

# Inhibited growth in the photosymbiont-bearing foraminifer *Marginopora vertebralis* from the nearshore Great Barrier Reef, Australia

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**ABSTRACT:** Growth rates of benthic photosymbiont-bearing foraminifera are strongly influenced by nutrient concentrations and temperature, yet little is known about the effect of terrestrially derived nutrients on growth rates of foraminifera in the field. This study investigates the growth response of *Marginopora vertebralis* to enhanced terrestrially derived nitrogen and phosphate under both field and laboratory conditions. The field studies were conducted around the Whitsunday Islands in the Great Barrier Reef (GBR), Australia, once during a peak flooding period and once during the dry season. During the flood season, dissolved inorganic nitrogen and particulate phosphate increased 2- to 5-fold from terrestrially derived runoff from the Proserpine River catchment compared to the dry season. Increased exposure to nutrients derived from terrestrial runoff negatively affected the growth rate of *M. vertebralis* in the field and in a comparative laboratory experiment. The field study showed a clear effect of site proximity to the river mouth and the intensity of the river discharge. Growth rates were higher in the reduced terrestrial runoff conditions at the offshore sites. When exposed to naturally enriched dissolved inorganic nitrogen and phosphate in the laboratory experiment, growth rate of *M. vertebralis* was reduced 51 and 37 %, respectively, compared with ambient controls. Eutrophication likely causes destabilisation in the coupling of photosynthesis and growth within the photosymbiont by restricting photosynthate translocation to the host. In addition to nutrient effects, we also tested the effect of summer (28°C) and winter (22°C) temperatures on foraminiferal growth in the laboratory. Growth rates were significantly lower in the warmer treatment than in the cooler treatment. Therefore, both increased temperatures and higher nutrient concentrations appear to negatively affect growth in photosymbiont-bearing benthic foraminifera. Overall, management of local stressors (in this case nutrient runoff) might ameliorate the impact of climate change (increased temperature) on calcifying photosymbiont-bearing organisms.

**KEY WORDS:** Water quality · Eutrophication · Whitsunday Islands · Foraminifera

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## INTRODUCTION

Landscape modification is influencing coastal marine systems globally (Pringle 2001). Since the European settlement of Australia's Queensland coastline in the mid-18th century, catchment areas adjacent to the Great Barrier Reef (GBR) have undergone extensive change. Widespread land clearing occurred in the catchment areas to facilitate agriculture (Furnas 2003). For example,

the Mackay-Whitsunday area on the central Queensland coast is classified as a priority management region because of a high proportion of the Proserpine River catchment being cleared and converted to beef grazing (33 %) and sugarcane cropping (23 %) (van den Berg et al. 2007). These land-use practices facilitate the export of sediment and nutrient-enriched river runoff, which significantly alters water quality in the adjacent coastal reef environments (Fabricius et al. 2005, Wooldridge et al. 2006).

Increases in the amount of sediment and nutrients entering the inshore zones of the GBR have led to major water quality declines. The Mackay-Whitsunday coast receives an estimated 80 tonnes of dissolved inorganic phosphate (DIP) and 2500 tonnes of dissolved inorganic nitrogen (DIN) per annum from the adjacent Proserpine, O'Connell and Pioneer rivers (Brodie 2006). Since pre-European settlement, there has been a 4-fold increase in dissolved nutrients (Neil et al. 2002, Moss et al. 2005), a 5- to 10-fold increase in sediment input (McCulloch et al. 2003), elevated concentrations of chlorophyll *a* (chl *a*) and an increase of total suspended solids (TSS) (Cooper et al. 2007). In the O'Connell and Proserpine River basins, total nitrogen and total phosphorus loads have increased by >4 and >10 times, respectively, relative to pre-European levels (National Land and Water Resources Audit 2002). Moreover, terrestrial runoff is enhanced during regular and episodic flood events in the monsoonal summer between December and May (Haynes & Michalek-Wagner 2000, Devlin et al. 2001). Coastal river runoff has led to a distinct cross-shelf water quality gradient, whereby turbid and nutrient-enriched waters dominate the inshore zones (Furnas 2003, Fabricius & De'ath 2004, Fabricius 2005, Brodie et al. 2007).

Temperature and the biochemical parameters of water quality correspond to biological gradients among marine organisms (van Woesik et al. 1999, Cooper et al. 2007). Metabolic rate, body size and ventilatory efficiency among marine organisms are regulated by temperature and oxygen consumption (Pörtner 2002), and in cases of high temperature, bleaching among photosymbiont-bearing species has been observed (Hoegh-Guldberg & Smith 1989, Talge & Hallock 2003). Meanwhile, stress induced by altered water quality affects a range of marine organisms such as fish (Wilson et al. 2006), corals (Kinsey & Davies 1979, Koop et al. 2001) and other invertebrates (Lewis & Snelgrove 1990, Preston & Doherty 1994, Idjadi & Edmunds 2006). Coral physiology (van Woesik et al. 1999, Fabricius 2005, Fabricius et al. 2005) and benthic foraminiferal community assemblages (Uthicke & Nobes 2008, Uthicke et al. 2010) are known to change depending on water quality within the Whitsunday region. Specifically, symbiont-bearing organisms suffer adverse metabolic and reproductive effects due to decreasing light penetration resulting either directly from sediment runoff (Fabricius 2005) or inorganic nutrient runoff, or indirectly through phytoplankton blooms caused by enhanced availability of inorganic nutrients (Cox et al. 2006). Quantifying the biological responses of local stressors is necessary to understand potential ways to reduce the susceptibility of organisms to global climate change (Wooldridge & Done 2009).

Here we investigate the growth response of the large benthic foraminifer (LBF) *Marginopora vertebralis* in various nutrient and temperature conditions. Our study consisted of 1 laboratory-controlled aquarium experiment and 2 repeated field studies (one in the dry months of winter and the other during the wet months of summer). Our multi-level design focused on isolating 2 main parameters associated with terrestrial runoff, temperature and nutrient concentration. In the laboratory, these parameters were manipulated to emulate flood and non-flood conditions, whereas field experiments were conducted to examine the system as a whole (e.g. Marques et al. 2005, Xu 2005). The specific aims of this study were to (1) establish the growth rate response of *M. vertebralis* to elevated levels of nutrients and temperature in a controlled environment, (2) investigate the growth rates in the field during 2 contrasting seasons (the cool dry season and the warm wet season) along a known water quality gradient in the Whitsunday region and (3) compare growth rates from the controlled experiment with those obtained from the field experiments.

