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Long-term effects of inorganic nitrogen enrichment on the reef-building corals *Stylophora pistillata* and *Acropora* spp.

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Abstract. The increase in the nutrient level has been suggested as a major cause of localized coral reef degradation. Although the response of corals to nutrients such as ammonium or nitrate is well-documented in laboratory studies, long-term effects of continuously high inorganic nitrogen concentrations on coral physiology remained obscure. To assess the long-term effects of elevated inorganic nitrogen concentrations on the survival and growth of coral colony, we monitored the colonies of two branching reef-building corals *Stylophora pistillata* and *Acropora* spp. that had been exposed to 40 μM of NH_4^+ and 30 μM of NO_3^- over a 12 months period in flow-through aquariums. Both corals showed different responses to nutrient enrichment in survival rate, zooxanthellae density and maximum quantum yield (*Fv/Fm*). These results suggest that although both *S. pistillata* and *Acropora* spp. can adapt to relatively high inorganic nitrogen levels, faster-growing *Acropora* spp. are more susceptible to the increase in nutrient levels. The results also suggest that nutrient enrichment is not an only cause for coral reefs degradation but could results in synergistic impacts when corals are exposed to other environmental stressors.

Key words: eutrophication, growth, inorganic nitrogen, reef-building coral

Introduction

Reef-building corals typically thrive in oligotrophic ocean characterized by low levels of inorganic nutrients. They have adapted to such unique environment by establishing obligate symbiosis with dinoflagellates (zooxanthellae) that can efficiently utilize light energy through photosynthesis (D'Elia and Wiebe 1990). Since nitrogen is a limiting factor for the growth of phytoplankton, symbiotic dinoflagellate *in vivo* take up dissolved inorganic nitrogen (DIN) from surrounding water as well as utilizing coral host metabolites as nutrient sources (Muscatine and Porter 1977; Muscatine et al. 1989).

This symbiotic relationship between corals and algae is easily disrupted by environmental changes which resulted in bleaching phenomena (Hoegh-Guldberg and Smith 1989). Anthropogenic nutrient enrichment impacts on reef-building corals in direct and indirect manners both of which eventually result in the degradation of coral reef ecosystems. Reduction in coral growth and calcification (Marubini and Davies 1996), negative effects on coral reproduction (Ward and Harrison 2000), increase in coral susceptibility to infectious disease and bleaching (Bruno et al. 2003) as well as alteration of benthic community structure in coral reefs (Wielgus et al. 2004) are some of the few documented results of increased nitrogenous levels in seawater.

In contrast to our consensus that reef-building corals can only thrive in low-nutrient concentration water, there are evidences that corals do grow well in relatively high-nutrient water (Atkinson et al. 1995). In addition, a 2-year, large-scale field experiment on the GBR, Elevated Nutrient on Coral Reef Experiment (ENCORE) did not find any major impact of elevated nutrient concentrations on coral reefs at the levels of nutrient loading in areas undergoing eutrophication (Koop et al. 2001). This suggests that there is a possibility of long-term accommodation to elevated nutrients levels by corals and their symbiotic algae, as pointed out by Szmant (2002).

Although effects of elevated nitrogenous levels on corals have been demonstrated in laboratories, most of these studies were short-term (2-3 months) experiments. Among them, many of these experiments aimed at examine the hypothesis of nutrient-limitation in the coral-algae symbiotic association. In this study, we aimed to investigate the effect of elevated nitrogenous level on a relatively long-term survival and physiological changes in two branching, reef-building corals.

Material and Methods

Two species of branching, reef-building corals *Stylophora pistillata* and *Acropora* spp. were used in this study. Colonies of these corals were collected from reefs in the northern Okinawa Island, Japan and

had been acclimatized for more than a year in the flow-through-outdoor tank in Sesoko Tropical Biosphere Research Center (University of the Ryukyus). In July 2007, coral samples were cut into a similar length (approximately 3 cm) and immediately attached to acrylic screws using superglue. The coral nubbins were then acclimatized in an outdoor tank with water flow and allowed to recover for 2 weeks prior to the experiment (Nakamura and Yamasaki 2005).

