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Rates of overgrowth by macroalgae and attack by sea anemones are greater for live coral than dead coral under conditions of nutrient enrichment

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Abstract

A mesocosm experiment was conducted to determine the effects of nutrient enrichment on competitive interactions between a hard coral, a green alga, and a sea anemone. In the low-nutrient controls, abundances of the green alga, *Codium edule*, and a sea anemone, *Mesactinia genesis*, remained low, and they coexisted with the live or dead scleractinian coral, *Acropora muricata*. Combined nitrogen and phosphorus additions markedly increased the photosynthetic efficiencies of zooxanthellae in *A. muricata*, the coverage of *C. edule*, and the asexual reproduction by *M. genesis*. After 35 d of nutrient addition, *C. edule* had begun to overgrow live *A. muricata*, but not dead coral. *A. muricata* finally died after 105 d, after being totally overgrown by *C. edule*. Within a few days of contact with live *A. muricata*, *M. genesis* was observed for the first time to have induced inflation of modified marginal aggressive organs known as acrorhagi tentacles, which it uses to attack neighboring coral. Nevertheless, *M. genesis* was not observed to attack *C. edule*, but moved away from it in the nutrient-enriched tanks. The hierarchy of competitive superiority under nutrient enrichment was in the order of *C. edule* > *M. genesis* > *A. muricata*. From this experiment, it was evident that nutrient enrichment inhibits corals' ability to compete with sea anemones and algae in Nanwan Bay, southern Taiwan.

Coral reefs are partially characterized by oligotrophic water. In the past few decades, however, human activities, including agriculture, urbanization, and tourism, have led to increased anthropogenic nutrient loading into coral reefs worldwide. Nutrient enrichment was found to increase algal photosynthesis (Littler et al. 1991), growth rates (Littler et al. 2006), and biomass (McClanahan et al. 2007). However, not all algal species are stimulated by nutrient enrichment. The growth of certain types of turf algae is frequently stimulated by nutrient enrichment, but this is not the case for frondose brown algae and coralline algae (McClanahan et al. 2007). When nutrient loading from land exceeds 'tipping-point' concentrations (Bell 1992, dissolved inorganic nitrogen and phosphorus [DIP] greater than 1.0 and 0.1 μmol L^{-1}, respectively), algal blooms may occur.

Nutrient enrichment also appears to favor symbiotic zooxanthellae within corals and sea anemone gastrodermal cells. Some benefits of high nutrient levels to corals are increased density of zooxanthellae and enhanced resistance to bleaching (McClanahan et al. 2003). However, excessive nutrients may inhibit coral growth (Koop et al. 2001; Littler et al. 2006) and calcification (Muscatine et al. 1989) when DIP concentrations exceed 0.01 μmol L^{-1} (Simkiss 1964). Finally, coral reproduction is also compromised by eutrophic conditions (Koop et al. 2001).

Nutrient enrichment may shift interactions between organisms within coral reefs from coexistence to competition, or from nutrient competition to competition for space, available light, or other limited resources. McCook et al. (2001) noted numerous examples of algae outcompeting coral either through utilization of allelochemicals or simply by overgrowth and consequent smothering. An indirect effect of algal overgrowth is the release of high levels of dissolved organic carbon, which leads to accelerated growth of microbes and pathogens living in the coral surface mucopolysaccharide layer, which can in turn induce coral disease and consequent mortality (Smith et al. 2006). Algal overgrowth was also found to reduce dissolved oxygen concentrations and inhibit the survival of coral recruits (Sammarco 1982). A phase shift to macroalgae-dominated reef systems sometimes occurs as a result of the combined effect of nutrient enrichment and herbivory reduction (Hughes 1994).

Competitive interactions between coral and sea anemones have been also observed. For instance, *Acropora* colonies extruded mesenterial filaments to damage the tissue of the corallimorpharian *Rhodactis rhodostoma* (Langmead and Chadwick-Furman 1999). The corallimorpharian also develops special bulbous marginal tentacles to attack coral tissue and overgrow coral. Some sea anemones may develop special aggressive organs (acrorhagi) to compete for space with coral (reviewed by Williams 1991). Under eutrophic conditions, results of competition include loss of coral cover, algal blooms, and a shift in the dominant communities of coral reefs (Littler et al. 2006). However, interactions among corals, macroalgae, and sea anemones under nutrient enrichment have not yet been examined. The effects of nutrient enrichment on these interactions may be critical processes that drive community composition and functioning of coral reef ecosystems.