## MATERIALS AND METHODS

**Study area.** The Whitsunday Islands comprise >40 closely spaced continental islands and >100 fringing reefs (Hopley et al. 1989, Heap et al. 1999) that are situated in the central wet tropic region of the GBR from 20° 00' to 20° 30' S and 148° 45' to 149° 15' E. A persistent environmental gradient occurs across the Whitsunday Islands, in which increased turbidity (leading to reduced light availability for benthic primary producers), increased inorganic nutrient levels and chl *a* content are positively correlated with proximity to terrestrial discharge (van Woesik et al. 1999, Rohde et al. 2006, Cooper et al. 2007). This study focuses on 5 fringing back reefs in the Whitsunday Island group (Fig. 1) with known water quality parameters from point source river runoff (Tables S1 & S2 in the supplement at [www.int-res.com/articles/suppl/m435p097\\_supp.pdf](http://www.int-res.com/articles/suppl/m435p097_supp.pdf)).

**Water quality indicator: *Marginopora vertebralis*.** We examined the photosymbiont-bearing benthic foraminifer *M. vertebralis* Qui & Gaimard 1830 (Fig. 2). This species belongs to the Subfamily Soritinae and has been recognized in the literature both morphologically (Ross 1972, Gudmundsson 1994, Lee et al. 2004) and with molecular small subunit (SSU) rRNA gene sequences (Garcia-Cuetos et al. 2005). Adults can grow up to 30 mm in diameter (Ross 1972). Geographical genetic flow, for up to 100 km apart, has been documented among populations of *M. vertebralis* within the central GBR (Benzie & Pandolfi 1991). All members of the Soritinae house *Symbiodinium* dinoflagellate

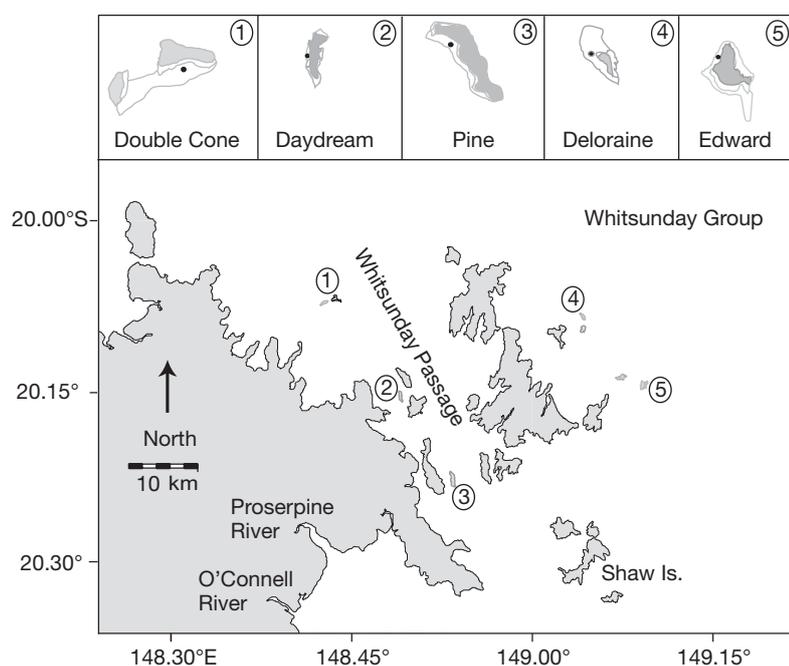


Fig. 1. Field experiment locations in the Whitsunday Islands, Great Barrier Reef, Australia. Terrestrial runoff and flood plumes from the Proserpine and O'Connell rivers' flow north through the Whitsunday Passage

photosymbionts (Langer & Lipps 1995, Lee et al. 1995). *M. vertebralis* populations are widely distributed among seagrass beds and in the epiphytic zones of the central Pacific (Smith 1968, Severin 1987, Langer & Hottinger 2000), and are known to reproduce asexually every 1 to 4 yr during late spring (Ross 1972, Fujita et al. 2000).

**Laboratory experiment: temperature and nutrient alterations.** *Marginopora vertebralis* were collected from Orpheus Island (18° 39' 23" S, 146° 29' 54" E) at 5 m water depth. The effects of temperature and dissolved inorganic nutrient concentration in the water were tested in a fully orthogonal 2-factor experiment, with 'Tank' as a nested factor (Fig. S1 in the supplement at [www.int-res.com/articles/suppl/m435p097\\_supp.pdf](http://www.int-res.com/articles/suppl/m435p097_supp.pdf)). For 42 d (from September to October 2008), 24 cylinders (500 ml) were set up in a 25°C temperature-controlled room at the Australian Institute of Marine Science (AIMS). To reduce crowding and facilitate identification of individuals, 5 *M. vertebralis* were placed in each cylinder. *M. vertebralis* were photographed at the beginning and end of the experiment. Unfiltered ambient seawater was pumped in from the ocean adjacent to the facilities at AIMS in Cape Ferguson, Queensland. The water was treated with 0.2 µM potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>), 2 µM of potassium nitrate (KNO<sub>3</sub>) or remained ambient. The added nutrients represent typical nutrient peaks experienced during flood plumes in the coastal reef zones (Schaffelke et al. 2003) as well as the range

found in the Whitsunday region during wet seasons (Cooper et al. 2007, Uthicke & Altenrath 2010). Two temperature treatments (22 and 28°C) represented average summer and winter sea surface temperatures in the central region of the GBR. Water temperatures remained within 22 ± 1 or 28 ± 1°C for the 22 and 28°C treatments, respectively, and were monitored hourly with temperature loggers. Salinity was recorded twice weekly with a refractometer and kept at 35 to 36 PSU. We simulated naturally low light conditions for all aquaria by administering a constant 6 to 9 mol photons m<sup>-2</sup> s<sup>-1</sup> for 12 h during the day using halogen lights that emit a light spectrum closely matched to that found in the field (Uthicke & Altenrath 2010).

**Field experiments: coastal water quality gradient.** TSS, dissolved and particulate nutrients, and chl *a* within the Whitsunday Island group vary naturally depending on the season and the proximity to point source, in addition to the flow conditions of the Proserpine and O'Connell rivers (Tables S1 to S3 in the supplement). Two field experiments were conducted: the first in the dry winter months, August to October 2008 (mean water temperature: 22°C), and the second in the wet summer months, January to February 2009 (mean water temperature: 28°C). The experimental duration for each experiment was 35 to 41 d. *Marginopora vertebralis* were collected from Shaw Island (20° 31' 02" S, 149° 04' 48" E) and photographed directly before deployment and after recovery. The specimens were deployed on the fringing back reefs of Double Cone, Pine, Daydream, Edward and Deloraine Islands (Fig. 1). The field sampling was not optimal as more replication would have been ideal; however, it was conducted in the best way possible given the logistical constraints. Using a method similar to that developed by Lee et al. (1983), 2 stakes were hammered into the substrate at a depth of 5 m below lowest astronomical tide, each with two 50 ml polypropylene tubes containing 5 *M. vertebralis* per tube. The tubes had four 8 × 2 cm slits and were covered by plankton mesh (500 µm mesh size) to allow water flow, a method previously used by Nobes et al. (2008) as well as Uthicke & Altenrath (2010).

