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Effects of phosphate on growth and skeletal density in the scleractinian coral *Acropora muricata*: A controlled experimental approach

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ABSTRACT

Phosphate contamination can negatively affect corals, modifying growth rates, skeletal density, reproduction, mortality, and zooxanthellae. We determined the effects of elevated phosphate on coral growth and density. Genetically distinct colonies of *Acropora muricata* were sub-divided and distributed among three 110-L aquaria, and exposed to phosphate levels of 0.09, 0.20, and 0.50 mg L^{-1} for four months. Total skeletal length, living tissue length, weight, branch production, and polyp extension were measured. Linear extension and tissue growth increased under all conditions. Growth rates were highest at a phosphate concentration of 0.50 mg L^{-1}. Weight increased through time, graded from low to high with phosphate concentration. Density decreased through time, and was significantly lowest in the high phosphate treatment. Phosphate concentration produced no visible effects of stress on the corals, as indicated by polyp extension and lack of mortality. It is suggested that the phosphate enhanced growth was due to increased zooxanthellar populations and photosynthetic production within the coral. Skeletal density reduction may be due to phosphate binding at the calcifying surface and the creation of a porous and structurally weaker calcium carbonate/calcium phosphate skeleton. Increased phosphate concentrations, often characteristic of eutrophic conditions, caused increased coral growth but also a more brittle skeleton. The latter is likely more susceptible to breakage and damage from other destructive forces (*e.g.*, bioerosion) and makes increased coral growth a poor indicator of reef health.

1. Introduction

1.1. Background

Coral reefs are one of the most productive and biodiverse ecosystems in the world (Jackson, 1991; Moberg and Folke, 1999; Reaka-Kudla, 1997; Roberts et al., 2002; Sebens, 1994). Unfortunately, coral colonies are disappearing at an alarming rate. Ten percent of the world's reefs are already seriously damaged (Miller, 1999; also see Knowlton and Jackson, 2008; Tamelander and Rajasuriya, 2008; Schutte et al., 2010), and sixty percent are predicted to exhibit serious damage within the next 20–40 years. Causes of this damage include increased sea surface temperatures (Baker et al., 2008; Hoegh-Guldberg, 1999; Wild et al., 2010), disease (Aeby et al., 2011; Aronson and Precht, 2001; Green and Bruckner, 2000; Patterson et al., 2002; Williams and Miller, 2005), overfishing of reef fish (Hay and Rasher, 2010; McManus 1997), mortality of grazing sea urchins in the Caribbean (Hughes, 1994; Hughes et al., 2010; Sammarco 1980, 1982a, 1982b), urban development (Finkl and Charlier, 2003; Hawkins and Roberts, 1994; Reopanichkul et al., 2010), exploitation for the aquarium trade (Laurance, 2010; Wood, 2001), and physical damage from divers (Guzner et al., 2010; Juhasz et al., 2010; Zakai and Chadwick-Furman, 2002).

Eutrophication can also cause coral mortality and sub-lethal effects on coral reefs (Hunter and Evans, 1995; Hunter and Stephenson, 1999; Lapointe et al., 1990, 2004; Rothenberger et al., 2008; Smith et al., 1981). Coastal waters can become eutrophic due to the addition of fertilizer (DeGeorges et al., 2010; Fishelson, 1973), sewage (Costa et al., 2000; DeGeorges et al., 2010), and other terrestrial run-off (Fabricius, 2005), increasing phosphorus and nitrogen concentrations in nearby reef systems (Costa et al., 2000; Lapointe et al., 2003, 2010). Human populations are increasing along coastal regions, with about two-thirds of the world's population (~3.9 billion people) now residing within 160 km of the coast. This number is estimated to increase to 6.2 billion by 2025 (Miller, 1999).

Corals possess endosymbiotic zooxanthellae (genus *Symbiodinium*) which receive metabolic wastes in the form of phosphorus, nitrogen, and carbon dioxide from the coral, while the coral receives oxygen and carbohydrates from the photosynthetic activities of the zooxanthellae (Baker, 2003; Barnes and Hughes, 1982; Furla et al., 2011; Trench, 1993; Yonge et al., 1932). The symbiotic relationship between coral and zooxanthellae has been well studied, although the complete details

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of this interaction are still unclear. The coral hosts are clearly dependent upon the zooxanthellae for survival, because the zooxanthellae transfer up to 99% of their photosynthetic products to the coral via translocation (Muscatine and Cernichiari, 1969;) of such products as glycerol, glucose, and alanine (Barnes, 1987; Falkowski et al., 1984; Muscatine and Cernichiari, 1969; Stambler, 2011). Although corals obtain up to 95% of their carbon requirements from the zooxanthellae (Muscatine, 1990; Muscatine et al., 1981), many species still must acquire additional nutrients and energy from zooplankton (Johannes et al., 1970). The degree of dependence upon zooxanthellar products varies according to coral species (Porter, 1976) and depth of the colony (Falkowski et al., 1984; Muscatine et al., 1989). For example, massive corals (*e.g. Montastraea*) can be more heterotrophic, while some branching corals (*e.g. Acropora*) rely mainly on zooxanthellae to meet their energy needs (Porter, 1976). Zooxanthellar photosynthesis and coral calcification are tightly linked (Al-Horani et al., 2003; Allemand et al., 2004; McConnaughey and Whelan, 1997; Suzuki et al., 1995).

Zooxanthellae require nutrients (P and N), dissolved inorganic carbon (DIC), and solar radiation for photosynthesis. Hermatypic corals require, minimally, DIC, ATP, and calcium for calcification.

The endosymbiotic zooxanthellae are concentrated in the oral endodermal cells surrounding the coelenteron of the coral polyp (Allemand et al., 2004). Changes in rates of calcification, photosynthesis, exchange of seawater through the mouth, and trans-epithelial ionic transport can affect the chemistry of the coelenteron (Gattuso et al., 1999). Expansion or retraction of coral polyps can affect calcium transport via the mouth. Calcium ions enter into the coelenteron via the mouth and/or by transport across the oral epithelium. Elevated phosphate levels in seawater may disrupt normal carbon cycle functions and concentrations of compounds produced and shared within the holobiont, resulting in abnormal growth. The chemistry of the coelenteron can differ from that of the surrounding seawater due to internal processes (*e.g.* photosynthesis, respiration, calcification; Al-Horani et al., 2003), as well as reduced flushing of water through the mouth (Wright and Marshall, 1991). Recently, it was determined that P/Ca ratios in coral skeletons provide a record of phosphate concentrations and thus nutrient availability in the water through geological time (LaVigne et al., 2008, 2010).

1.2. Effects of phosphate enrichment on corals

Average phosphorus concentrations on natural reefs are generally low (0.01 mg L^{-1}; Kleypas et al., 1999), but there appears to be a trend towards increasing values in recent years. For example, concentrations up to 0.266 mg L^{-1} have been documented on some reefs in the Southern Gulf of Mexico (Cruz-Piñón et al., 2003). Elevated phosphate levels are known to affect several aspects of coral health, such as growth rate (Bucher and Harrison, 2002; Ferrier-Pagès et al. 2000; Koop et al., 2001; Renegar and Riegl, 2005; Steven and Broadbent, 1997), skeletal density (Koop et al., 2001; Rasmussen et al., 1993), reproduction (Harrison and Ward, 2001; Koop et al., 2001), mortality (Koop et al., 2001; Walker and Ormond, 1982), disease susceptibility (Bruno et al., 2003), and zooxanthellar density (Koop et al., 2001; Steven and Broadbent, 1997; Takabayashi, 1996). Phosphate can stimulate phytoplankton population growth, potentially reducing the availability of light to corals (Walker and Ormond, 1982), or stimulating growth of benthic filamentous algae that can overgrow corals (Done, 1992). Overgrowth by filamentous algae can be particularly detrimental in areas where populations of grazers such as fish and sea urchins have been dramatically reduced by overfishing or disease (Hughes and Connell, 1999).

