noon. Electricity was generated only in the presence of sunlight. The electricity was supplied for 6 months (from January until June 2000) and was cut off thereafter. Gelatinous accretions were formed during daytime when electricity was present. These then hardened when electricity was absent (nighttime). Full accretion was achieved after four months of alternating live and dead phases of electricity. The full accretion on the cathodic surface hindered the interaction between the two electrodes. Algae later recruited onto the newly formed substrate indicating that the accretion process had already ceased. This led to the decision to terminate the electrical input permanently. It took 2 years for full accretion to be achieved in the experiment done by Schuhmacher and Schillak (1994), while this level was reached in our experiment in less than a year. In both experiments, the electricity input was terminated after full accretion took place.

The main variation in the electrical regime was primarily due to the difference in day length from January to March and from April to June. The first 3 months have shorter day lengths than the last three. The period when electricity for the treated grids was cut off constituted the post mineral accretion phase which also lasted for 6 months.

The untreated set-up followed the same design described above except that there was no electricity supplied and the ‘‘anodes’’ used were made of wood. This was to simulate the shading effect produced by the anodes.

Nearby natural colonies served as the controls. Branches in each of the colonies were tagged using cable ties and labeled with Dymo™ tapes. Sample size was 120.

2.4. Collection and preparation of samples

A total of 260 thumb-sized nubbins of *P. cylindrica* were collected and placed in aerated bins for staining. The nubbins were first acclimatized for 24 h at 28 °C. They were stained using 10 ppm Alizarin Red S dye for 48 h under direct sunlight with water temperature maintained at 28 °C using seawater ice. The seawater and dye were replaced every 24 h.

The stained nubbins were then distributed among the grids (each grid having approximately 40 nubbins) with roughly equal spacing in between. The nubbins were inserted in the mesh with the growing tip perpendicular to the plane of the mesh. All were tagged and labeled at the bases, the latter also to serve as reference points for growth measurement. The control colonies were not stained due to difficulty in containing the stain in situ.

The following procedures pertain to all nubbins. After 6 months, approximately half were collected and sacrificed to examine the immediate effects of mineral accretion (reported in Sabater and Yap, 2002). The remainder was left in place for a further 6 months (total observation period of 1 year) to detect longer-term effects of the treatment.

2.5. Measurements of longitudinal growth and survival

Longitudinal growth was measured to determine the rate of vertical extension of the coral nubbins and control branches through time. Longitudinal growth was measured from the tagged base to the tip using a measuring tape accurate to 1 mm. Measurements were carried out every 2 months after the electricity was switched on and continued after it was cut off.

Survival was also monitored every two months. The number of mortalities in each grid per monitoring period was counted and the data were subjected to survival analysis (see later part). Transplants with 90% tissue loss were considered dead. Lost transplants, due to either physical or biological causes, were likewise counted as mortalities.

2.6. Girth growth measurements

The assumption of the presence of an ion concentration gradient where the concentration decreases perpendicularly from the plane of the cathode was the basis for the examination of girth growth. The hypothesis was that girth growth at the basal region would be faster due to increased ion concentration brought about by mineral accretion. Nubbins were cross-sectioned at the basal region using a high-speed rotating tile cutter to expose the Alizarin stain. Measurements were made using a vernier caliper accurate to 0.02 mm from the outer edge of the stain towards the edge of the nubbin. Girth growth measurements were made only for the treated and untreated nubbins since the controls were not stained. As mentioned above, this procedure was performed on half of the samples after 6 months, and on the remainder after 1 year.

2.7. Corallite properties

Corallite properties consisting of corallite size (in millimeters) and density were examined under a stereoscope equipped with a micrometer eyepiece and a net micrometer (area covered by the grid = 40.96 mm^2). Corallites at the tip (growing end), middle (estimated at an equal distance from the tip and basal region) and base (adjacent to the attachment region of the nubbin) were randomly selected, counted and measured. Measurements of the corallite sizes were done by measuring from the opposite ends of the corallite walls. The number of corallites was counted within the area bounded by the net micrometer.

2.8. Environmental parameters

The following environmental parameters were monitored during each field visit: temperature, salinity, turbidity, sedimentation rate and water movement. Details of measurements are provided in Sabater and Yap (2002).