**Organic carbon and nitrogen measurements.** Total foraminiferal carbon and nitrogen content was determined from a subset of individuals (n = 6 to 8 per site) from each field experiment. Prior to analysis, the samples were rinsed under freshwater and dried in a 60°C oven. A high resolution balance was used to weigh all

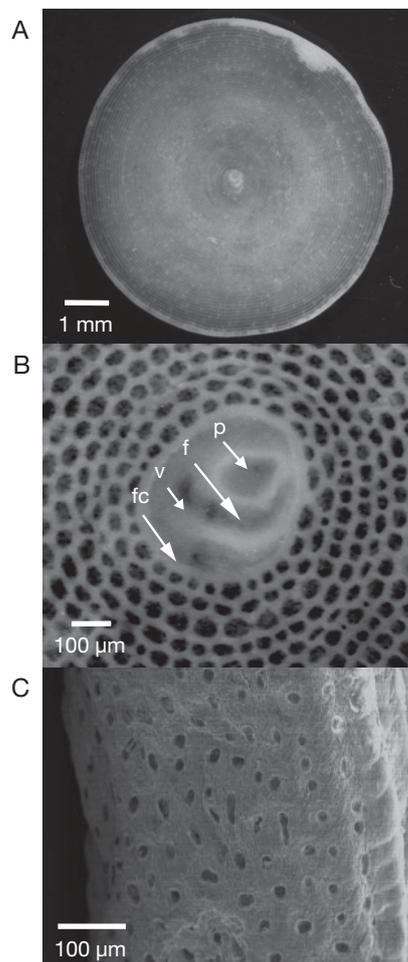


Fig. 2. *Marginopora vertebralis*. Key morphological characteristics: (A) biconcave, discoid tests with cyclic chamber formation and a central embryonic apparatus, (B) an embryonic apparatus (megalosphere) with p = proloculus, f = flexostyle, v = volhof and fc = flabeliform chamber; and (C) numerous and closely packed median apertures between marginal apertural rows with a raised rim (peripheral view)

individuals prior to homogenizing the test ( $\text{CaCO}_3$  shell) with a smooth agate mortar and pestle, and storing the test at  $-18^\circ\text{C}$ . From  $\sim 4$  mg of the fine powder, organic carbon and total nitrogen were determined using a Shimadzu TOC-V Analyser with a SSM-5000A solid sample module. Inorganic carbon was dissolved in a  $2 \text{ mol l}^{-1}$  HCl solution and evaporated on a hot plate. Two in-house standards, coastal sediment (3.40% C, 0.067% N) and Round 40 (0.86% C), were used to monitor the analytical quality. Calibration was carried out with MESS-1 certified reference material (2.99% C, 0.19% N).

**Water column measurements.** Surface water was sampled at each location to measure total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), dissolved organic carbon (DOC), particulate nitrogen

(PN), particulate phosphorus (PP), particulate organic carbon (POC), chl *a* and TSS. Subsamples for TDN and TDP were immediately filtered through a  $0.45 \mu\text{m}$  filter cartridge (Sartorius MiniSart N) into acid-washed screw-cap test tubes and stored at  $-18^\circ\text{C}$  (following the procedure of Cooper et al. 2007, Uthicke & Altenrath 2010). Using the standard wet chemical method for inorganic nutrients (Parsons et al. 1984), TDN and TDP samples were prepared by persulphate digestion (Valderrama 1981) before entering a segmented flow analyzer (Bran+Luebbe AA3). For particulate nutrients and chl *a*, subsamples were filtered through pre-combusted glass fibre filters (Whatman GF/F) and stored at  $-18^\circ\text{C}$ . PN was determined by high temperature combustion using an ANTEK 707/720 Nitrogen Analyzer (Furnas et al. 1995). After digestion in 5% potassium persulphate, PP was determined spectrophotometrically as inorganic  $\text{PO}_4$  (Parsons et al. 1984). Following acidification with concentrated phosphoric acid, POC was determined by high temperature combustion ( $950^\circ\text{C}$ ) using a Shimadzu TOC-V carbon analyzer with a SSM-5000s solid sample module. Chl *a* concentrations were measured fluorometrically (Turner Design 10AU) after grinding the filters in 90% acetone (Parsons et al. 1984). The amount of TSS was determined by weighing TSS on pre-weighed  $0.4 \mu\text{m}$  polycarbonate filters (47 mm in diameter, GE Water and Process Technologies) and determining the differences between pre- and post-filtered weights after drying overnight in a  $60^\circ\text{C}$  oven.

**Measurement methods.** For both field and laboratory experiments, each individual was photographed using a Canon EOS40D digital camera, with a Leica MZ16A  $1.0\times$  lens attached to a Leica MS5 microscope. Before each image was taken, algal growth on the surface of the foraminifer was removed. Surface area of each foraminifer was measured from the digital images by using Image-Pro 5 (Media Cybernetics). Dead foraminifera, indicated by a loss in symbiont colour, were excluded from the analysis.

**Data analysis.** Growth rates of the foraminifera were calculated from the change in area measured; we assumed a constant increment of growth per day. The relative growth rate per day ( $S_r$ ) was calculated as:

$$S_r = \left( \frac{S_e - S_i}{S_i} \right) \times \frac{1}{t} \quad (1)$$

where  $S_i$  is the initial surface area,  $S_e$  is the end surface area and  $t$  is the number of days in the experiment.

All growth data were  $\log_{10}$ -transformed. Replicated cylinders in the laboratory experiment ( $n = 8$ ) were a nested factor for the analysis. Similarly, the 4 holding containers at each of the 5 fringing reef locations were

likewise nested within the main factor Site. Because there was some mortality in the dry winter field experiment (<5%) and in the laboratory-controlled experiment (<8%), an unbalanced nested ANOVA was used with a type II partial sum of squares (Langsrud 2003). There was no mortality in the wet summer field experiment, so a fully orthogonal nested ANOVA with a type III sum of squares was used. For the laboratory-controlled experimental analysis, nutrients and temperature were analysed as fixed factors. Based on nutrient data collected here and in previous studies from the last 5 yr (Cooper et al. 2007, Uthicke & Altenrath 2010), the sites were grouped into low nutrient (Edward and Deloraine Islands) and high nutrient (Pine, Daydream and Double Cone Islands) categories to test the effect of proximity to coast and time, using a factorial ANOVA. We used a non-parametric Kruskal-Wallis analysis to test for differences between the growth rates obtained in the field and those in the laboratory. A Tukey's honestly significant difference (HSD) post hoc analysis was run to investigate the significant pairwise comparisons. All statistical tests were performed using the general linear model procedure in STATISTICA 9 (StatSoft).

Using PRISM version 5.0a (GraphPad Software), exponential non-linear regressions were calculated to express the water quality trends over distance from river discharge. This was calculated using the mean and standard error for DIN, PP, POC and TSS water column parameters; the sites were ordered according to distance from river discharge. Principal components analysis (PCA) was used to examine the relationship among site, year and water quality variables in PRIMER 6.1.10 (Primer-E). Data are presented as means  $\pm$  SE unless otherwise indicated.