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Nanwan Bay is located at the southern tip of Taiwan (21°57′N, 120°44′E) within Kenting National Park. It is a semienclosed embayment bounded by two capes with well-developed fringing reefs distributed along the shoreline. Dense thickets of scleractinian corals of the genus *Acropora* were formerly dominant in the coral reefs of Nanwan Bay. Over the past 15 yr, seasonal outbreaks of the green alga *Codium edule* have been documented in the coral reefs (Dai 1997). Many *Acropora* colony patches have been degraded and persistently replaced by the sea anemones *Condylactis* sp. and *Mesactinia genesis* (Chen and Dai 2004). In 2003, coverage by macroalgae and sea anemones reached 31% and 50%, respectively, of the area of biomass coverage in some patch reefs of Nanwan Bay (Tkachenko et al. 2007). Sea anemone outbreaks have only been reported on a few coral reefs (Chadwick-Furman and Spiegel 2000; Okey 2004), but the conditions leading to the cause and their ensuing effects are still unknown.

The most likely causes of coral degradation, algal increases, and sea anemone outbreaks in the coral reefs of Nanwan Bay are sewage discharges, overfishing, and sedimentation (Meng et al. 2008). Large quantities of untreated waste and human feces are carried by sewer drains into Nanwan Bay, producing excessive nutrient loading (Lin et al. 2007). In this article, we focused on an ecosystem-level experiment to test how nutrients affect competitive interactions among a green alga, a sea anemone, and a hard coral. A mesocosm approach was adopted in order to observe changes in the community structure of a relatively complex coral reef ecosystem under nutrient enrichment. We hypothesized that nutrient enrichment would inhibit corals' ability to compete with sea anemones and macroalgae in Nanwan Bay.

Methods

Coral reef mesocosm facility—The coral reef mesocosm facility is located at the National Museum of Marine Biology and Aquarium (NMMBA), approximately 10 km northwest of Nanwan Bay. The facility consists of six tanks designed to serve as living models of fringing reefs of Nanwan Bay. Each elliptical tank measures 3.0 m long by 2.0 m wide, with an area of 5.14 m^2 and 1.0-m–deep water covering a 3-cm–thick layer of sandy sediment (Fig. 1). Additionally, associated organisms collected from nearby coral reefs are maintained within the mesocosms.

The experiment was carried out over the course of 4 months (August–December 2006). Seawater for the tanks was pumped from nearby coral reefs. Equal volumes (180 L h^{-1}) of water were added to each tank twice a day at a rate sufficient to provide an exchange of 10% d^{-1} of the volume, which is the approximate flushing rate of Nanwan Bay (H.-J. Lee pers. comm.). Liang et al. (1978) observed that ambient currents in Nanwan Bay, from which the algae and coral were collected, were normally <20 cm s^{-1}. Water in the tanks was thus well mixed by two pumps (120 L min^{-1}) and produced near-surface and near-bottom currents of 15–20 and 5–10 cm s^{-1}, respectively. Water temperatures in the tanks were maintained at 25.0–26.0°C using a heat-exchanger cooling system to simulate field conditions in spring (25.4°C ± 0.02°C). The photosynthetically active radiation (PAR) intensity from 08:00 to 17:00 h at a 5.0-m depth in coral reefs of Nanwan Bay was 165 ± 1.6 μmol photons m^{-2} s^{-1} from January to April 2007 (T.-Y. Fan unpubl.). The PAR intensity at a 0.5-m depth of the tanks was maintained at 266 ± 5.0 μmol photons m^{-2} s^{-1} from 07:00 to 17:00 h (a 10 : 14-h light : dark photoperiod) and was provided by metal halide lamps (HPIT 400 W, Philips). The PAR intensity reaching the corals in the mesocosms was comparable to field conditions in spring.

Fig. 1. The coral reef mesocosm facility located at the National Museum of Marine Biology and Aquarium, southern Taiwan.