2.9. Data processing and statistical analyses

The bi-monthly linear extension rate of each nubbin was computed to determine the temporal trend in growth from month 1 to 6 (mineral accretion phase) and from

month 7 to 12 (post mineral accretion phase). An analysis of variance with repeated measures (ANOVAR) was used to determine differences in linear growth across time and between treatments and blocks. "Treatments" are as described previously under Experimental design, while blocks are groupings of grids at the same depth. ANOVAR was chosen because the test is robust and powerful as long as the sphericity criterion is met (Potvin et al., 1990). A repeated multiple contrast test was used to determine between which levels (in this case, time) the differences in growth rate lay.

A linear regression analysis was performed to determine the effect of the measured environmental parameters on the linear extension of the corals in each treatment. The bi-monthly average of each environmental factor was computed and compared against the bi-monthly growth data.

The girth growth data were a combination of data from the nubbins gathered from the mineral accretion phase (reared for 6 months) and those from the post mineral accretion phase (reared for 12 months). These were tested for normality and homoscedasticity. A univariate three-factor ANOVA was used to determine differences between phases (mineral accretion and post mineral accretion phase), treatments and blocking (Zar, 1984). A type IV sum of squares was used because the data set is unbalanced and has missing cells (Shaw and Mitchell-Olds, 1993). The data were blocked according to depth to determine if there were differences due to this factor (Newman et al., 1997). A Tukey-type multiple comparisons test was used to determine the groupings in case of significant differences between blocks and treatments.

The Kaplan–Meier product limit estimate (Lee, 1992) was used to compare the trends in survival of corals in each treatment over time. The distributions were compared using non-parametric tests.

Data on corallite size and number did not achieve normality and homoscedasticity from any transformation, therefore, a two-way non-parametric ANOVA (Zar, 1984) was used to detect treatment effects. A non-parametric Tukey-type multiple comparisons test was used to determine the groupings which were significantly different. All statistical analyses were carried out using SPSS 9.0 for Windows.

3. Results

3.1. Longitudinal growth across time

The data on temporal trends in linear extension rate conformed to the sphericity requirement of ANOVAR (Mauchly's criterion = 0.901, $df = 14$, $P = 0.778$). There were significant differences in linear extension rates across time (Table 1A.1). Time also showed significant interaction effects with block and treatment. This indicates that the growth differences in some of the blocks and treatments did not respond in the same manner across time. The treated nubbins had relatively high linear extension rates during the first two bi-monthly intervals (with mineral accretion) after which they decreased (Fig. 3). The untreated nubbins showed variable growth over time. The control nubbins had relatively stable linear growth rates.

Table 1
ANOVAR of linear extension rate with repeated measures against time

(A) ANOVAR table of linear extension rate

Source	Type IV sum of squares	*df*	Mean square	*F*	Significance
(A.1) Within-subjects factor					
Time	3.596	5	0.719	8.709	0.000
Time × Blocking	2.324	10	0.232	2.815	0.002
Time × Treatment	5.589	10	0.559	6.768	0.000
Time × Blocking × Treatment	3.678	20	0.184	2.227	0.002
Error (Time)	39.641	480	0.083		
(A.2) Between-subjects factor					
Intercept	15.559	1	15.559	1092.709	0.000
Blocking	0.076	2	0.038	2.684	0.073
Treatment	0.014	2	0.007	0.475	0.623
Blocking × Treatment	0.066	4	0.016	1.153	0.337
Error	1.367	96	0.014		

(B) Contrast profile (repeated) of linear extension across the 6 bi-monthly intervals[a]

Levels	Time	Time × Blocking	Time × Treatment	Time × Blocking × Treatment
Level 1 vs. Level 2	0.000	0.002	0.024	0.108
Level 2 vs. Level 3	0.892	0.011	0.000	0.024
Level 3 vs. Level 4	0.454	0.138	0.013	0.043
Level 4 vs. Level 5	0.312	0.628	0.005	0.263
Level 5 vs. Level 6	0.860	0.032	0.002	0.004

Sphericity requirement was met (Mauchly's criterion = 0.901). Significance values were sphericity assumed. Part A: ANOVAR table. Part B: Contrast profile between six successive bi-monthly intervals of longitudinal growth measurements using repeated contrasts. All values are significance levels.