## RESULTS

### Laboratory growth rates under the influence of temperature and nutrients

The laboratory experiments identified significant differences in foraminiferal growth between the nutrient treatments and the water temperature treatments, with no interaction between these effects (Fig. 3B, Table 1). Increased temperature had an additive negative effect on growth in both the nutrient-enriched water treatments and the control treatment. The highest growth rate was observed in the ambient (control) nutrient treatment ( $2.43 \pm 0.14 \mu\text{m}^2 \text{d}^{-1}$ ); this was significantly higher than in the nutrient-enriched DIP ( $1.20 \pm 0.07 \mu\text{m}^2 \text{d}^{-1}$ ) and DIN treatments ( $1.53 \pm 0.07 \mu\text{m}^2 \text{d}^{-1}$ ), as shown by the post hoc pairwise comparisons ( $p < 0.05$ ). Thus, growth rates of *Marginopora*

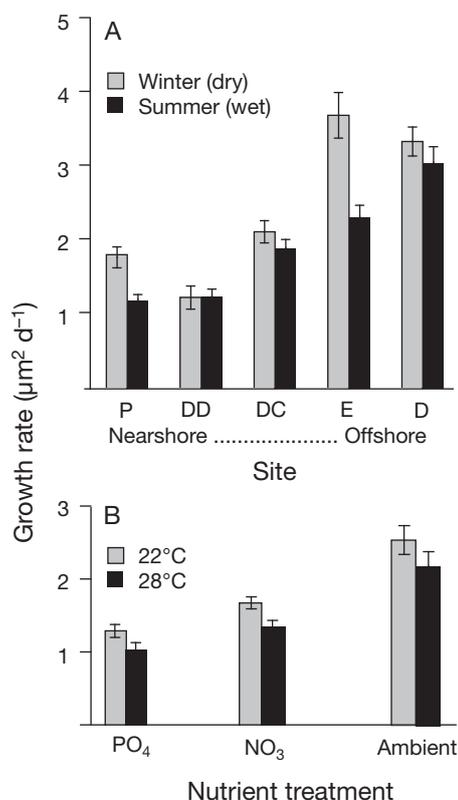


Fig. 3. *Marginopora vertebralis*. Daily growth (mean  $\pm$  SE) at the Whitsunday Islands, Great Barrier Reef, Australia. (A) The field experiment sites are ordered along the water quality gradient extending nearshore from Pine Island (P), Daydream Island (DD), Double Cone Island (DC), Edward Island (E) to Deloraine Island (D). (B) The laboratory experiment used the following temperature and nutrient treatments to emulate flooding conditions in the winter (22°C) and summer (28°C): PO<sub>4</sub> = 0.2  $\mu\text{M}$  KH<sub>2</sub>PO<sub>4</sub> added to seawater; NO<sub>3</sub> = 2  $\mu\text{M}$  KNO<sub>3</sub> added to seawater; ambient = ambient nutrient seawater

Table 1. Statistical results of the laboratory-controlled growth experiments of *Marginopora vertebralis*. Data were analysed with an unbalanced 2-factor (Nutrients and Temperature) nested ANOVA, with a type II partial sum of squares. All growth rates were log<sub>10</sub>-transformed and calculated relative to the initial foraminiferal surface area. \*Significant results ( $p < 0.05$ )

Effect	SS	df	MS	F	p
Nutrients	2.02	2	1.01	48.84	<0.001*
Temperature	0.11	1	0.11	5.31	0.024*
Nutrients $\times$ Temperature	0.65	20	0.03	1.58	0.077
Aquarium effect	0.06	1	0.06	2.76	0.100
Error	1.80	87	0.02		

*vertebralis* were reduced by 51% in the DIP enriched treatment and by 37% in the DIN enriched treatment compared with the ambient control. The mean growth

rate at 28°C was 17% lower ( $1.54 \pm 0.10 \mu\text{m}^2 \text{d}^{-1}$ ) than that at 22°C ( $1.84 \pm 0.10 \mu\text{m}^2 \text{day}^{-1}$ ) (Fig. 3B, full results are presented in Table S3 in the supplement).

### Field experiment under the influence of water quality

Both field experiments showed significant differences among sites (Table 2, Fig. 3A). There was a significant division between the growth rate of *Marginopora vertebralis* from Deloraine and Edward islands compared with that at Pine and Double Cone islands from both sampling periods. Daydream Island exhibited no significant difference to any other site in the summer and only a significant difference with Double Cone and Edward in the winter (Table S4 in the supplement at [www.int-res.com/articles/suppl/m435p097\\_supp.pdf](http://www.int-res.com/articles/suppl/m435p097_supp.pdf)). Growth rates were highest at the offshore site of Deloraine Island with a mean rate of  $3.32 \pm 0.20 \mu\text{m}^2 \text{d}^{-1}$  in the winter and  $3.05 \pm 0.21 \mu\text{m}^2 \text{d}^{-1}$  in the summer, whereas at the nearshore site of Pine Island, the mean growth rate of *M. vertebralis* was  $1.76 \pm 0.14 \mu\text{m}^2 \text{d}^{-1}$  during the winter and  $1.23 \pm 0.07 \mu\text{m}^2 \text{d}^{-1}$  in the summer.

In a second analysis, we grouped samples from each reef *a priori* according to proximity to coast (Pine, Daydream and Double Cone islands as the high nutrient group; Deloraine and Edward islands as the low nutrient group) and time period (Table 2). A significant interaction between the factors of Proximity and Time was also present in the 2-factor ANOVA. This was caused by an additive effect when summer flooding reduced the growth rate (low nutrient:  $2.67 \pm 0.46 \mu\text{m}^2$

Table 2. Statistical results of *Marginopora vertebralis* field growth comparisons from 5 fringing back reefs in the Whitsunday region, Great Barrier Reef, Australia. The experiment was replicated once in the dry winter and once in the wet summer. For the last analysis, the sites were grouped into low nutrient (Edward and Deloraine islands) and high nutrient (Pine, Daydream and Double Cone islands) categories to test the effect of proximity to coast and time, using a factorial ANOVA. All growth rates were  $\log_{10}$ -transformed and calculated relative to the initial foraminiferal surface area.

\*Significant results ( $p < 0.05$ )

Effect	SS	df	MS	F	p
Dry winter					
Site	3.614	4	0.904	32.279	<0.001*
Nested effect	0.739	15	0.049	1.760	0.057
Error	2.127	76	0.028		
Wet summer					
Site	2.353	4	0.588	37.449	<0.001*
Nested effect	0.363	15	0.024	1.539	0.111
Error	1.257	80	0.016		
Proximity to shore	107.083	1	107.083	165.840	<0.001*
Time	13.261	1	13.261	20.538	<0.001*
Proximity to shore × Time	3.848	1	3.848	5.959	0.016*
Error	123.975	192	0.646		

$\text{d}^{-1}$ , high nutrient:  $1.45 \pm 0.39 \mu\text{m}^2 \text{d}^{-1}$ ) compared with the dry winter period (low nutrient:  $3.51 \pm 0.50 \mu\text{m}^2 \text{d}^{-1}$ , high nutrient:  $1.69 \pm 0.50 \mu\text{m}^2 \text{d}^{-1}$ ). The presence of the significant interaction supports the statement that growth rates are on average lower in the nearshore reefs during the flooding season.