The effects of phosphate on coral condition and/or growth have been studied both in the lab and the field (Ferrier-Pagès et al., 2000; Kinsey and Davies, 1979; Koop et al., 2001; Rasmussen, 1986, 1988; Rasmussen et al., 1993; Renegar and Riegl, 2005; Stambler et al., 1991). The results of past studies, however, were not consistent, particularly with respect to coral growth. Growth is often considered an indicator of coral health (Neudecker, 1983; Rosen, 1981) and is commonly quantified via measurement of linear extension (Jinendradasa and Ekaratne, 2002) or changes in buoyant weight (Davies, 1989). Although the buoyant weight technique is attractive because it allows for non-destructive measurement of complete colony growth, it was not used in our study, because it may produce highly variable data (Bucher et al., 1998).

Koop et al. (2001) performed a study monitoring effects of nutrient enrichment on coral reefs in the field (called Effect of Nutrient Enrichment on Coral Reefs Experiment — ENCORE), where phosphate levels were manipulated over two one-year periods on the Great Barrier Reef. In the first year, low doses of phosphate (0.218 mg L^{-1}, declining to 0.0475 mg L^{-1} PO_4^{3-} within 2–3 h) were introduced to a semi-enclosed "micro-atoll". In the second year, higher phosphate concentrations were used (0.484 mg L^{-1} declining to 0.228 mg L^{-1} PO_4^{3-} at the end of low tide). Elevated phosphate concentrations did affect coral health, but these effects varied between coral species and experimental phosphate levels. For example, linear extension rates increased to 49 mm/yr in *Acropora longicyathus* at a phosphate concentration of 0.484 mg L^{-1}, but not at a lower concentration of 0.218 mg L^{-1} (Bucher and Harrison, 2002; Koop et al., 2001). No increases in growth were observed in the other coral species considered (Koop et al., 2001; Takabayashi, 1996). Similarly, Stambler et al. (1991) used phosphorus concentrations similar to Koop et al.'s (2001; 0.009 mg L^{-1}, 0.048 mg L^{-1}, 0.190 mg L^{-1}) and found no changes in linear extension rate in *Pocillopora damicornis*.

Bell et al. (2007) re-considered Koop et al.'s (2001) work and found that waters associated with their experimental reefs were already above the defined target experimental values, placing the results of this experiment in question. In considering algal growth, Bell et al. felt that this was the reason that there was no significant response in algal growth observed to the nutrient additions (they note anecdotally, however, that algal growth increased on the crests and sides of these reefs, indicating that nutrient enrichment does indeed affect algal growth).

In past studies, a buoyancy technique (Davies, 1989) was used to estimate calcification rates in *Acropora longicyathus* (Bucher and Harrison, 2002; Koop et al., 2001) and *A. palifera* (Koop et al., 2001; Steven and Broadbent, 1997). Under the highest phosphorus concentrations, both of these species exhibited increased calcification rates. *A. aspera* did not (Koop et al., 2001), indicating a species-specific response. More variability was seen when calcification rates were documented to decrease in *Stylophora pistillata* and *P. damicornis* exposed to high phosphorus concentrations (Koop et al., 2001; Takabayashi, 1996). Furthermore Ferrier-Pagès et al. (2000) found that fragments of *S. pistillata* exhibited a 60% decrease in estimated calcification rates at phosphorus concentrations of 0.266 mg L^{-1}. Similarly, Renegar and Riegl (2005) observed decreased rates of calcification in *Acropora cervicornis* exposed to phosphate concentrations of 0.393 mg L^{-1}, with no effect at 0.204 mg L^{-1}. Exposure to increased phosphorus concentrations caused a significant reduction in both skeletal density (*i.e.*, greater porosity) and calcification rates in *A. longicyathus* (Bucher and Harrison, 2002; Koop et al., 2001). On the other hand, the same concentrations caused significant increases in density in other coral species (Koop et al., 2001; Takabayashi, 1996).

In a field study, Risk and Sammarco (1991) reported that the coral *Porites lobata* exhibited increasing density with distance from shore on the Great Barrier Reef. Inshore corals were characterized by a lower skeletal density than those offshore. They also demonstrated a higher skeletal porosity inshore. Scanning electron microscopy (SEM) examination of skeletal micro-architecture revealed that the difference in coral density was not due to a decrease in the number of septa and dissepiments, or a change in the spacing between them; rather, it was due to the differences in porosity and the thickness of the calical characters. Risk and Sammarco (1991) suggested that this inshore–offshore cline could have been caused by higher

levels of inshore nutrients, primarily phosphate which has been suspected to hinder calcification (Kinsey and Davies, 1979; Sammarco and Risk, 1990).

In the present study, we utilized a set of laboratory microcosms to examine the effects of phosphate on corals. We controlled heavily for a variety of potential confounding factors, including phytoplankton populations (removed by filtration), filamentous algae (removed with grazing snails), light — diurnal and nocturnal (controlled with precise timing and monitoring of radiant exposure), temperature (maintained at a constant of 25 °C), PO_4 concentration (maintained with daily monitoring and adjustment), pH (maintained at 8.2), O_2 concentration (maintained at saturated level), salinity (maintained at 35 ppt with an automatic top-up system), and nitrogenous compounds (kept in check using a bacterial filter). In addition, we employed regular and frequent water quality sampling with a high level of accuracy and precision, large tanks (to decrease micro-scale variance), a small number of fragments per tank (to decrease crowding and fouling effects), frequent feeding of corals (to reduce potential suppressed growth rates common in captivity), genetic diversity of colonies (confirmed using DNA analysis), using weight and total skeletal linear length to calculate density, and running the experiment for an extensive period of time — ~4 months.

Using this controlled experimental approach, we attempted to determine the effects of increased phosphate concentration in seawater on the hermatypic zooxanthellate scleractinian coral *Acropora muricata* (see Wallace, 1999 for discussion on the taxonomy for this species formerly known as *Acropora formosa*) in a controlled laboratory environment. We did this by adding phosphate to aquarium microcosms at three different levels and monitoring total skeletal length, living tissue length, weight, number of branches, and degree of polyp extension. Through analyses of these variables, we demonstrate that, although skeletal growth and weight increase in these corals under high phosphate conditions, skeletal density decreases, potentially compromising skeletal integrity. We also include a discussion of possible physiological mechanisms driving this response.

2. Materials and methods

2.1. Target species

The coral species used in this experiment was the branching coral *Acropora muricata*, one of the most rapidly growing stony corals recorded, with an average annual linear extension rate of up to 121 mm/yr (Jinendradasa and Ekaratne, 2002). This Indo-Pacific species can be readily purchased. The five colonies used here came from Fiji and were obtained from Live Aquaria (Rhinelander, WI).

2.2. Experimental design

The experiment followed a mixed model, repeated measures, replicated, unbalanced orthogonal design. It was run for 115 days and consisted of exposing fragments of *Acropora muricata* to varying phosphate concentrations (0.09, 0.20, 0.50 mg L^{-1}) — concentrations similar to those used in previous studies (Ferrier-Pagès et al., 2000; Koop et al., 2001; Renegar and Riegl, 2005). Five coral colonies were fragmented into 27 sub-colonies (Fig. 1). The resulting ramets (Futuyma, 1998) were randomly distributed among three 110 L treatment tanks (Fig. 2).

2.3. Procedure and processing of coral

We confirmed through molecular genetic analysis using Amplified Fragment Length Polymorphisms (AFLPs) that our colonies were all genetically distinict. Samples were processed at the Dept. of Pharmaceutical Sciences, Univ of Buffalo, NY using the protocols described in Atchison (2005), Brazeau et al. (2005) and Atchison et al. (2008)

Live corals were maintained in the Climate Control Laboratory, Department of Biological Sciences, Nicholls State University (Fig. 2). Coral colonies were fragmented into ramets using bone cutters, secured onto retractable PVC piping via epoxy putty (AquaStick®), and distributed randomly into separate treatment tanks attached to a PVC support-base and labeled using a soldering iron (Fig. 3).