[a] Numbers reported are significance values.

Table 1B shows the contrast profile of linear extension across time. Significant differences occurred only during the early stages of the mineral accretion phase. The increase in linear extension tends to become insignificant after the second bi-monthly interval. There were significant interactions between blocks and time, and treatment and time across all levels.

Blocking and treatment did not have any significant effect on linear extension (Table 1A.2) after 12 months (6 months with mineral accretion and 6 months without). There were no significant interactions between treatments and blocks.

None of the five environmental parameters measured showed a statistically significant effect on growth of the treated nubbins (Table 2). The untreated nubbins, on the other hand, appeared to be significantly affected by changes in salinity and sedimentation while the control corals appeared to be affected by changes in temperature, turbidity and sedimentation.

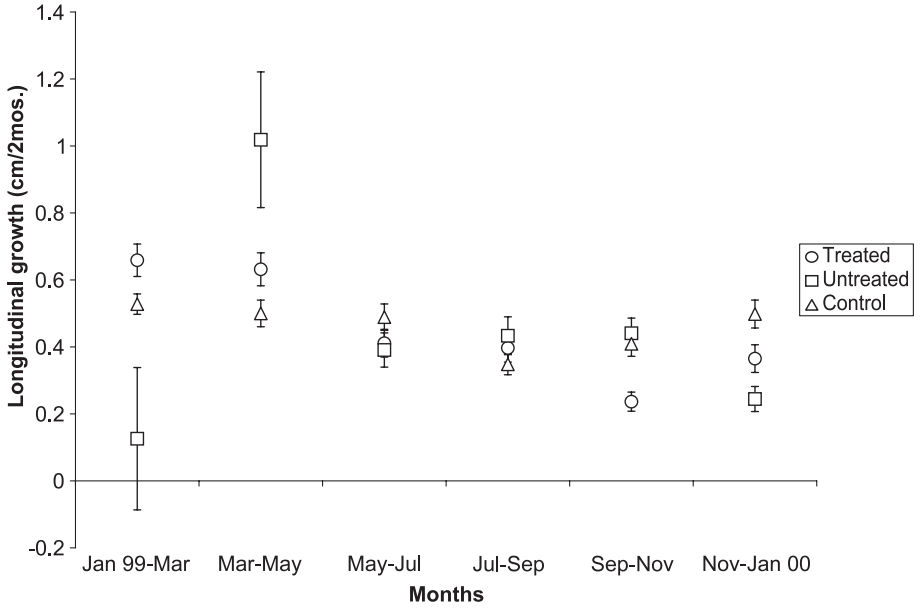

Fig. 3. Temporal trend in longitudinal growth of the treated, untreated and control nubbins. The mineral accretion phase covered the first three bi-monthly intervals, followed by the post mineral accretion phase after power was cut off. Error bars are standard errors.

3.2. Girth growth

Fig. 4 shows the girth growth of treated and untreated nubbins during the mineral accretion and post mineral accretion phases. Treated nubbins consistently showed higher girth growth in both phases compared with the untreated nubbins. Note that the rates reported for the mineral accretion phase were from the first batch of nubbins reared for 6 months while the rates for the post mineral accretion phase were from the remaining nubbins which were reared for a further 6 months. ANOVA showed significant differences

Table 2
Results of regression of linear growth of *P. cylindrica* under treated, untreated and control conditions against temperature, salinity, turbidity, sedimentation rate and water motion

Treatments	Important variables	Adjusted R^2	Unstandardized β coefficient	Standardized β coefficient	t	F significance
Treated		0.000	− 0.357		− 4.478	0.011
Untreated	Salinity	0.990	2.19	0.995	14.238	0.005
	Sedimentation		1.398	1.429	20.448	0.002
Control	Temperature	0.998	12.660	1.174	24.053	0.026
	Turbidity		0.906	0.948	23.964	0.027
	Sedimentation		− 0.254	− 1.174	− 30.749	0.021

Data are from January 2000 to January 2001. Only significant results are given.