Table 3. *Marginopora vertebralis*. Summary of the mean growth rates ( $\pm$ SE) of *M. vertebralis* from the Whitsunday region, Great Barrier Reef, Australia, from field and laboratory experiments. The last column indicates which growth rates were significantly higher (>) or lower (<) than the growth rate in the specific treatment as shown by Tukey's HSD post hoc pairwise comparisons after Kruskal-Wallis non-parametric tests ( $H_{15} = 186.4881$ ,  $p < 0.001$ ,  $n = 309$ ); the full table (Table S4) is available in the supplement at [www.int-res.com/articles/suppl/m435p097\\_supp.pdf](http://www.int-res.com/articles/suppl/m435p097_supp.pdf). DIN:  $2 \mu\text{m KNO}_3$  added to seawater; DIP:  $0.2 \mu\text{m KH}_2\text{PO}_4$  added to seawater; DW: dry winter; WS: wet summer; MSE: mean square error

Treatment	n	MSE	df	Growth rate ( $\mu\text{m}^2 \text{d}^{-1}$ )	Significant post hoc results
Ambient 22°C	20	0.02	88	$2.54 \pm 0.20$	> Pine & Double Cone
Ambient 28°C	16	0.02	88	$2.29 \pm 0.21$	> Pine (WS only) & Double Cone
DIN 22°C	20	0.02	88	$1.68 \pm 0.08$	< Edward (DW only) & Deloraine
DIN 28°C	18	0.02	88	$1.35 \pm 0.09$	< Deloraine & Edward
DIP 22°C	20	0.02	88	$1.30 \pm 0.09$	< Deloraine & Edward
DIP 28°C	19	0.02	88	$1.09 \pm 0.10$	< Deloraine, Edward, Daydream & Ambient 22°C
Deloraine DW	20	0.03	76	$3.32 \pm 0.20$	> DIN 22 & 28°C; DIP 22 & 28°C
Edward DW	18	0.03	76	$3.68 \pm 0.31$	> DIN 22 & 28°C; DIP 22 & 28°C
Daydream DW	19	0.03	76	$2.11 \pm 0.15$	> DIP 28°C
Double Cone DW	19	0.03	76	$1.22 \pm 0.16$	< Ambient 22 & 28°C
Pine DW	20	0.03	76	$1.76 \pm 0.14$	< Ambient 22°C
Deloraine WS	20	0.02	80	$3.05 \pm 0.21$	> DIN 22 & 28°C; DIP 22 & 28°C
Edward WS	20	0.02	80	$2.31 \pm 0.16$	> DIN 28°C; DIP 22 & 28°C
Daydream WS	20	0.02	80	$1.89 \pm 0.12$	> DIP 28°C
Double Cone WS	20	0.02	80	$1.24 \pm 0.10$	< Ambient 22°C & 28°C
Pine WS	20	0.02	80	$1.23 \pm 0.07$	< Ambient 22°C & 28°C

### Organic carbon and nitrogen measurements

The mean C:N ratio was significantly higher in the winter ( $13.5 \pm 1.5$ ) compared to the summer ( $10.0 \pm 0.6$ ) (Fig. 4A, Table 4). The total organic carbon (TOC) content in the foraminifera was constant among sites and differed between the sampling years; on average, TOC percent composition was  $0.117 \pm 0.011\%$  during the summer and  $0.106 \pm 0.013\%$  in the winter (Fig. 4B, Table 4). The total content of nitrogen has an interactive effect of site and year (Table 4). The highest mean percent nitrogen content occurred at Double Cone ( $0.014 \pm 0.002\%$ ) and Pine islands ( $0.013 \pm 0.002\%$ ) during the summer and the lowest percent nitrogen content was at Deloraine Island in the winter ( $0.007 \pm 0.001\%$ ) (Fig. 4C).

### Water column measurements

Water quality parameters were measured at each site during the 2 field sampling events. Increased loading of PP, DIN, TSS and TOC was observed at the high nutrient sites during the summer sampling period relative to the low nutrient sites (Fig. 5, Table S3). A constantly low concentration of PP was recorded at all sites in the winter, with a mean concentration of  $0.072 \pm 0.019 \mu\text{mol l}^{-1}$ . PP increased in the summer at all sites, with the greatest concentration at nearshore Pine Island ( $2.017 \pm 0.279 \mu\text{mol l}^{-1}$ ) and the lowest at the offshore site at Deloraine Island ( $1.222 \pm 0.225 \mu\text{mol l}^{-1}$ )

Table 4. Total organic carbon (TOC) and total organic nitrogen (TON) from subsamples of *Marginopora vertebralis* retrieved from the 5 sites in the Whitsunday region, Great Barrier Reef, Australia. An unbalanced 2-factor ANOVA was used with a type II partial sum of squares to examine the interaction of Site and Year on the C and N stoichiometry in *M. vertebralis*. \*Significant results ( $p < 0.05$ )

Effect	SS	df	MS	F	p
<b>C:N molar mass</b>					
Site	31.14	4	7.79	1.40	0.245
Year	208.91	1	208.91	37.57	<0.001*
Site × Year	31.30	4	7.83	1.41	0.243
Error	333.67	60	5.56		
<b>TOC molar mass</b>					
Site	0.005	4	0.001	2.449	0.056
Year	0.002	1	0.002	4.443	0.039*
Site × Year	0.003	4	0.001	1.532	0.204
Error	0.031	60	0.001		
<b>TON molar mass</b>					
Site	0.0001	4	0.0000	3.943	0.007*
Year	0.0003	1	0.0003	59.669	<0.001*
Site × Year	0.0001	4	0.0000	3.209	0.019*
Error	0.0003	60	0.0000		

(Fig. 5A). The amount of DIN followed the same yearly trend, with consistently low concentrations at all sites in the winter and a mean of  $0.161 \pm 0.041 \mu\text{mol l}^{-1}$ . In the summer, DIN concentrations increased closer to