2.4. Water chemistry

Phosphate levels (mg L^{-1}) in the seawater were measured three times per week (average) to maintain target concentrations. Ammonia (N; mg L^{-1}), nitrite (NO_2; mg L^{-1}), pH, temperature (°C), and salinity (ppt) were recorded at these times. Nitrate–N (mg L^{-1}) and calcium (mg L^{-1}) levels were measured at weekly intervals (average). Phosphate levels were measured using Hach® Phos Ver3 reagents (Cat. 2125-99). In cases where phosphate concentrations were low, phosphate was added and re-tested after 30 min. Phosphate levels were adjusted using sodium phosphate ($Na_3PO_4.12H_2O$;

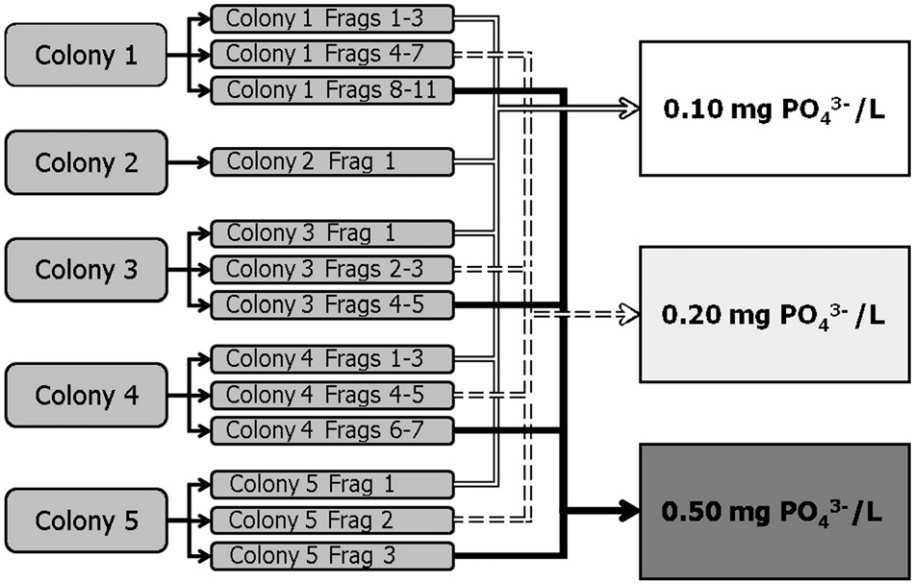

Fig. 1. Schematic of experimental design. Five genetically distinct coral colonies were fragmented and distributed into three tanks holding varying experimental phosphate levels.

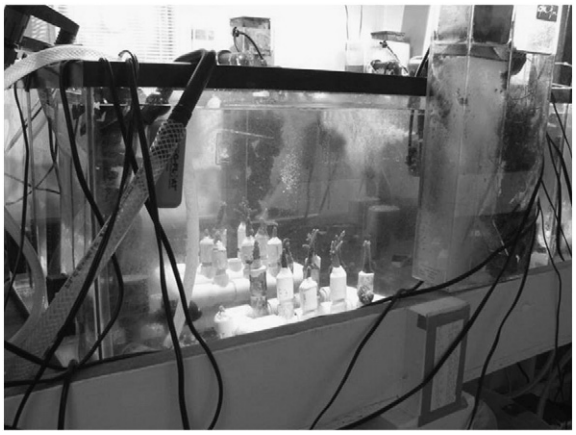

Fig. 2. Experimental tank showing PVC support structure with coral fragments. Tank fitted with three powerheads, filter attachment, protein skimmer and live rock/algal nitrogen control system (from Fiji; upper right), heaters, salinity stabilization system, and UV sterilizer. Lighting provided via two 400 W metal halide bulbs and two 160 W VHO actinic bulbs and cooling fans. Timers simulated a natural 12:12 h, light: dark photoperiod, duration — 5 h of high intensity metal halide lighting and 12 h of actinic lighting per day.

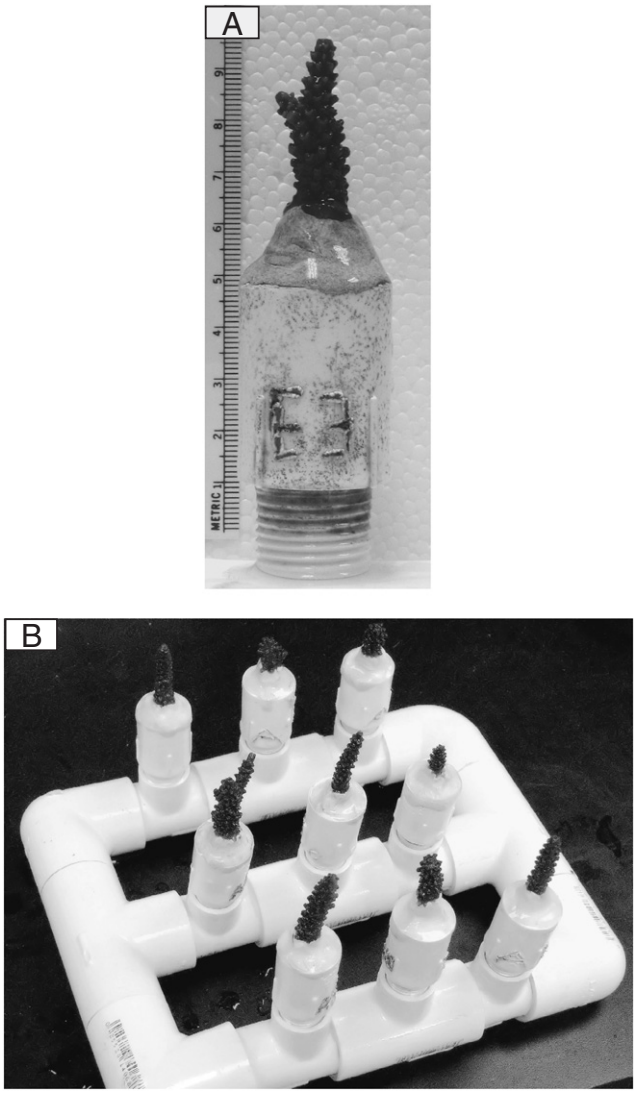

Fig. 3. (A) PVC insert with coral fragment. Measurements taken from the coral included total skeletal linear length (TSL; mm), living linear length (LL; mm), weight (g), number of branches, and polyp extension. (B) PVC support structure for corals, with holders inserted.

Acros Organics®, NJ, Product # 42440). pH levels were maintained between 8.2 and 8.4, using a synthetic saltwater buffer (Reef Buffer, Seachem®). Ammonia and nitrite levels were measured using Jungle Labs® test kits, and Hach reagents (Nitra®Ver 5) were used to measure nitrate levels. (Calcium levels were measured using test kits by Salifert and supplemented using a calcium additive; Reef Complete, Seachem®.) Saltwater (Instant Ocean®) was prepared with water from a reverse-osmosis deionization unit (RODI; Air Water Ice®, Extreme Typhoon 150 III), and salinity was maintained between 34 and 35 ppt, being measured with a handheld refractometer.