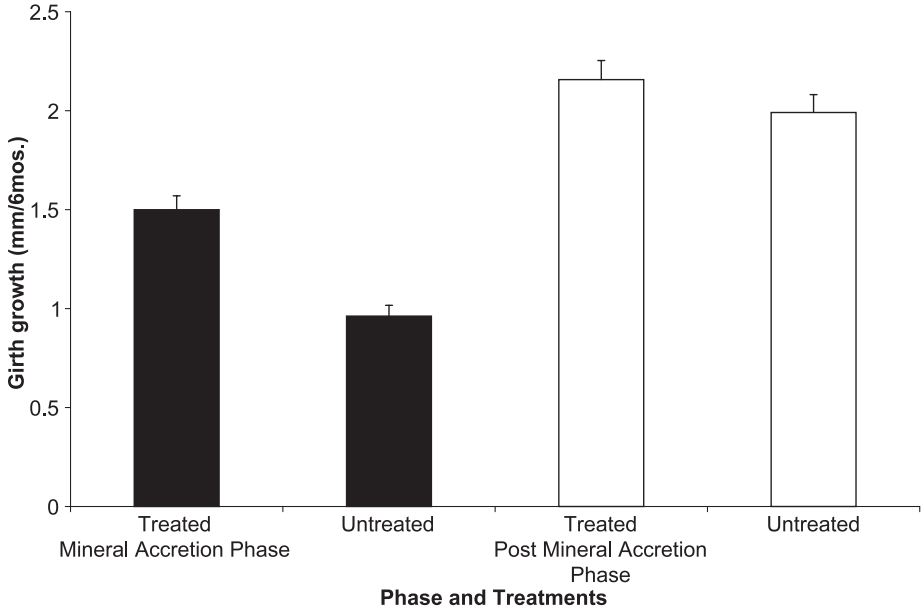

Fig. 4. Mean girth growth rate (mm/6 months) of the treated and untreated nubbins during the mineral accretion and post mineral accretion phases. Error bars are standard errors.

in growth rates between phases and treatments (Table 3). Differences in girth growth rates between treatments were higher during the mineral accretion phase compared with the post mineral accretion phase. There were significant phase by treatment, phase by block and treatment by block interactions.

Table 3
Univariate three-factor ANOVA of girth growth of the treated and untreated nubbins during the mineral accretion and post mineral accretion phases

Dependent variable: girth growth rate					
Source	Type IV sum of squares	*df*	Mean square	*F*	Significance
Between subjects					
Phase	2.931	1	2.931	123.696	0.000
Blocking	0.025	2	0.012	0.519	0.596
Treatment	0.740	1	0.740	31.228	0.000
Phase × Blocking	0.155	2	0.078	3.278	0.040
Phase × Treatment	0.320	1	0.320	13.517	0.000
Blocking × Treatment	0.187	2	0.093	3.940	0.021
Phase × Blocking × Treatment	0.018	2	0.090	0.378	0.686
Error	4.715	199	0.024		
Total	15.878	211			
Corrected total	9.250	210			

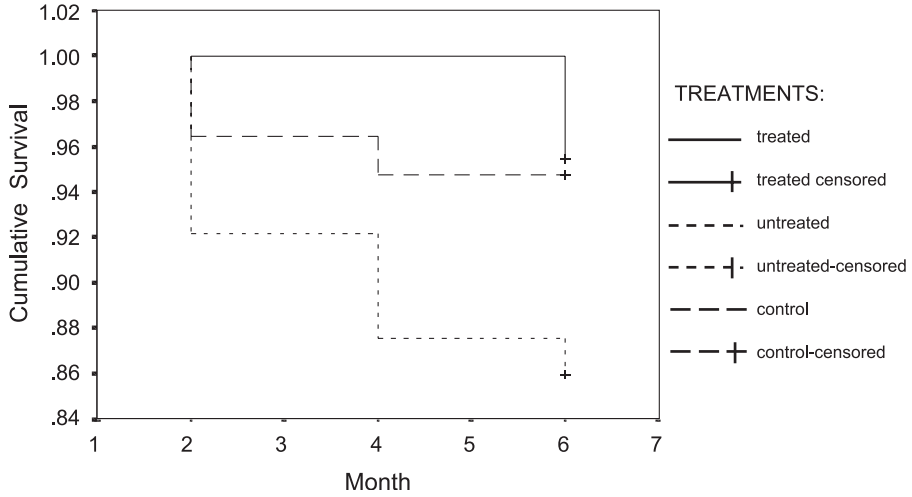

Fig. 5. Cumulative survival rates of *P. cylindrica* previously exposed to mineral accretion (treated), totally unexposed (untreated) and control nubbins 6 months after power was cut off.