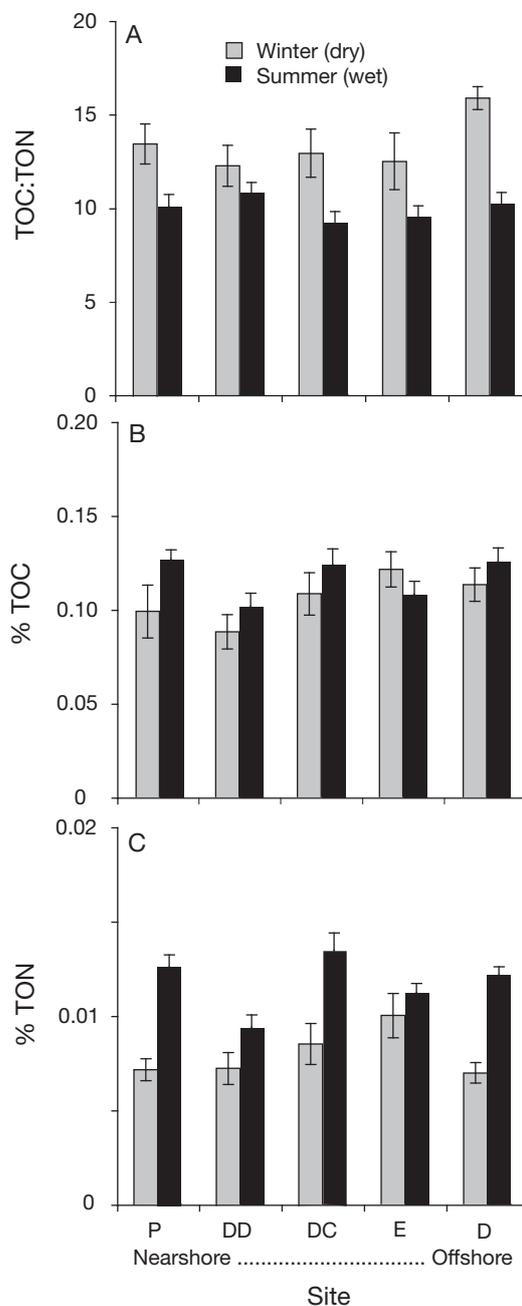


Fig. 4. Total organic carbon (TOC) and total organic nitrogen (TON) from subsamples of *Marginopora vertebralis* retrieved from 5 fringing back reefs in the Whitsunday region, Great Barrier Reef, Australia, in field experiments. Panels show means  $\pm$  SE of (A) the ratio of TOC:TON, (B) percentage of TOC and (C) percentage of TON in skeletons of *M. vertebralis*. The x-axis follows the water quality gradient from nearshore to offshore, starting at Pine Island (P) and then continuing to Daydream Island (DD), Double Cone Island (DC), Edward Island (E) and Deloraine Island (D)

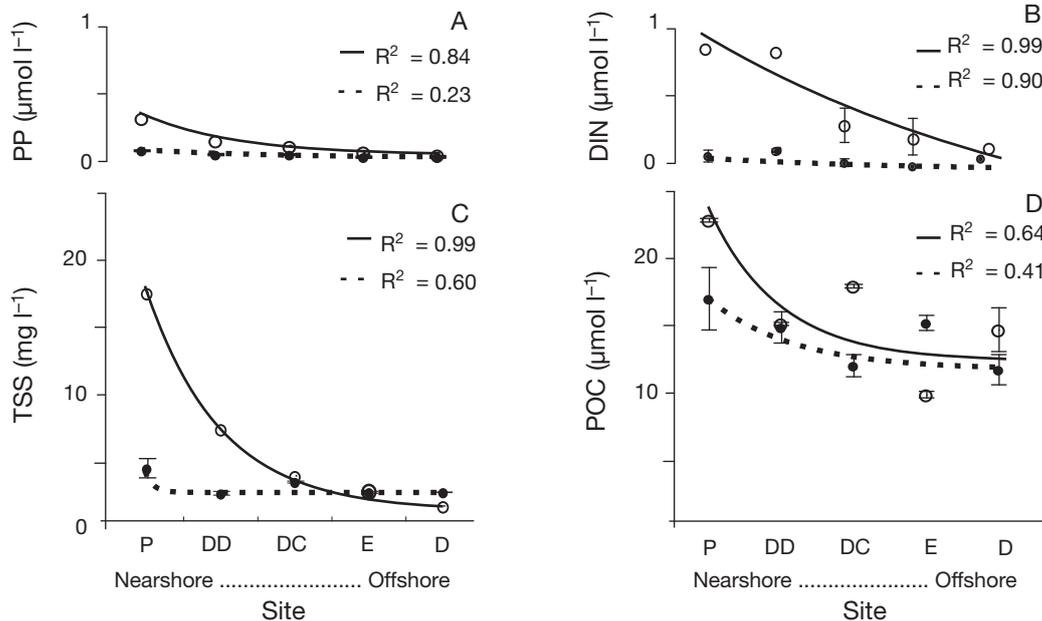


Fig. 5. Summary of the 4 primary water quality variables measured at each site during the summer (open circles, solid line) and winter (closed circles, dashed line) experimental periods at the Whitsunday Islands, Great Barrier Reef, Australia. The x-axis follows the water quality gradient extending from nearshore to offshore, starting at Pine Island (P) and then continuing to Daydream Island (DD), Double Cone Island (DC), Edward Island (E) and Deloraine Island (D). The y-axis shows the mean ( $\pm$ SE) water column concentrations of (A) particulate phosphorus (PP), (B) dissolved inorganic nitrogen (DIN), (C) total suspended solids (TSS) and (D) particulate organic carbon (POC)

the river runoff ( $0.917 \mu\text{mol l}^{-1}$  from Pine Island;  $0.236 \mu\text{mol l}^{-1}$  from Deloraine Island) (Fig. 5B). Compared to winter, the TSS measured in the summer increased by ~5-fold in the nearshore waters surrounding Pine Island, by ~4-fold at Daydream Island, only a slight increase was observed at both Edward and Double Cone islands and a ~3-fold decrease was observed at Deloraine Island (Fig. 5C). The content of POC had a similar year–site trend as the other water quality parameters (Fig. 5D). The content of POC in the water column at Pine Island was  $16.51 \pm 3.99 \mu\text{mol l}^{-1}$  in the winter and  $23.59 \pm 0.22 \mu\text{mol l}^{-1}$  in the summer. To a lesser extent than the high nutrient sites, the low nutrient site at Deloraine Island also exhibited increased POC in the summer compared with the winter ( $13.70 \pm 2.79 \mu\text{mol l}^{-1}$  in the summer;  $10.10 \pm 0.22 \mu\text{mol l}^{-1}$  in the winter). The PCA further illustrates the relationship of the water column variables among the sites during the 2 sampling times with a distinct separation between the summer and the winter (Fig. 6). The PCA shows that Deloraine, Edward and Double Cone islands displayed a negative association with DIN, PP, POC and TSS during both years whereas the nearshore Pine and Daydream islands,

during the wet summer sampling period, had the closest association with these parameters.