2.5. Equipment

A. muricata colonies require a high degree of lighting, strong turbulent water flow, high calcium concentrations, and a steady temperature (Borneman, 2001). Water flow was provided by two powerheads (Maxi-Jet® 1200) and powered by a wave-making unit (Maxi-Jet® Natural Wavemaker System). Powerheads were timed to turn on and off at 20-s intervals, creating a pulsing flow of current. Magnetic powerhead holders (Algae Free® Sure Grip 50) were used to secure the powerheads to the glass. Powerheads (Hagen® Aqua Clear 50) fitted with a quick filter attachment provided additional water flow debris filtering. Protein skimmers (CPR® Bak-Pak 2R) removed excess waste accumulation, particularly organic matter. Heaters (Marineland® Visi-Therm Stealth 250 W) were used to maintain temperatures at 25–26 °C. Tanks were covered with glass to reduce evaporation and introduction of airborne contaminants. Salinity was maintained between 34 and 35 ppt by float switches which triggered pumps to transfer RODI freshwater housed in a 55-gal holding tank to the main tanks. An ultraviolet (UV) sterilizer (Coralife® Turbo Twist 9 W) controlled free-floating algae that could decrease light penetration to the corals.

Three light systems were used to provide PAR. The first was broad-spectrum (broad wavelength) high-intensity lighting derived from a 400 W metal halide ballast (PFO®) and two 10,000 K bulbs (Ushio®). Second, two 160 W actinic VHO bulbs (UVL lighting® T12) and a VHO (PFO®) ballast produced light in the 420 nm range to accommodate the energetic/photosynthetic needs of the zooxanthellae (Jeffrey and Haxo, 1968). Fans were installed to reduce excessive heat from the lights. Timers were used to simulate a natural 12:12 h light:dark photoperiod, with a duration of 5 h of high intensity metal halide lighting within the twelve-hour actinic lighting day. Two lunar lighting units (R2 Solutions® Moonlight 11000 MC) were also kept on continually.

2.6. Biotic nutrient controls

Live rock from Fiji was placed into the protein skimmers to serve as a biological filter, providing a high surface area for nitrifying bacteria. Algae associated with the live rock also helped to keep nitrogen (ammonia, nitrite, and nitrate) levels low. Turbo snails (*Turbo fluctuosa*) were introduced into the tanks to help prevent algal accumulation on the coral and tank. Because zooxanthellae photosynthate may be nutritionally limiting for corals (Johannes et al., 1970), supplemental food was provided to the corals to increase coral growth and survival (see Ferrier-Pagès et al., 2003; Lewis, 1974) three times per week, using Cyclop-Eeze (Liquid Life Argent®). The powerfilters (Aqua Clear®) were turned off during feeding for 30 min.

2.7. Data collection

Data were collected on the corals six times over a 115-day period. Measurements included total skeletal linear length (TSL; mm), living tissue linear length (LL; mm), weight (g), number of branches, and degree of polyp extension (extended *vs.* not extended). The threshold for counting new growth as a new branch was 5 mm. Three replicate

readings were taken of TSL (mm), LL (mm), and weight (g) during each sampling period to determine the precision of the measurement.

Wet weight was recorded for each coral fragment. The PVC support was dried and total weight of the block with coral was weighed on a digital Mettler scale. Polyp extension was measured three times per week, and recorded as fully extended, partially extended, or no extension.

After data collection, the positions of the PVC coral support structures were rotated in a uniform fashion within the tank to control for any potential position effects. During sampling, corals were photographed (front and back) for record-keeping purposes.

2.8. Length measurements

We made direct measurements of colony length. The variables were total skeletal length and living tissue length, measured using digital calipers.

2.9. Data analysis and graphics

Data were logged and collated using EXCEL 2003 and analyzed using SAS 2003 and BiomStat 3.3, using standard parametric *a priori* and *a posteriori*/*post-hoc* statistical tests (Sokal and Rohlf, 1981). Data were further analyzed and graphed using SigmaPlot 10.0. Diagrams were created using MS PowerPoint 2003 and Paint 2003.

3. Results

3.1. Control of experimental water quality

Mean phosphate levels in the tanks were 0.09 ± 0.018, 0.20 ± 0.024, and 0.50 ± 0.059 mg L^{-1} (mean ± 95% conf. intervals; target levels = 0.10, 0.20, and 0.50 mg L^{-1}; Fig. 4). Seawater temperature was maintained at 25 °C, pH 8.2, and 34–35‰ (Table 1). Calcium levels were maintained at 400 mg/L, not being permitted to fall below a mean level of 355–360 mg L^{-1}. (It is known that calcium levels > 360 mg L^{-1} have no enhancing effects on coral calcification rates; Tambutté et al., 1996.) No ammonia was detected at any stage of the experiment, and nitrite and nitrate levels were negligible or absent during most of the experiment. Water quality parameters occasionally differed significantly between treatments (Table 1) but always remained well within the natural range known to support healthy coral growth (Kleypas et al., 1999).

3.2. Growth

Corals exposed to elevated phosphate levels exhibited increased growth rates. Corals maintained at the highest phosphate concentration (0.50 mg L^{-1}) had the highest total skeletal linear (TSL) growth rates (Fig. 5A). Total TSL growth at the highest phosphate concentration was 14.0 ± 6.20 mm over a period of 115 days, significantly greater than TSL growth at the lower phosphate levels (Fig. 5B). No difference was found in TSL between the 0.09 and 0.20 phosphate mg L^{-1} treatments (3.9 ± 2.58 and 4.8 ± 5.35 mm, respectively). Between a point sixty-five days prior to initiation of the experiment and addition of phosphate to the tanks (t_0), fragments (ramets, or clones) of Colony A exhibited low or negligible TSL growth rates in all experimental tanks (Fig. 6). Once phosphate levels were increased, fragments exposed to the highest levels exhibited significantly greater growth rates.

Phosphate-enhanced growth through time, as measured by living length (LL) of coral tissue, was also significantly higher in the 0.50 mg L^{-1} treatment than at lower concentrations (Fig. 7A). Total growth with respect to LL among the three treatments was 14.6 ± 5.43 mm of the highest phosphate concentration treatment (Fig. 7B) and was significantly greater than growth in the other two treatments (4.6 ± 2.14 mm at 0.09 mg P L^{-1}, and 5.8 ± 4.80 mm at 0.20 mg P L^{-1}).

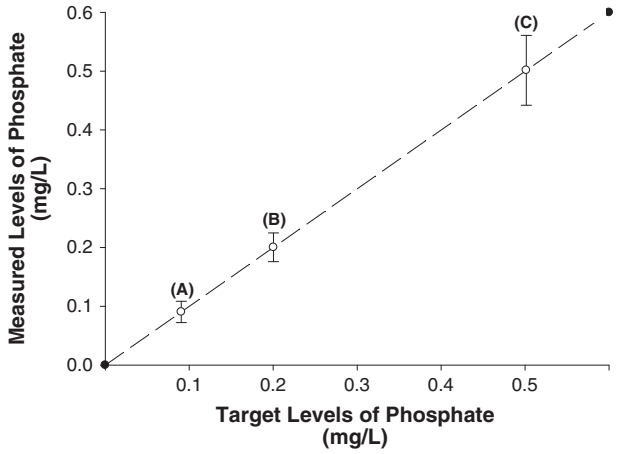

Fig. 4. Relationship between actual experimental concentrations of phosphate (0.09, 0.20, 0.50 mg L^{-1}) and target concentrations (0.10, 0.20, 0.50 mg L^{-1}). Mean shown with 95% confidence levels. Significant difference in phosphate concentrations between the 0.50 mg phosphate L^{-1}, 0.20 mg L^{-1}, and 0.09 mg L^{-1} treatments ($p < 0.05$, one-way ANOVA; $p < 0.05$, Tukey's *post hoc* test; treatments that do not share a common letter are significantly different).

Weight of the corals increased significantly with time in all treatments (Fig. 8). Those corals subjected to the highest concentrations of phosphate (0.50 mg L^{-1}) accumulated weight at the highest rate. Weight accumulation was significantly different between all treatments.