3.3. Survival

The trends in cumulative survival for each treatment during the post mineral accretion phase are shown in Fig. 5. The treated and control nubbins each had three mortalities from an initial number of 66 and 57, respectively, 6 months after the termination of

Table 4
Comparison of the survival trends between the previously treated, untreated and control nubbins

(A) Mean and median survival time

Nubbins	Survival time (6 months maximum)			
	Mean	S.E.	Median	S.E.
Treated	6.00	0.00	[a]	[a]
Untreated	5.59	0.15	[a]	[a]
Control	5.82	0.10	[a]	[a]

(B) Non-parametric comparisons

Treatment pair	Non-parametric comparisons						Verdict
	Logrank		Breslow		Tarone–Ware		
	Statistic	Significance	Statistic	Significance	Statistic	Significance	
Treated × Untreated	3.70	0.0543	3.91	0.0479	3.81	0.0510	Treated ≥ Untreated
Treated × Control	0.05	0.8247	0.06	0.8065	0.05	0.8156	Treated = Control
Untreated × Control	2.57	0.1092	2.52	0.1126	2.54	0.1108	Control = Untreated

Part A: Mean and median survival time over 6 months after exposure (post mineral accretion phase). Part B: Non-parametric comparisons between each treatment pair to determine the survival status of the nubbins in each treatment relative to other treatments.

[a] Cumulative survival curve did not cross the 0.50 mark.

electrochemical deposition. The untreated nubbins exhibited the lowest survival having nine mortalities from an initial number of 64.

Treated nubbins had significantly higher survival than untreated ones (Table 4) over the long term. Survival analysis showed no significant differences between the treated and control nubbins and between the control and untreated nubbins.

3.4. Corallite diameter

Fig. 6 shows the trend in average corallite size from the tip of the nubbin to the base per replicate in each treatment 6 months after mineral accretion was terminated. All treatments followed the same trend where the corallite size increases from the tip towards the base.

Treated nubbins had an average corallite size of 0.73, 0.84 and 0.89 mm at the tip, middle and basal region, respectively. The untreated nubbins had an average size of 0.75, 0.87 and 0.93 mm, while the controls had values of 0.75, 0.89 and 0.96 mm, respectively.

There were significant differences between treatments and regions as well as a significant treatment by region interaction (Table 5). Multiple comparisons showed that the differences occurred between all treatments and all regions. The corallites of the control nubbins were significantly larger than those of the untreated nubbins. The untreated nubbins, on the other hand, had significantly larger corallites than the treated nubbins.