Experiments were carried out with coral specimens prepared from parent colonies originating from coral reefs in Nanwan Bay, which had been maintained for over a year at the NMMBA. In March 2006, three growth forms of hard corals were added, including branching corals (*Acropora muricata*, *Porites* sp., *Stylophora pistillata*, *Seriatopora hystrix*, *Pocillopora damicornis*, and *Montipora stellata*), solitary corals (*Fungia* spp.), and massive corals (*Porites* sp., *Faviid* spp., and *Heliopora coerulea*). After 3 months of acclimation, each tank also received green algae (*Avrainvillea erecta*, *Caulerpa racemosa*, *Chaetomorpha crassa*, *Codium edule*, *Halimeda discoidea*, *Ulva intestinalis*, and *Ulva reticulata*), red algae (*Amansia glomerata*, *Galaxaura obtusata*, *Gracilaria coronopifolia*, *Halymenia floresia*, *Laurencia brongniartii*, *Mastophora rosa*, *Peyssonnelia rubra*, and *Plocamium telfairiae*), brown algae (*Lobophora variegate* and *Turbinaria conoides*), 40 sea anemones (*Mesactinia genesis*), 2 damselfish (*Pomacentrus bankanensis* and *Pomacentrus pavo*), 2 giant clams (*Tridacna* sp.), 2 gold-ringed cowries (*Cypraea annulus*), 2 hermit crabs (*Paguroidea* sp.), and 5 sea cucumbers (*Holothuria leucospiolota*). Coverage of uncolonized sand in the mesocosms was 60% of the bottom area, which was comparable to the 56% in Nanwan Bay (P.-J. Liu unpubl.). Coverage of corals and macroalgae in terms of the area of biomass coverage in the tanks was 25.0% ± 2.1% and 5.0% ± 0.3%, respectively. The biomass of herbivorous fish in the tanks was about 25.6 ± 0.34 g wet weight m^{-2}, which was close to the 21.0 g wet weight m^{-2} recorded in Nanwan Bay (P.-J. Liu unpubl.). All of these macroalgae and animals naturally co-occur in coral reefs of Nanwan Bay,

and they complement the natural assemblage of the infauna collected with the sediments, rocks, and plankton added with the seawater, including hydroids, polychaetes, sea anemones, sponges, isopods, amphipods, symbiotic coral crabs, shrimp, tunicates, and chitons.

Experimental design—A factorial design was applied to examine the effects of nutrient enrichment on competitive interactions of a green alga and sea anemone with a hard coral under low levels of herbivory and carnivory. Three tanks were assigned to be controls and received only the daily addition of relatively low-nutrient seawater ($NO_3^- + NO_2^-$: 1.96 ± 0.12 μmol L^{-1}, PO_4^{-3}: 0.05 ± 0.02 μmol L^{-1}), while the other three were enriched with nitrogen (5.50 mmol NO_3^- m^{-2} d^{-1}) and phosphorus (0.48 mmol PO_4^{-3} m^{-2} d^{-1}) at an N : P molar ratio of 12, which was intended to mimic anthropogenic inputs to coral reefs in Nanwan Bay (Lin et al. 2007). Nutrient addition was initiated 6 months after transplantation of corals in March, when the status of the communities and water environment in each tank appeared stable. Dissolved nutrients were added daily at noon to the water column from stock solutions of $NaNO_3$ and KH_2PO_4.

Three types of placement in quadruplicate were established in baskets within each tank. The first type of placement experimentally induced contact of the branching scleractinian coral, *A. muricata*, with the sea anemone, *M. genesis*, and the green alga, *C. edule*, by placing them side by side (E-CAS in enriched tanks and C-CAS in control tanks). The second type of placement induced contact of *C. edule* and *M. genesis* with dead *A. muricata* for comparison and to examine their interactions after coral decline (E-AS in enriched tanks and C-AS in control tanks). The third placement consisted of isolated *A. muricata* as the control (E-C in enriched tanks and C-C in control tanks).