Relative density (RD) was calculated according to the following formula:

$$RD = [(W_t/W_{max})/(TSL_t/TSL_{max})] - [(W_i/W_{max})/(TSL_i/TSL_{max})] + 1,$$

where RD = Relative Density, W = wet weight of individual fragment in g, TSL = total skeletal length in mm, i = initial measurement,

Table 1
Physico-chemical attributes of the experimental seawater. Mean +/− s.d. shown for pH, temperature (°C), salinity (‰), calcium (mg L^{-1}), ammonia–N (mg L^{-1}), nitrite (mg L^{-1}), and nitrate–N (mg L^{-1}). Calcium levels were returned to 400 mg L^{-1} when required; mean weekly levels shown. Data analyzed by one-way ANOVA ($\alpha = 0.05$) followed by Tukey's *post hoc* test. Means within a category designated by a common letter are not significantly different ($p > 0.05$).

Variable	Phosphate level	Mean SD	N
pH	0.09 mg/L	8.22 ± 0.100^{A}	71
	0.20 mg/L	8.26 ± 0.094^{B}	71
	0.50 mg/L	8.25 ± 0.105^{AB}	71
Temperature	0.09 mg/L	25.35 ± 0.692^{A}	56
	0.20 mg/L	25.27 ± 0.586^{A}	56
	0.50 mg/L	25.30 ± 0.686^{A}	56
Salinity	0.09 mg/L	34.39 ± 1.073^{A}	56
	0.20 mg/L	34.57 ± 0.912^{A}	56
	0.50 mg/L	35.00 ± 0.638^{B}	55
Calcium	0.09 mg/L	360.88 ± 50.536^{A}	17
	0.20 mg/L	367.06 ± 32.262^{A}	17
	0.50 mg/L	375.29 ± 39.979^{A}	17
Ammonia	0.09 mg/L	$0.00 \pm N/A^{A}$	15
	0.20 mg/L	$0.00 \pm N/A^{A}$	14
	0.50 mg/L	$0.00 \pm N/A^{A}$	16
Nitrite	0.09 mg/L	0.03 ± 0.165^{AB}	45
	0.20 mg/L	$0.00 \pm N/A^{A}$	41
	0.50 mg/L	0.09 ± 0.212^{B}	44
Nitrate	0.09 mg/L	0.13 ± 0.168^{A}	14
	0.20 mg/L	0.11 ± 0.196^{A}	14
	0.50 mg/L	0.13 ± 0.198^{A}	14

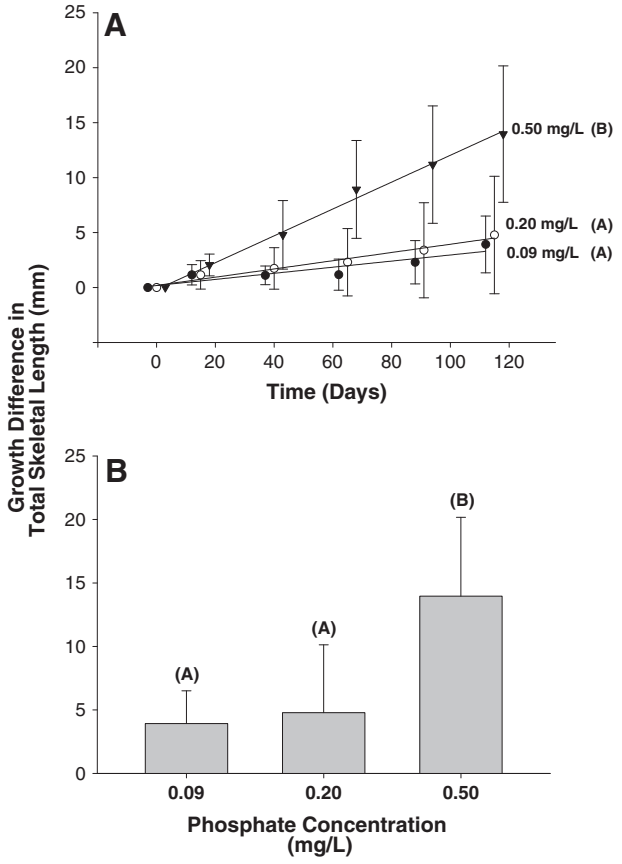

Fig. 5. (A) Changes in total skeletal length (TSL; mm) in *Acropora muricata* under varying phosphate concentrations through time (days). Mean shown with 95% confidence levels. TSL growth is significantly higher at 0.50 mg phosphate L^{-1} than in the 0.20 mg L^{-1} and 0.09 mg L^{-1} treatments ($p<0.05$, repeated measures one-way nested ANOVA; treatments that do not share a common letter are significantly different). 0.09 mg L^{-1} treatment: $p<0.01$, linear regression, $Y=0.028X+0.185$, $r=0.915$ (Pearson's product moment correlation coefficient, Sokal and Rohlf 1981); 0.20 mg L^{-1} treatment: $p<0.001$, $Y=0.038X+0.191$, $r=0.985$; 0.50 mg L^{-1} treatment: $p<0.001$, $Y=0.122X-0.185$, $r=0.997$. (B) Overall growth response indicated by total skeletal length (TSL_6-TSL_0; mm) in *Acropora muricata* under different experimental concentrations. Mean shown with 95% confidence levels. TSL total growth gained is significantly higher at 0.50 mg phosphate L^{-1} than in the 0.20 mg L^{-1} and 0.09 mg L^{-1} treatments ($p<0.05$, one-way ANOVA; $p<0.05$, Tukey's *post hoc* test).

t = measurement at time t, and max = maximal observation for each variable per coral fragment. Relative density of corals from the highest phosphate concentration was found to be significantly lower than corals from the other two lower phosphate concentration treatments (Fig. 9).

There was no significant difference in production of new branches between the corals in the various phosphate enrichment treatments (1.1 +/− 0.69, branch production +/− 95% conf. limits at 0.09 mg L^{-1} phosphate concentration; 1.3 +/− 0.57 at 0.20 mg L^{-1}; 2.3 +/− 2.37 at 0.50 mg L^{-1}).

No significant differences were detected in frequency of polyp extension among the experimental treatments (1.0 ± 0.01 — mean extension +/− 95% conf. limits — at 0.09 mg L^{-1} phosphate concentration; 1.0 ± 0.01 at 0.20 mg L^{-1}; 1.0 +/− 0.00 at 0.50 mg L^{-1}).

4. Discussion

There are sources of variance that we have eliminated here that are present in other studies, particularly field studies. Koop et al.'s (2001) experiment yielded some valuable results, but it was understood that experimental levels of nutrients added to the reef varied

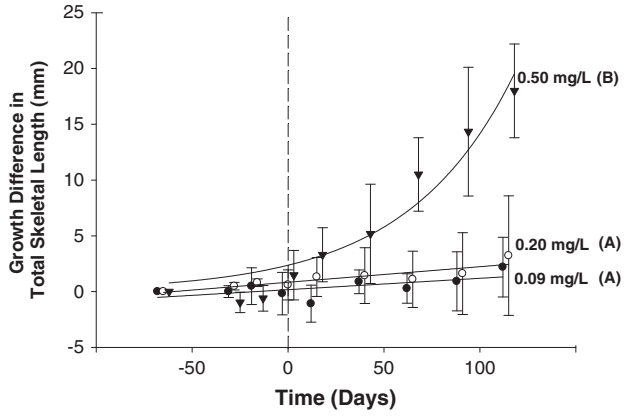

Fig. 6. Changes in total skeletal length (TSL; mm) in *Acropora muricata* in Colony A under varying phosphate concentrations through time (days). Pre-experimental growth rates shown as well. Mean shown with 95% confidence levels. Colony A TSL growth is significantly higher at 0.50 mg phosphate L^{-1} than the 0.20 mg L^{-1} and 0.09 mg L^{-1} treatments ($p<0.05$, repeated measures one-way nested ANOVA; treatments that do not share a common letter are significantly different). 0.09 mg L^{-1} treatment: $p<0.05$, linear regression, $Y=0.010X+0.174$, $r=0.658$ (Pearson's product moment correlation coefficient, Sokal and Rohlf 1981); 0.20 mg L^{-1} treatment: $p<0.001$, $Y=0.014X+0.837$, $r=0.889$; 0.50 mg L^{-1} treatment: $p<0.001$, $Y=1.018^{X}*2.365$, $r=0.967$.