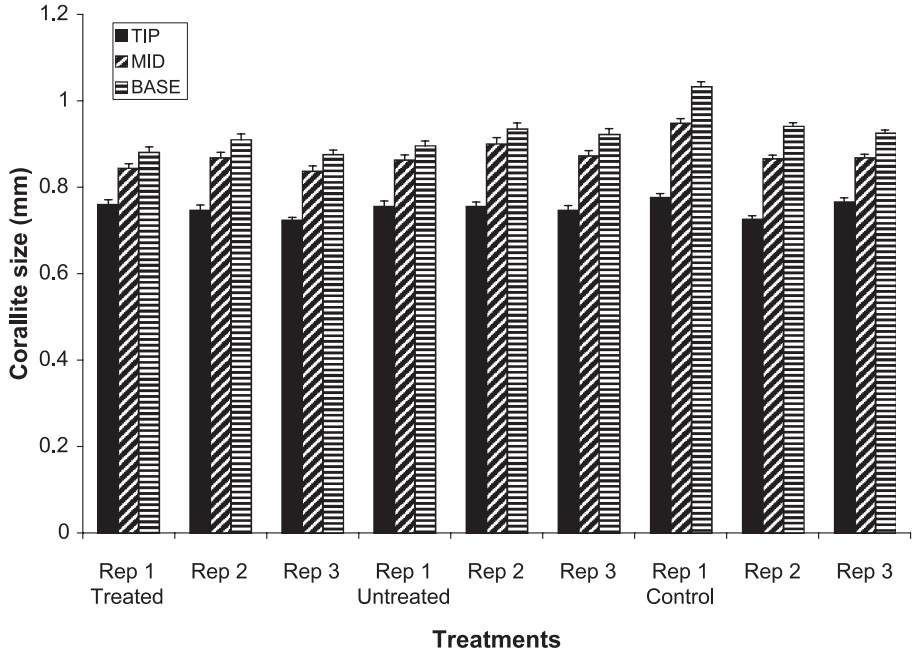

Fig. 6. Average corallite sizes of each region (tip, mid, base) per replicate of nubbins previously exposed for 6 months to mineral accretion (treated), totally unexposed (untreated) and control colonies after a further 6-month observation period. Error bars are standard errors.

Table 5
Non-parametric two-factor ANOVA of the corallite diameters of the treated, untreated and control nubbins reared for 6 months after a 6-month exposure to mineral accretion

Part A. Non-parametric two-factor ANOVA

Source	Sum of squares	*df*	*H*	$\chi^2_{0.05,\,v}$	Significance
Cells	72,710,056,335	8			
Treatment	13,440,830.13	2	44.4879	5.991	$P \ll 0.05$
Region	114,291,447.8	2	697.1158	5.991	$P \ll 0.05$
Treatment × Region	72,582,324,057	4	293,136.1	9.488	$P \ll 0.05$

Part B. Non-parametric multiple comparisons

Comparison	Difference	S.E.	*q*	$q_{0.05,\,inf}$	Conclusion
Control × treated	112,990.5	2360.225	47.87277	3.314	control ≫ treated
Control × untreated	64,493.5	2360.225	27.32515	3.314	control ≫ untreated
Untreated × treated	48,497	2360.225	20.54762	3.314	untreated ≫ treated
Base × tip	438,761.5	2360.225	185.8982	3.314	base ≠ tip
Base × mid	122,139.5	2360.225	51.74909	3.314	base ≠ tip
Mid × tip	316,622	2360.225	134.1491	3.314	mid ≠ tip

Part A: ANOVA results. Part B: Tukey-type non-parametric multiple comparisons of the corallite diameters among treatments and among regions of the nubbins.

3.5. Number of corallites

An increasing corallite size from tip to base results in a corresponding decrease in the number of corallites within a fixed area. This trend was observed in all the treatments (Fig. 7).

There were significant differences in the density of corallites between treatments and between regions (Table 6). The treated nubbins had significantly higher densities of corallites than the untreated nubbins followed by the control nubbins.

4. Discussion

The deposition of minerals onto a cathode substrate through seawater electrolysis presumably enabled the increase in the concentration of mineral ions in a small boundary layer above the cathode which could be utilized by the coral for skeleton formation (Meyer and Schuhmacher, 1993; Hilbertz and Goreau, 1996; Sabater and Yap, 2002). The increase in mineral ion concentration within the vicinity of the transplants created a gradient with high concentration outside the coral polyp and the coelenteric cavity. This could have induced a diffusional influx of mineral ions into the coelenteron via the paracellular pathway brought about by the "leaky" nature/permeability of the cnidarian epithelium (Tambutté et al., 1995, 1996; Bénazet-Tambutté et al., 1996a,b). This would have increased the availability of ions for active uptake via the transcellular route of calcification. These processes would explain the significant increase in the girth and longitudinal growth rates of *P. cylindrica* (Sabater and Yap, 2002).