### Comparison of laboratory and field results

A Kruskal-Wallis test was used to compare the growth data from the laboratory and field experiments (Table 3, Table S4). The highest growth rates were

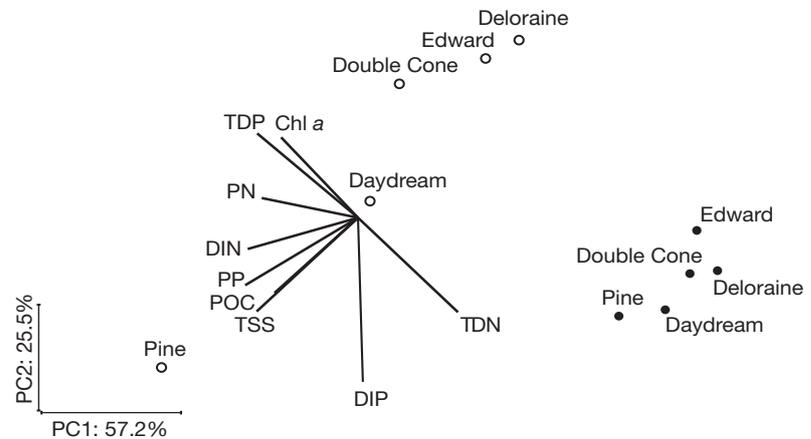


Fig. 6. Principal components analysis (PCA) of the water quality variables at the Whitsunday Islands during the dry winter (●) and wet summer (○). Chl a: chlorophyll a; DIN: dissolved inorganic nitrogen; DIP: dissolved inorganic phosphate; PN: particulate nitrogen; POC: particulate organic carbon; PP: particulate phosphorus; TDN: total dissolved nitrogen; TDP: total dissolved phosphorus; TSS: total suspended solids

observed at Deloraine and Edward islands ( $3.32 \pm 0.20$  and  $3.68 \pm 0.31 \mu\text{m}^2 \text{d}^{-1}$ , respectively) in the winter. The growth rates from *Marginopora vertebralis* at Edward and Deloraine islands are comparable to those from the ambient treatment in the laboratory experiment ( $2.54 \pm 0.2 \mu\text{m}^2 \text{d}^{-1}$  at  $22^\circ\text{C}$ ;  $2.29 \pm 0.2 \mu\text{m}^2 \text{d}^{-1}$  at  $28^\circ\text{C}$ ) and are significantly greater than the growth rates from the DIP and DIN treatments. The growth rate of *M. vertebralis* at Daydream Island was comparable to that of the laboratory treatments and was significantly greater than that of the  $28^\circ\text{C}$  DIP treatment. The reduced growth rates at Pine and Double Cone islands during both sampling times were equivalent to those observed in the DIN and DIP treatments and significantly lower than that observed in the ambient nutrient treatment from the laboratory experiment.

## DISCUSSION

The field and laboratory experiments presented here characterise the effects of enhanced nutrients from terrestrial sources and temperature on  $\text{CaCO}_3$  production by *Marginopora vertebralis*. Understanding the ecological response of photosymbiont-bearing benthic foraminifera to eutrophication is highly relevant because they are widely distributed throughout reef biotypes (Langer & Hottinger 2000), they are ecological indicators of water quality (Hallock et al. 2003) and they contribute significant proportions of  $\text{CaCO}_3$  to the shallow marine sediment (Scoffin & Tudhope 1985). Slower growth rates of *M. vertebralis* were associated with increased summer temperatures and additional nutrient input within the nearshore region of the Whitsunday Islands. These findings indicate that nearshore water, which is seasonally enriched with terrestrially derived nutrients, reduces the growth rate of *M. vertebralis*. In order to explore the cross effects of temperature, DIN and DIP, a manipulative experiment was conducted on *M. vertebralis*. Results from the 2 sampling events in the field strongly mimic those from the laboratory experiment. Below, we first discuss the results in regard to possible oxidative stress caused by the thermal adjustment and aerobic demand (Pörtner 2002). Secondly, we raise the hypothesis that *M. vertebralis* growth rates are altered by the destabilisation of photosymbiosis through the release from nutrient limitation, similar to photosymbiont-bearing corals (Dubinsky & Berman-Frank 2001).

### The effect of temperature

Thermal tolerance and oxygen consumption are determining factors that regulate metabolic rate, body

size and ventilatory efficiency among marine organisms (Pörtner 2002). Thermal stress among coral reef foraminifera can influence community composition and species distributions (Langer & Hottinger 2000). Diatom-bearing hyaline foraminifera respond to heat stress ( $\sim 32^\circ\text{C}$ ) physiologically by housing fewer symbionts, through a reduced area in lipid bodies and greater areas of vacuolar endoplasm and ectoplasm (Talge & Hallock 2003). However, it may be more accurate to compare the foraminiferal response to that in corals due to the symbiont similarity between hermatypic corals and dinoflagellate-bearing porcelainous foraminifera. For example, among corals, elevated sea surface temperature induces bleaching (Hoegh-Guldberg & Smith 1989, Lesser et al. 1990, Glynn et al. 1993) and ultimately regulates internal energy budgets, which affect growth, fecundity and life history evolution (Pörtner & Knust 2007). Our results suggest that the summer temperature of  $28^\circ\text{C}$  is near the upper limit before the onset of thermal stress for *Marginopora vertebralis*. Although these temperatures are not lethal, the physiological capacity to maintain growth appears to be compromised. This is of interest as ongoing anthropogenic threats, such as climate change, may be more severe in regions with diminished water quality (Wooldridge & Done 2009), and therefore act in a synergistic manner influencing growth patterns.

### The effect of eutrophication

Within coral reef ecosystems, the availability of nutrients, essential for host and symbiont metabolism, varies throughout the water column. The success of photosymbiont-bearing organisms, such as corals and large benthic foraminifera, has occurred in high-light and oligotrophic conditions and is attributed to the photosymbiont translocation of photosynthate to the host (Muscatine & Lenhoff 1983, Muscatine et al. 1984). Photosynthetically driven  $\text{CO}_2$  reduction provides energy-rich carbohydrates used to support metabolic activities and cell growth. Ter Kuile & Erez (1987) noted an internal competition between the foraminiferal host and its photosymbiont for these carbohydrates. The uncoupling of the algal production of carbohydrates and cell growth is largely governed by nutrient limitation within the photosymbiont (Dubinsky & Berman-Frank 2001). As described by Redfield (1958), the atomic ratio of C:N in aquatic primary producers is  $\sim 6.6$ . Coupling occurs between photosynthesis and cell growth within the algal photosymbiont as the proportion of nutrients becomes closer to the Redfield ratio within the photosymbiont (Dubinsky & Berman-Frank 2001); therefore, the carbohydrates are

retained by the photosymbiont instead of being translocated to the host. Previous studies have observed high symbiont density in foraminifera as a response to eutrophication (e.g. Talge & Hallock 2003), similar to trends observed in corals (Falkowski et al. 1993, Steven et al. 1997). This mechanism, as observed in corals (Dubinsky & Berman-Frank 2001), may explain reduced skeletal growth rates in the foraminiferal host due to reduced energy supply.