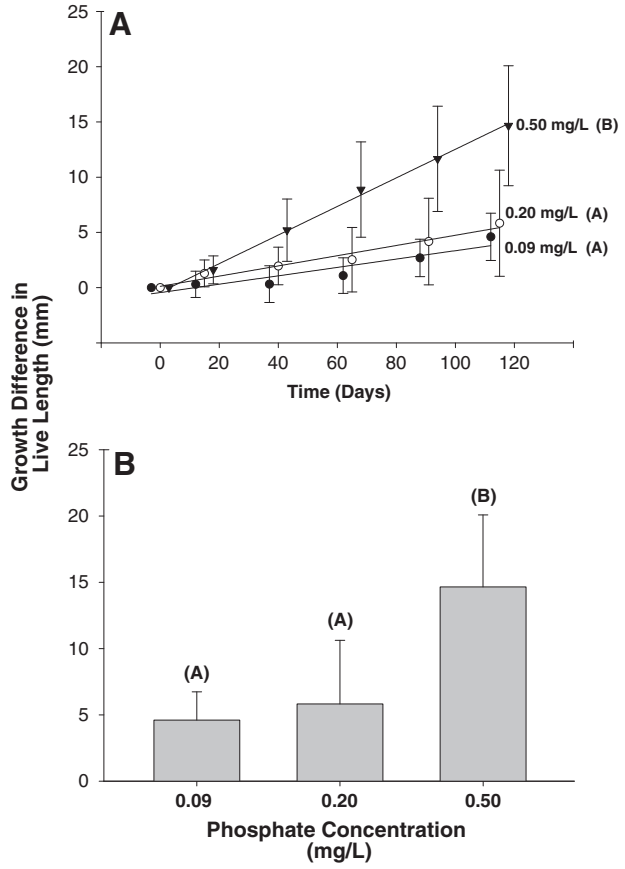

Fig. 7. (A) Changes in living tissue length (LL; mm) in *Acropora muricata* under varying phosphate concentrations through time (days). Mean shown with 95% confidence levels. LL growth is significantly higher at 0.50 mg phosphate L^{-1} than in the 0.20 mg L^{-1} and 0.09 mg L^{-1} treatments ($p<0.05$, repeated measures one-way nested ANOVA; treatments that do not share a common letter are significantly different). 0.09 mg L^{-1} treatment: $p<0.01$, linear regression, $Y=0.038X-0.445$, $r=0.932$ (Pearson's product moment correlation coefficient, Sokal and Rohlf 1981); 0.20 mg L^{-1} treatment: $p<0.001$, $Y=0.046X+0.119$, $r=0.982$; 0.50 mg L^{-1} treatment: $p<0.001$, $Y=0.129X-0.416$, $r=0.999$. (B) Overall growth response in living tissue length through time (LL_6-LL_0; mm) in *Acropora muricata* under different phosphate concentrations. Mean shown with 95% confidence levels. LL gained is significantly higher at 0.50 mg phosphate L^{-1} than in the 0.20 mg L^{-1} and 0.09 mg L^{-1} treatments ($p<0.05$, one-way ANOVA; $p<0.05$, Tukey's *post hoc* test).

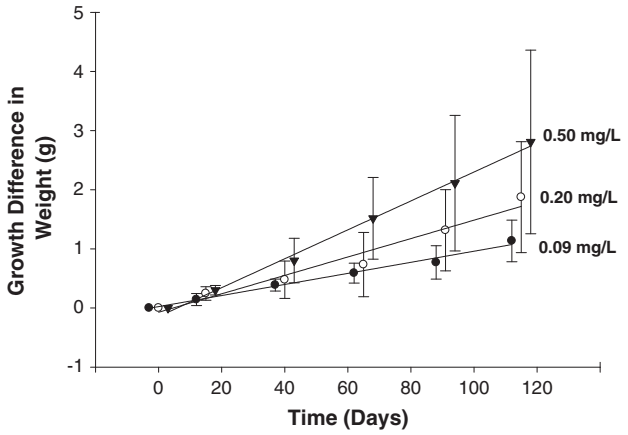

Fig. 8. Changes in weight (g) in *Acropora muricata* under varying phosphate concentrations through time (days). Mean shown with 95% confidence levels. Significant difference in weight-gain between the 0.50 mg phosphate L^{-1}, 0.20 mg L^{-1}, and 0.09 mg L^{-1} concentrations ($p<0.05$, repeated measures one-way nested ANOVA). 0.09 mg L^{-1} treatment: $p<0.001$, linear regression, $Y=0.009X+0.024$, $r=0.994$, (Pearson's product moment correlation coefficient, Sokal and Rohlf 1981); 0.20 mg L^{-1} treatment: $p<0.001$, $Y=0.016X-0.071$, $r=0.983$; 0.50 mg L^{-1} treatment: $p<0.001$, $Y=0.024X-0.149$, $r=0.998$.

with the tide because of natural flushing. To avoid such problems, we took advantage of a controlled laboratory environment. Experimental levels of phosphate here were maintained very close to pre-defined target levels throughout the experiment through frequent monitoring, removing this potential source of variance. In addition, the coral colonies used here were determined to be genetically distinct, and showed no significant differences in growth rate prior to the addition of phosphate. This indicates that any treatment effects observed were not due to differences between the colonies. Thus, the differences in coral growth reported here are due primarily to differences in phosphate concentration and not to differences in other parameters such as genetics or aquaria conditions.

There was no difference in coral growth rates in the 0.09 and 0.20 mg L^{-1} phosphate concentration treatments, but at 0.50 mg L^{-1}, a significant difference did emerge. This indicates that a phosphate-enhanced growth effect is manifested somewhere between 0.20 and 0.50 mg L^{-1}.

The graded response in weight accumulation which occurred in corals exposed to the experimental treatments indicates that, of the variables considered here, weight is the most sensitive to phosphate concentration. Our density measurements indicate that the rate of weight increase did not keep pace with the rate of skeletal extension, resulting in a more rapidly growing, but less dense coral skeleton at the highest phosphate concentration. The skeleton was becoming less dense, more porous, and most likely weaker. Most of the weight increase was, of course, due to accretion of calcium carbonate. A threshold effect was also detected in this case, occurring between 0.20 and 0.50 mg L^{-1} phosphate. Calcium carbonate deposition is clearly more tightly linked to ambient phosphate concentration than skeletal extension or live tissue length.

Acropora species appear to be tolerant of high phosphate levels and exhibit increased growth under these conditions, when compared to other corals (Bucher and Harrison, 2002; Koop et al., 2001; Steven and Broadbent, 1997). Bucher and Harrison (2002) suggest that *Acropora* growth may be less affected than other coral species when exposed to an increase in inorganic nutrient levels, in the absence of stresses such as reduced salinity, elevated sediments, or pollutants. Inter-specific variation in responses may also be due to different clades of *Symbiodinium* sp. Other potential factors include the presence (sometimes in high abundances) or absence of known or unknown enzymes (*e.g.*, Ca^{2+}-ATPase, carbonic anhydrase).

4.1. Variation in results from earlier studies

Our study supports recent field studies (Bucher and Harrison, 2002; Koop et al., 2001; Steven and Broadbent, 1997) that demonstrated an increase in growth of *Acropora* species under conditions of increased phosphate levels, similar to those used here. On the other hand, Kinsey and Davies (1979) hypothesized that phosphate can inhibit coral growth and may cause a decrease in skeletal density.