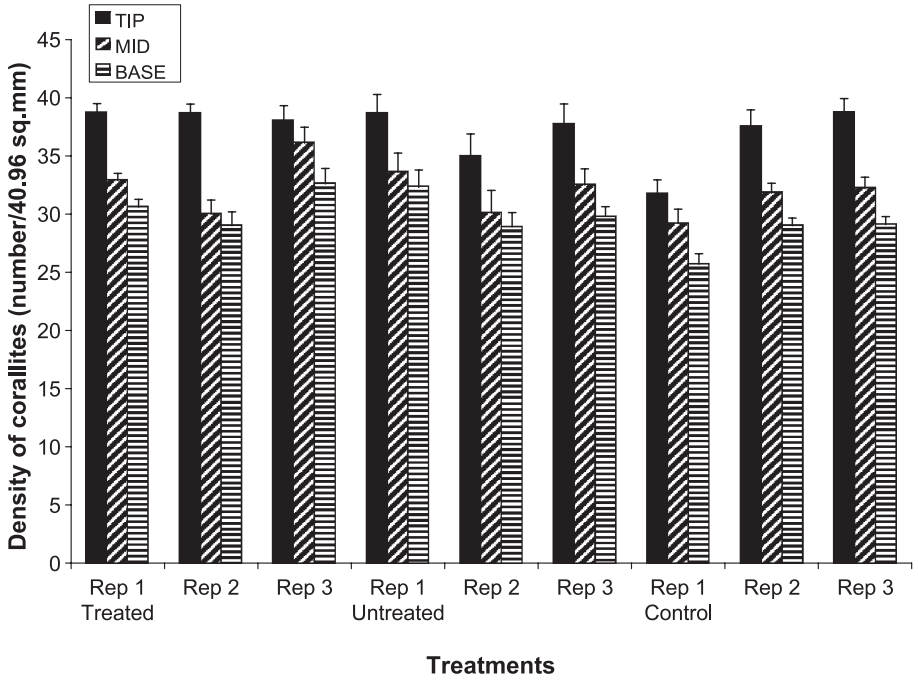

Fig. 7. Mean density of corallites in each region (tip, mid, base) per replicate of nubbins previously exposed for 6 months to mineral accretion (treated), totally unexposed (untreated) and control colonies after a further 6-month observation period. Error bars are standard errors.

Through time, the cathode becomes saturated by the accreted minerals resulting in a decreasing interaction between the two electrodes until the accretion stops. Thus, the effect of mineral accretion on growth dissipates through time. This explains why significant increases in longitudinal growth were found only for the first two bi-monthly intervals during the mineral accretion phase after which growth rates were no longer significantly different over time. The mineral accretion treatment did not have a significant effect on growth at the end of the 12-month experiment. Growth enhancement, therefore, occurred only when the electric field was present which caused an increase in the dissolved mineral ions around the coral transplants utilized for skeleton formation and mineral accretion for substrate formation.

The short-term nature of growth enhancement can also be seen in the data for overall girth growth. There were significant differences between phases and between treatments. The significant phase by treatment interaction strongly indicates that significant differences in girth growth brought about by the treatments were true only for a certain phase. Close examination of Fig. 4 reveals that the difference in growth rates between the treated and untreated nubbins was greater during the accretion phase as compared to the post accretion phase. The differences in growth rates between treatments were no longer significant in the post mineral accretion phase. The presumed increase in mineral ion concentration in the basal regions of the treated nubbins triggered the increase in the

Table 6
Non-parametric two-factor ANOVA of the number of corallites per unit area of the treated, untreated and control nubbins reared for 6 months after a 6-month exposure to mineral accretion

Part A. Non-parametric two-factor ANOVA

Source	Sum of squares	*df*	*H*	$\chi^2_{0.05,\ \upsilon}$	Significance
Cells	74,447,120	8			
Treatment	102,743	2	11.9497	5.991	$P \ll 0.05$
Region	384,825.3	2	96.3994	5.991	$P \ll 0.05$
Treatment × Region	73,959,552	4	6017.33	9.488	$P \ll 0.05$

Part B. Non-parametric multiple comparisons

Comparison	Difference	S.E.	*q*	$q_{0.05,\ inf}$	Conclusion
Treated × control	3596.5	143.0909	25.1344	3.314	treated ≫ control
Treated × untreated	1484	143.0909	10.371	3.314	treated ≫ untreated
Untreated × control	2112.5	143.0909	14.7633	3.314	untreated ≫ control
Tip × base	10,111	143.0909	70.6614	3.314	Tip ≠ base
Tip × mid	6599	143.0909	46.1175	3.314	Tip ≠ mid
Mid × base	3512	143.0909	24.5438	3.314	Mid ≠ base

Part A: ANOVA results. Part B: Tukey-type non-parametric multiple comparisons of the density of corallites among treatments and among regions of the nubbins.

number of corallites by budding of the polyps which in turn increased the number of calcifying individuals for coral growth (Sabater and Yap, 2002). The absence of the ion enrichment in the post mineral accretion phase slowed down growth at the basal region to the level of the growth of the untreated nubbins.