Twenty four samples of each coral species were selected for the experiments. Twelve samples of each species were placed in both nutrient-enriched and control flow-through tanks (12 cm wide x 53 cm length x 7.5 cm height). Water flow rate was maintained at > 2 cm s^{-1}. Two replicates tanks were set-up for each treatment. Samples were attached to a side wall of the tanks to expose the lateral sides of coral nubbins to sunlight. The nutrient-treated tanks received seawater enriched with NH_4^+ and NO_3^- to give a final concentration of 40 μM and 30 μM, respectively. The control tanks received no nutrient supplement. The concentration of NH_4^+ and NO_3^- in the control tanks were 0.40 μM and 0.80 μM, respectively.

During the period from August 2007 to August 2008, maximum quantum yield of PS II (*Fv/Fm*) were measured weekly in coral nubbins after 15 min sunset. Buoyant weights were measured at the beginning of the experiment and at the end of the experimental period. Nutrient concentrations and flow rate were checked periodically and all tanks were cleaned every week to minimize the proliferation of algae. Data loggers (StowAway, Tidbit) were deployed to record the water temperature variability over the experimental period.

At the end of the experiment, tissue was removed from corals with a WaterPik (Johannes and Wiebe 1970), homogenized and centrifuged at 15,000 x g for 5 min (10°C). The supernatant was then discarded and the remaining zooxanthellae pellets were resuspended in a 10 ml of filtered seawater. Centrifugation was repeated 3 times. Samples were then divided into subsamples for both haemocytometer counts and pigment determination. Chlorophylls were extracted with 90% acetone and kept at 4°C for 48 h in darkness prior to the spectrophotometric determination. Chlorophyll *a* was calculated using the equations described by Jeffrey and Humprey (1975). Coral surface area was estimated using the aluminum foil method (Marsh 1970). ANOVA and *t*-test were used to analyze the data and to evaluate the differences in experimental treatments.

Results

During the course of the experiment, coral nubbins were exposed to a wide temperature ranges: summer 31°C-25°C, autumn 28°C-23°C, winter 23°C-17°C, spring 24°C-19°C. Visible bleaching in *S. pistillata* in both nutrient-enriched and control tanks was observed during summer 2007 and 2008 but not in *Acropora* spp. All bleached samples recovered from bleaching in October 2007. Coral infected by filamentous algae following the bleaching event was 33% in nutrient-enriched samples. However, no filamentous algae infection was observed in the control samples.

Concentrations of NH_4^+ and NO_3^- in enriched tanks were 100-fold and ~38-fold, respectively of those in the control tanks. Differences in coral morphology were observed between the nutrient-enriched samples and the control samples. Corals exposed to elevated nutrient concentrations exhibited darker colouration, larger polyp size, and longer polyp extension period. All coral samples survived throughout the experimental period except *Acropora* spp. that was exposed to elevated nutrient concentrations. Mortality rate was 25%.

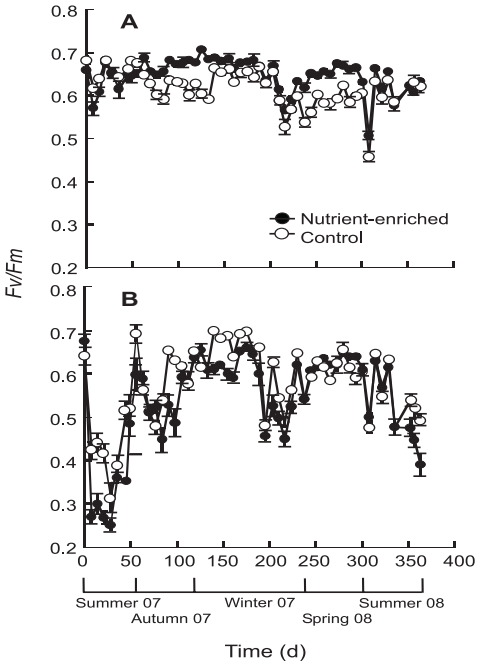

Figure 1: *Fv/Fm* changes in symbiotic algae under nutrient-enriched (filled circle) and control (open circle) conditions throughout the experimental period. (A) *Acropora* spp (B) *S. pistillata* (n = 12, SE = error bars).

Significant difference in *Fv/Fm* between nutrient-enriched samples and control samples were observed throughout the experimental period as shown in Fig 1 (ANOVA, $p<0.01$). *S. pistillata* exhibited a large fluctuation of *Fv/Fm* during the experiment period whereas *Acropora* spp. showed relatively constant

values. Larger fluctuations were observed during summer as well as winter.