In some earlier experiments, increased phosphate concentrations caused a reduction in coral calcification rates (Ferrier-Pagès et al., 2000), particularly in *A. cervicornis* (Renegar and Riegl, 2005). Variation in results may have been caused by the use of different experimental techniques, *e.g.*, the use of small tanks (*e.g.* 5–8 L) with a high density of coral fragments. Here, we used large tanks (110 L) and a small number of small fragments in each, eliminating crowding and water quality problems. Adding food to eliminate any nutritional limitations (Johannes et al., 1970) also helped to support healthy coral growth.

Determining weight via a simple wet-weight technique has, we believe, benefited this study.

Past studies have often used the buoyant weight technique (Davies, 1989). Bucher et al. (1998), however, have questioned the accuracy of that technique due to variations in coral skeletal micro-density found between species and regions. In addition, it assumes that the micro-density of the coral skeleton is equivalent to that of pure aragonite (2.94 g/cm^3). Significant variations in skeletal density have been found between *Acropora* species characterized by differences in chemistry or skeletal micro-architecture from different regions.

4.2. Mechanisms underlying effects of phosphate enrichment on coral growth

It is possible that the observed accelerated growth under phosphate-enriched conditions may be due to increased zooxanthellar populations and photosynthetic production within the coral. An increase in zooxanthellar populations within corals is known to occur under conditions of increased phosphate concentrations (Koop et al., 2001; Steven and Broadbent, 1997; Takabayashi, 1996). Phosphate is also known to increase chlorophyll levels (Bucher and Harrison, 2002; Koop et al., 2001) and photosynthetic rates (Ferrier-Pagès et al., 2000).

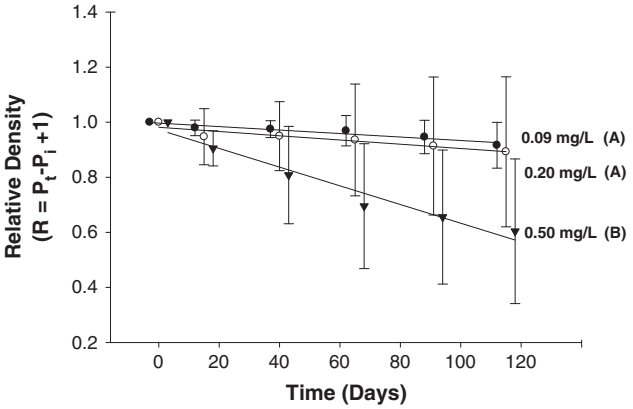

Fig. 9. Changes in relative density of *Acropora muricata* under varying phosphate concentrations through time (days). Relative density = P_t $[(W_t/W_{max})/(TSL_t/TSL_{max})]-P_i$ $[(W_i/W_{max})/(TSL_i/TSL_{max})]+1$, where i = initial, and t = measurement at time t. Mean shown with 95% confidence levels. Significant difference in relative density between the 0.50 mg phosphate L^{-1} *vs.* the 0.20 mg L^{-1} and 0.09 mg L^{-1} treatments ($p<0.05$, repeated measures one-way nested ANOVA; treatments that share a common letter are not significantly different). 0.09 mg L^{-1} treatment: $p<0.01$, linear regression, $Y=-0.0006X+0.997$, $r=0.958$, (Pearson's product moment correlation coefficient, Sokal and Rohlf 1981); 0.20 mg L^{-1} treatment: $p<0.01$, $Y=-0.0008X+0.982$, $r=0.932$; 0.50 mg L^{-1} treatment: $p<0.001$, $Y=-0.0034X+0.973$, $r=0.979$.

Increasing phosphate levels may have "fertilized" the zooxanthellae, stimulating their population growth. The stimulation of zooxanthellar cell production and coral growth by elevated phosphate levels has been reported by other investigators (Bucher and Harrison, 2002; Koop et al., 2001). As zooxanthellar production increases, the coral most likely receives additional translocation products such as photosynthetically derived ATP, which in turn allows for an increase in calcification and growth. Such an increase in ATP could enhance calcification by fueling active transcellular transport mechanisms (Al-Horani et al., 2003; Tambutté et al., 1996). An increase in ATP production could increase calcification by transporting additional calcium into the extracytoplasmic calcifying fluid (ECF) via the Ca^{2+}-ATPase-mediated $Ca^{2+}/2H^{+}$ exchange which occurs at the calicoblastic epithelium (Fig. 10A). Therefore, an increase in calcium ions within the ECF could lead to increased calcification and growth rates.

An increase in the zooxanthellar population could disrupt the equilibrium of products exchanged between the host and symbiont. An influx of H^{+} ions produced by calcification can result in acidification of the environment. Furla et al. (1998) suggested that, under normal conditions, a stable intra-coelenteron pH is maintained by OH^{-} ions derived from photosynthesis. Tanaka et al. (2007) proposed that if photosynthesis increases, an alkalizing effect could occur within the coelenteron, increasing calcification in order to generate additional H^{+} ions in order to neutralize elevated OH^{-} levels resulting in high growth rates. As a result, elevated levels of calcium ions may be transported to the calcification site.

The phosphate-enhanced coral growth we observed here may be due to an increase in active trans-cellular calcium transport resulting from expendable energy reserves, or calcification enhanced by alkalization within the coral (Fig. 10B).

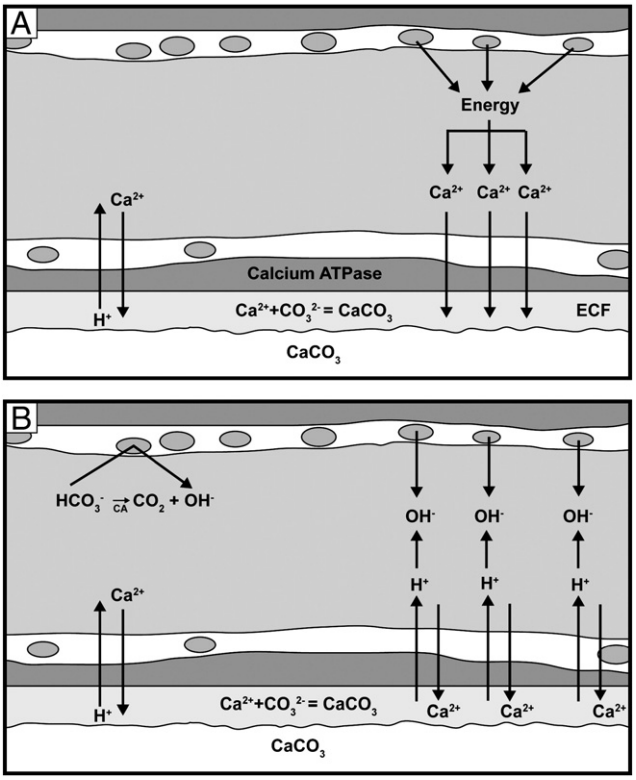

Fig. 10. (A) Mechanism by which coral growth could be stimulated via phosphate-enhanced zooxanthellar production. Additional photosynthetically-derived ATP could allow for an increase of active calcium transport to the extracytoplasmic calcifying fluid (ECF), thereby stimulating increased calcification and growth rates (see Allemand et al. 2004; Furla et al. 2000; Gattuso et al. 1999; McConnaughey and Whelan 1997 for descriptions of the calcification process). (B) Mechanism by which phosphate-enhanced zooxanthellar population density can cause intra-coelenteric alkalization due to an increase in HCO_3^- derived photosynthesis. Corals maintain an optimum intra-coelenteron pH by increasing the influx of H^{+} ions produced by calcification. As a result of this, increased calcium ions are transported to the area of calcification, resulting in faster growth (see Tanaka et al. 2007; also see Weis et al. 1989; Furla et al. 1998).