The organic carbon and nitrogen contents measured here in *Marginopora vertebralis* (as a holobiont) were not carbon-limited at the site level, although a slightly higher organic carbon content was measured during the flooding period in the summer. In contrast, nitrogen content showed an interactive effect of site and year, where nitrogen content increased mostly from the nearshore sites of Double Cone and Pine islands in the summer. In this case, the nitrogen content was driving the change in the C:N ratio, as the carbon content was uniform. A distinctly lower C:N ratio was measured in *Amphistegina radiata* and *Heterostegina depressa* during the wet summer (Uthicke & Altenrath 2010), similar to the ratio found in *M. vertebralis* in this study. Even though the C:N values were higher in the dry winter in *M. vertebralis* (~13), they never matched that of *A. radiata* and *H. depressa* (~20) from the same region. Our measurements are comparable to those of Muscatine et al. (1989), who measured a coral C:N ratio between 4 and 19, with a clear reduction when the water was enriched with nitrogen. The symbiosis found in corals is comparable to that in *M. vertebralis*, as they both house similar dinoflagellate algae (*Symbiodinium* spp.). For corals, the release from nutrient limitation is expected to destabilize the translocation of carbon from symbiont to the host and, in exchange, benefit symbiont growth (Dubinsky & Berman-Frank 2001). Therefore, it is possible that the same mechanism is driving a reduction in the amount of carbon being transferred from the symbiont to the foraminiferal host when waters are enriched with concentrated nutrients. However, given the physiological differences of symbiosis among corals (intercellular) and foraminifera (intracellular), future investigation is required.

A number of environmental parameters within coastal marine zones are influenced by river runoff. Long-term (>5 yr) monitoring results for the Whitsunday water column (Cooper et al. 2007) are comparable to broad-scale observations of environmental parameters from coastal waters adjacent to the major GBR catchments (Brodie et al. 2007). Excessive input of nutrients is known to shift the microbial benthic communities in coastal marine systems (Meyer-Reil & Köster 2000). Benthic foraminiferal composition has provided a reliable indication of water quality shifts

(Hallock et al. 2003, Schueth & Frank 2008, Uthicke & Nobes 2008). Specifically, the FORAM Index (Hallock et al. 2003) uses proportional abundance of 3 functional groups (photosymbiont bearing, opportunistic and smaller heterotrophic taxa) to calculate the 'suitability' of water quality to support reef growth. Within the Whitsunday region, ecosystem shifts are related to eutrophication (van Woesik et al. 1999, Fabricius et al. 2005, Cooper et al. 2007). In particular, benthic foraminifera from the GBR shift from photosymbiont-bearing species (i.e. mixotrophic) towards a greater abundance of heterotrophic species as water quality decreases (Uthicke & Nobes 2008, Uthicke et al. 2010). Shifts in proportional abundances may transpire from slower host growth among photosymbiont-bearing foraminifera because of a longer growth period before reproduction, thereby shifting the proportional abundance of photosymbiont-bearing LBF in the community over time.

### Effects on CaCO<sub>3</sub> production

Photosymbiont-bearing LBF contribute a significant portion of calcareous sediment to the reef environment (Scoffin & Tudhope 1985). Any changes in the growth rates of LBF have the potential to substantially alter the annual CaCO<sub>3</sub> budget within coral reefs (Maxwell 1968, Hallock et al. 1986, Langer et al. 1997, Fujita et al. 2000). In summary, different growth rates of *Marginopora vertebralis* under a flood plume scenario (warmer water and peak DIP and DIN) from the laboratory treatments caused a 22 to 55% yr<sup>-1</sup> reduction of CaCO<sub>3</sub>. From the field experiments, closer proximity to point source runoff (i.e. highly affected by terrestrial runoff) could equate to a 45 to 65% yr<sup>-1</sup> loss of CaCO<sub>3</sub> from *M. vertebralis*. The slower growth and loss of CaCO<sub>3</sub> production in LBF could also occur in other photosymbiont-bearing calcareous marine organisms, such as calcareous algae and corals, when the symbionts are released from nutrient limitation. Therefore, sediment composition is not only influenced by the input of terrestrial sediments and organic matter, but also by the declining CaCO<sub>3</sub> production rate.

### Observed variability and environmental complexities

Earlier growth studies on foraminifera have observed variability in the response of species in relation to additional dissolved inorganic nutrients. From laboratory experiments, Lee et al. (1980) concluded that all symbionts within benthic foraminifera are naturally nitrogen and phosphate limited. In contrast, Ter Kuile

et al. (1987) recorded a 5-fold increase in the growth rate of *Amphistegina lessonii* and a doubling of *Amphisorus hemprichii* when cultured in higher DIP and nitrate. Similar results have been observed in diatom-bearing *Heterostegina depressa* and *Amphistegina radiata* as a result of high concentrations of dissolved and particulate nutrients (Uthicke & Altenrath 2010). This variation can be attributed to different calcification mechanisms and carbon budgets between species (Ter Kuile & Erez 1991) and differences in the symbiont assemblages within the host (Leutenegger 1983).

There are a number of environmental complexities to consider that may account for a proportion of the site variability observed in the present study. The reasoning behind conducting accompanied laboratory experiments was to control for likely environmental conditions that influence the Whitsunday region and most inshore reefs on the GBR; such influences include localised hydrodynamics (Debenay et al. 2005), salinity (Reddy & Rao 1984, Caron et al. 1987) and light (Talge & Hallock 2003, Nobes et al. 2008). Growth rates in the laboratory experiments were slightly lower than those observed in the field. However, experimental trends were in line with field observations, with increasing growth rates occurring at ambient nutrient levels and tropical winter temperatures. The range of DIN and DIP concentrations from the terrestrially influenced nearshore locations in the field was above the beneficial level for optimal growth rates of *Marginopora vertebralis*. The growth rates from the offshore reefs at Edward and Deloraine islands were within the range of the ambient conditions measured in the aquaria. The cooler dry winter had consistently higher growth rates than the warmer wet summer, similar to the trend seen in the temperature treatments in the laboratory experiment.

## CONCLUSIONS

Human populations in coastal zones have altered landscapes, terrestrial runoff and nutrient fluxes at accelerating rates over the past century (Vitousek et al. 1997, Rabalais 2002, Galloway et al. 2003, Cooper et al. 2007, Dinsdale et al. 2008). The present study highlights a quantitative approach to understanding the biological response of shifting environmental parameters through multilevel testing of laboratory experiments with a field analogy. The current use of LBF has proved successful in describing one response to enhanced nutrients and temperature in a terrestrially influenced marine ecosystem. It has recently been suggested that one management option to improve reef resilience to global change is to reduce local stressors

by improving water quality (Marshall & Johnson 2007, Wooldridge & Done 2009). Here we show direct evidence that reduced nutrient runoff can have beneficial outcomes for the growth success of benthic photosymbiont-bearing foraminifera. Nutrient enrichment and increased temperature clearly had an additive effect; thus, improving local conditions can ameliorate additive environmental stresses such as ocean acidification and increased temperature. In future studies, ecophysiological examination of the photosymbiont cell density and pigment will clarify the proposed mechanism of symbiosis destabilization.

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