Zooxanthellae density in different species of corals responded differently nutrient-enrichment (Fig 2). In the case of *S. pistillata*, significantly higher zooxanthellae density was observed for samples incubated in nutrient-enriched seawater compared to those in control (*t*-test, $p<0.001$). In contrast, in *Acropora* spp., control samples exhibited higher zooxanthellae density than the nutrient-treated samples (*t*-test, $p<0.001$).

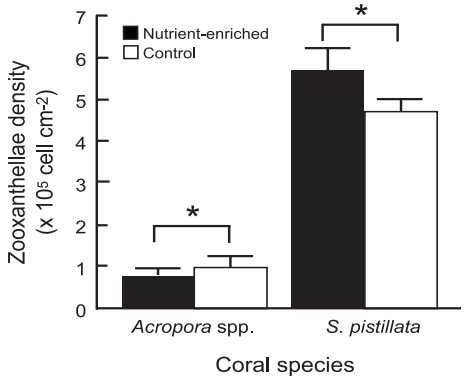

Figure 2: *Acropora* spp. and *S. pistillata*. Number of zooxanthellae per surface area of coral nubbins incubated in nutrient-enriched seawater and control seawater (n = 6, SE = error bars).

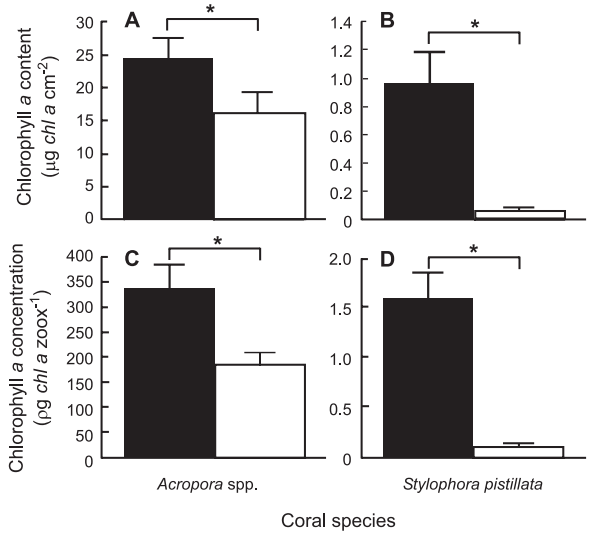

Figure 3: *Acropora* spp. and *S. pistillata*. (A and B) Chlorophyll *a* content per surface area and (C and D) Chlorophyll *a* per zooxanthella for coral nubbins incubated in nutrient-enriched seawater and control seawater (n = 6, SE = error bars).
Note: The y-axis scales are different for *Acropora* spp. and *S. pistillata*.

The most distinct effect of elevated nutrient concentration on the symbiotic algae was the increase in chlorophyll *a* content as shown in Fig 3. Chlorophyll *a* per surface area and chlorophyll *a* per algal cell were significantly greater for nutrient-treated samples compared to control samples (*t*-test, $p<0.001$). The difference between nutrient-treated samples and control samples were 10-fold higher in *S. pistillata* than in *Acropora* spp. Chlorophyll *a* content of *S. pistillata* was significantly lower compared to *Acropora* spp. (ANOVA, $p<0.001$) even though zooxanthellae density of the former was significantly higher than the later (Fig 2, ANOVA, $p<0.001$). These results are concomitant with the low *Fv/Fm* recorded in summer 2008 for *S. pistillata* as shown in Fig 1B.

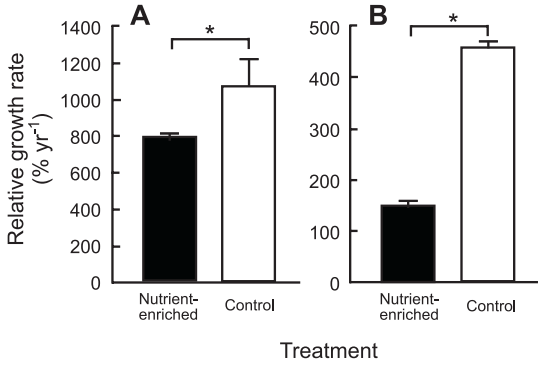

Figure 4: Differences in the relative growth rate over a 12 months period for (A) *Acropora* spp. (B) *S. pistillata* incubated in nutrient-enriched seawater (filled bar) and control seawater (open bar). (n = 6, SE = error bars).
Note: The y-axis scales are different for *Acropora* spp. and *S. pistillata*.