The progression of accretion during the early phase inhibited the settlement of filamentous and fleshy algae because of the high-pH environment within the vicinity of the cathode and the burial of successful colonizers by the minerals being deposited (Schuhmacher and Schillak, 1994). Intentional removal of electricity or even negative feedback inhibition in the cathode–anode interaction results in settlement and recruitment of blue-green and fleshy algae and other fouling organisms (Schuhmacher and Schillak, 1994; Schuhmacher et al., 2002). The settlement of algae (mostly filamentous macro-cyanobacteria) on the treated grids after removal of the electricity apparently affected the coral transplants. The absence of electricity in the post mineral accretion phase caused the disappearance of the high ion concentration at the site of calcification (Sabater and Yap, 2002) and of the high-pH environment that enhances biomineralization (Vago et al., 2001). This opened the newly formed substrate to colonization by algae (Schuhmacher and Schillak, 1994) which compete with corals for space. The decrease in growth rate could also be seen in the girth growth data where the difference in growth between the treated and untreated nubbins is smaller during the post mineral accretion phase. The basal area (where girth measurements were obtained) was the most affected region of the nubbin because it is adjacent to the substrate where the algae were found. Tanner (1997) demonstrated that competition could decrease the fitness (where survivorship, growth and reproduction are the major components) of corals. Competition with algae thus causes a significant decrease in growth, recruitment and reproduction (Tanner, 1995; Miller and Hay, 1996).

Measurement of the prevailing environmental parameters is essential to determine external factors affecting growth aside from the treatments being applied. None of the environmental parameters measured accounted for the variability in the growth rates of the treated nubbins. Only the untreated and control nubbins appeared to be significantly affected by environmental factors considered in the study. It is likely that the enhanced accretion process masked the effects of the environmental factors on coral growth. The chemical processes that occurred in the treated grids had a more significant effect on growth of the coral transplants than the physical factors.

The treated and control nubbins fared better than the untreated nubbins in terms of survival. Firm attachment to the substrate provided by the enhanced mineral accretion afforded the transplants a better chance of survival (van Treeck and Schuhmacher, 1997; Lindahl, 1998; Ammar et al., 2000; Schuhmacher et al., 2002).

In a previous study (Sabater and Yap, 2002), it was shown that the corallite sizes of the treated nubbins increased from tip until the mid region but decreased from the mid to the basal region of the nubbin. A reverse trend was observed for the density of corallites which decreased from the tip to the middle region but increased from mid-region to base. The untreated and control nubbins showed a different trend with size increasing from tip to base while the reverse was true for corallite density. These results indicate that the division of polyps at the basal region of corals in the treated grids was accelerated by the presence of mineral ion enrichment or the electric field. Comparing these results to the data presented in this paper on corallite size and number, the trend for the treated nubbins is seen to have changed. The phenotypic alteration in corallite size and numbers disappeared in the absence of an electric field. Upon further examination of the data, the sizes of the basal corallites of the nubbins previously exposed to mineral accretion were still slightly smaller (0.89 mm average) as compared to the untreated and control nubbins (0.93 and 0.96 mm, respectively). Thus, the previously small and numerous corallites had grown and matured which resulted in a trend in size and density similar to those of the untreated and control corals after 1 year. The effect, therefore, of mineral accretion on the corallite characteristics is only temporary.

The phenotypic characteristics of corals adjust to changes in the external environment. Some factors that affect the phenotypic characteristics of coral colonies are water turbulence, depth (as a function of light and water motion) and turbidity (Willis, 1985; Bruno and Edmunds, 1997; Vago et al., 1998). This is the first study to show that when corals are exposed to mineral ion enrichment, they exhibit phenotypic plasticity in terms of basal corallite size and density. The ion enrichment triggered the asexual reproduction of the basal polyps with an effect on girth growth and density (unpublished data) of the coral transplants. We hypothesize that the energy normally allocated for the active uptake of ions needed in skeleton formation was re-channeled towards reproduction in the presence of ion enrichment brought about by mineral accretion.

In conclusion, the overall effect of the mineral accretion technique on coral growth was evident only during the active accretion phase. After termination of mineral accretion, the subsequent recruitment of fleshy algae which compete with the coral transplants further decreased their growth rate. The technology conferred a significant advantage, however,

on the transplanted corals by enhancing their survival through firm attachment to the substrate.

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