4.3. Phosphate concentrations and skeletal density

Phosphate has been referred to as a "crystal poison" to the calcifying environment and the causative factor of coral density loss under nutrient enrichment (Kinsey and Davies, 1979; Risk and Sammarco, 1991; Simkiss, 1964). Our results support recent studies that have demonstrated an increase in porosity and a decrease in density under phosphate-enriched conditions (Koop et al., 2001). This condition may cause the coral to be vulnerable to breakage or more susceptible to natural wave action and colonization by internal bioeroders, further decreasing its skeletal integrity (Risk et al., 1995; Sammarco and Risk, 1990). A rapid "lean" linear growth and a trend towards the production of numerous branches would allow for increased surface area for photosynthesis and feeding. Although this trend would appear to be advantageous, as mentioned above, less heavily calcified corals may become brittle and more prone to breakage, and thus represent an unhealthy state.

We are suggesting that coral density may be decreased due to phosphate ions distorting the orderly array of ions on the crystal surface. Simkiss (1964) has proposed that phosphate may be binding at the site of calcification. Recent studies have confirmed this suggestion finding a correlation between phosphate within the coral skeleton and in the surrounding waters (Montagna et al. 2006). Raistrick's (1949) hypothesis suggested that each phosphate group is capable of replacing one carbonate ion in the crystal lattice (Simkiss, 1964). In this case, crystal distortion will occur because the foreign metaphosphate ions do not conform to the three-dimensional structure normally characteristic of the crystal lattice. As a result of this distortion, coral skeletal integrity may be compromised due to the production of a porous and structurally weaker calcium carbonate/calcium phosphate skeleton (Fig. 11). Although recent research suggests that this "anionic substitution" is responsible for only a small proportion of phosphate incorporated into the skeleton, the incorporation mechanism is still unclear and may vary between genera and forms of saltwater phosphorus (LaVigne et al., 2008; LaVigne et al., 2010).

To summarize, we suggest that a phosphate-induced increase in zooxanthellar populations may cause an increase in photosynthate and expendable energy reserves, and in turn permit increased calcification/growth. The increased photosynthesis would create a higher demand for carbonic anhydrase, thus increasing the pH in the intracoelenteric space. This would also create a deficit of carbonate available for calcification which in turn would allow the phosphate ions to be used as a supplement to the carbonate ions while simultaneously helping to maintain an optimum pH. A lighter skeleton containing higher levels of calcium phosphate may then be formed, resulting in an increase in coral skeletal porosity.

4.4. Coral health in an eutrophic environment — experimental evidence for the Janus effect

Understanding the effects of nutrients on coral growth is important for maintaining the health of a coral reef ecosystem. It has been demonstrated here and elsewhere that corals can grow faster under high phosphate conditions (Bucher and Harrison, 2002; Koop et al., 2001; Steven and Broadbent, 1997). Higher coral growth rates were once considered to be a positive indicator of coral health (Neudecker, 1983; Rosen, 1981). Edinger et al. (2000) have suggested the opposite, however. Although individual corals on eutrophic reefs may grow faster, colonies are often susceptible to increased bioerosion pressure as well as competition for space with non-calcified organisms (also see Birkeland, 1987;

Edinger and Risk, 1994; Glynn, 1988; Hallock and Schlager, 1986; Risk and Sammarco, 1991; Risk et al., 1995; Sammarco and Risk, 1990). In fact, they claim that under nutrient enrichment conditions, coral growth rates and reef growth rates become decoupled. Edinger and Risk (1994; Edinger et al., 2000) have deemed this phenomenon "the Janus effect", where an increase in coral growth rates cannot compensate for the additional pressures associated with eutrophic conditions affecting accretion rates, and reef growth is inhibited. In addition, coral skeleton integrity may be further compromised due to the production of a structurally inferior calcium phosphate skeleton. As a result, corals may be more prone to breakage resulting from human and storms. Exposed skeletons may allow for algal colonization and a potential shift in community structure.

In our study, the highest phosphate concentration produced no visible effects of stress on the corals, as indicated by polyp extension. In addition, no coral mortality was observed. Phosphate levels tested were much higher than those often found on natural reefs. This implies that increased phosphate alone may not lead to widespread reef mortality. The apparent health of the reef, however, may decline as conditions become more eutrophic reefs, resulting in high bioerosion rates, high phytoplankton populations (Walker and Ormond, 1982), and increased benthic filamentous algal populations (Done, 1992). Thus, it would appear that reefs in nutrient-rich waters may survive the symptoms of global climate change, but their lack of resilience to mechanical stress may ultimately lead to disintegration — *i.e.*, sea-level rise and increased storm frequencies and intensities, but only with difficulty, due to their weakened condition.

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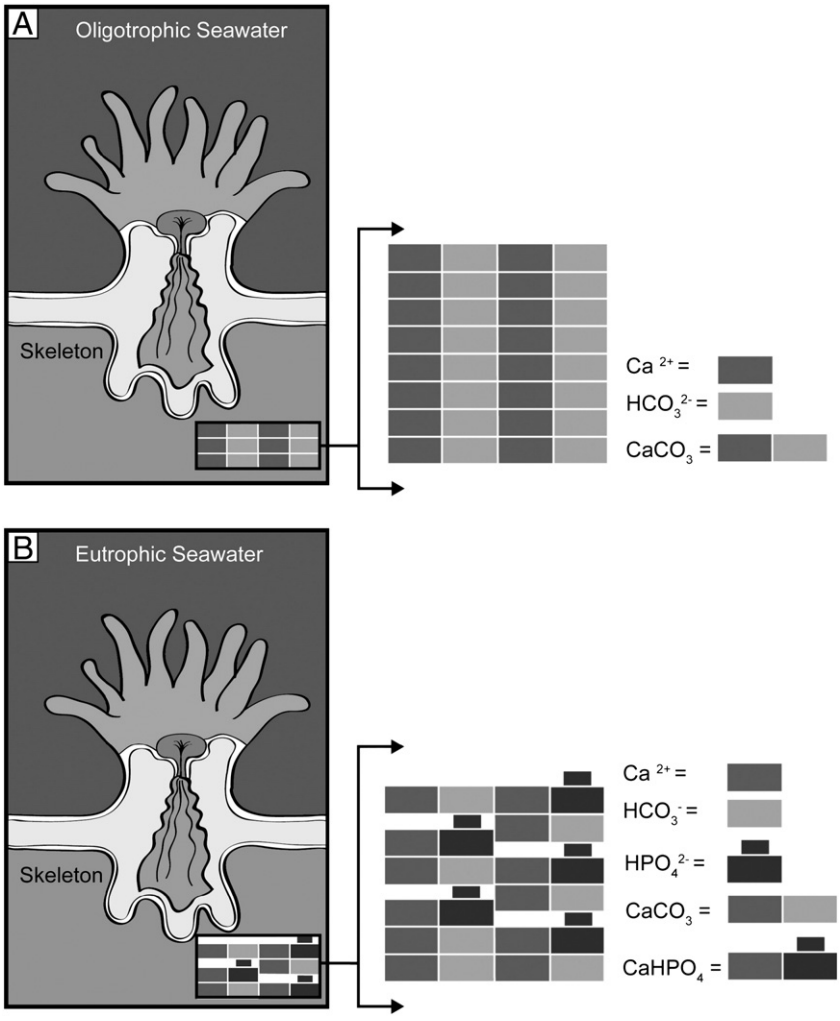

Fig. 11. Proposed mechanism by which an increase in coral growth and porosity may increase under conditions of increased phosphate, in a DIC-limited environment. (A) In an oligotrophic environment, calcium and carbonate bind to form a dense calcium carbonate skeleton. (B) Under a phosphate-enriched conditions, zooxanthellar populations increase, decreasing available carbonate available for coral calcification. Coral growth could continue if calcium binds with newly available phosphate, thereby complementing the depleted carbonate levels. Crystal distortion could occur since the foreign phosphate ions would not be expected to conform to the precise three-dimensional structural fit in the normal crystal lattice. As a result, a porous and structurally weaker calcium carbonate/calcium phosphate skeleton would be produced. Such a scenario would allow skeletal growth to continue, but with decreased density.

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