Significantly higher growth rate was observed in control samples than in nutrient-enriched samples (Fig 4, ANOVA, $p<0.001$). Differences in the relative growth rate (RGR) between control sample and nutrient-treated sample for *Acropora* spp. and *S. pistillata* were 1.4-fold and 3.1 fold, respectively.

Discussion

The present study has demonstrated that *Acropora* spp. and *S. pistillata* can survive in conditions of relatively high nutrient levels. However growth rates of corals were negatively affected in the nutrient-enriched treatment. In the short-term (1-2 months) experimental period, more than 50% decrease in the growth of corals exposed to 20 µM of NO_3^- or 20 µM of NH_4^+ was reported (Ferrier-Pagès et al. 2000; Marubini and Davies 1996). However, corals cultured in high-nutrient seawater at the Waikiki Aquarium, Hawaii show similar growth rates compared to those in the field (Atkinson et al. 1995). Coral reefs have formed and flourished under a wide range of natural nutrient regime (Szmant 2002). Given ample time, it is likely that coral and its symbiotic algae have the ability to adapt to relatively high nutrient levels.

Both *Acropora* spp. and *S. pistillata* showed higher chlorophyll *a* content in nutrient-treated samples although the zooxanthellae density was slightly lower in nutrient-enriched samples compared to control samples in *Acropora* spp. General response of corals towards nutrient enrichment include increase in algal density and pigmentation. This resulted in darker colouration in coral tissue (Hoegh-Guldberg and Smith 1989; Muscatine et al. 1989). The symbiotic algae in host can increase their density of thylakoids under elevated nutrient treatment (Berner and Izhaki 1994). This is likely in the case of *Acropora* spp. In contrast, *S. pistillata* might have suffered photoinhibition near the end of the experimental period which resulted in low chlorophyll *a* content.

Effect of nutrient enrichment on coral varies according to coral species. *Acropora* spp. and *S. pistillata* responded differently to nutrient enrichment. Mortality was observed in *Acropora* spp. but not in *S. pistillata*. Although the latter suffered bleaching during summer 2007 (during high SST period), it appears that they are more tolerant to elevated nitrogen level compared to the former. Elevated nutrient concentrations seem to lower the *Fv/Fm* of *S. pistillata* but may have a positive effect on *Acropora* spp. The results suggest that fast-growing *Acropora* spp. may be more susceptible to elevated nutrient levels compared to the slower-growing *S. pistillata*. This result agreed with those reported by Schlöder and D'Croz (2004).

The concentrations of NH_4^+ and NO_3^- used in this study are orders of magnitude higher than in nature as well as on polluted coral reefs. The concentration of NH_4^+ used was lower than the toxic level reported by Hoegh-Guldberg (1994). This is useful for the investigation of effects of elevated nitrogenous levels on the dynamics of coral-symbiotic association, changes in the biochemical composition of algae and long-term survival of corals.

While there are evidences that nutrient enrichment causes negative impact on coral reefs, most of the affected sites have restricted water circulation and the effects are localized (Szmant 2002). Coral nubbins were exposed to constant water flow in this study, which could have lessened the toxic effect of high inorganic nitrogen levels combined with high water temperature (Bouchard and Yamasaki 2008). Water flow reduces photodamage of algal photosynthesis as well as facilitates recovery of coral from bleaching (Nakamura et al. 2003; Nakamura et al. 2005). In general, nutrient enrichment is not the sole factor causing coral reef degradation. Corals exposed to elevated nutrient level become more susceptible to other stressors. This can lead to the decline in coral health and coral reef ecosystem.

Further studies are required in the synergistic impacts of elevated nutrient levels and other environmental stressors on the physiology of corals and coral-symbiotic association. This study suggests that effects of elevated nutrient to corals are species-dependent. Faster growing species may suffer more than slow growing one. This may be due to the differences in adaptation capability of the symbiosis system to changing environment.

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