Response—Water temperature, salinity, pH, dissolved oxygen (DO) concentrations, and PAR intensity were continually monitored in each tank using YSI 600XLM multiparameter monitoring sensors and HOBO temperature and light data loggers (Onset Computer Corporation). The HOBO loggers were positioned at the bottom of each tank. Chlorophyll *a* concentrations in the water column were determined twice a week in each tank with a spectrophotometer by immediately filtering water samples in triplicate through Whatman GF/F filters and then extracting them in 90% acetone for 24 h at 4°C in the dark (Parson et al. 1984). Water samples for nutrient analyses were collected in triplicate twice each week from each tank and were filtered through Whatman GF/F filters. Concentrations of NO_3^-, NO_2^-, NH_4^+, and PO_4^{-3} were determined colorimetrically by a flow injection analytical method (Strickland and Parsons 1972; Pai et al. 1990).

In order not to disturb the interactions among *C. edule*, *M. genesis*, and *A. muricata* during the experimental period, changes in the abundance of *C. edule* were monitored by taking photos and measuring the vertical projected coverage by Image-Pro Plus. Chlorophyll fluorescence measurements have proved to be useful in assessing photosynthetic efficiency (Krause and Weis 1991). The photosynthetic efficiencies of *C. edule* and *A. muricata* were monitored every 2 weeks in each tank by measuring the chlorophyll fluorescence of photosystem II (PS II) using a submersible pulse amplitude–modulated (Diving-PAM) fluorometer (Waltz). The fluorescence parameters of *Fo* (initial chlorophyll fluorescence after acclimating corals and algae in darkness for 20 min when all reaction centers are open), *Fm* (maximal chlorophyll fluorescence after dark acclimation for 20 min when all reaction centers are closed following a saturating flash of light), and *Fv* : *Fm* (maximal quantum yield of PS II, where $Fv = Fm - Fo$) were measured. The maximal quantum yield of PS II (*Fv* : *Fm*) has been used to assess bleaching susceptibility and the response to stressors, such as microbial activities in some coral species (Smith et al. 2006).

Statistical analysis—A repeated-measures ANOVA was employed to determine whether the effects of time, treatments (nutrient-enriched vs. control), physical contact with live *A. muricata*, and their interactions were significant with regard to environmental variables and the responses of *C. edule*, *M. genesis*, and *A. muricata* (SAS 2004). Fisher's least significant differences (LSD) test was used to perform post hoc means comparisons for significance effects (Sokal and Rohlf 1995). Before the analyses, data were power-transformed (Clarke and Warwick 1994) to ensure that they conformed to the assumptions of the parametric statistics.

Results

Environmental factors—During the study period, water temperature was maintained at 25.8°C ± 0.1°C and salinity at 34.1–34.8 in the mesocosms (Table 1). pH values averaged 8.54 ± 0.04 in the enriched tanks and 8.40 ± 0.04 in the controls. DO concentrations showed a distinct diurnal change from 8.0 ± 0.2 mg L^{-1} at night to 11.0 ± 0.3 mg L^{-1} in the day. No significant effects of time or treatment were detected on water temperature, salinity, pH values, DO concentrations, or PAR intensities monitored in the mesocosms during the study period (repeated-measures ANOVA, $p > 0.05$ for each variable). The chlorophyll *a* concentration averaged 0.09 ± 0.003 mg m^{-3} in the controls, but was significantly higher and reached 0.46 ± 0.03 mg m^{-3} in the enriched tanks (repeated-measures ANOVA: $F_{1,4} = 177.1$, $p < 0.001$).

Before the start of the experiment, $NO_3^- + NO_2^-$ and PO_4^{-3} concentrations were low and showed no significant differences among tanks (Table 1). During the experimental period, mean $NO_3^- + NO_2^-$ and PO_4^{-3} concentrations remained low in the controls (Fig. 2). In the enriched tanks, there was a spike in concentrations 1 h after nutrient addition ($NO_3^- + NO_2^-$: 5.12 ± 0.20 μmol L^{-1}; PO_4^{-3}: 1.19 ± 0.096 μmol L^{-1}), decreasing to lower levels as the nutrients were consumed in the evening ($NO_3^- + NO_2^-$: 2.81 ± 0.13 μmol L^{-1}; PO_4^{-3}: 0.55 ± 0.08 μmol L^{-1}). $NO_3^- + NO_2^-$ and PO_4^{-3} concentrations were significantly higher in the enriched tanks relative to the controls (repeated-measures ANOVA: $F_{1,4} = 15{,}780$ for $NO_3^- +$

Table 1. Physical and chemical characteristics of and nutrient fluxes (mean ± standard error [SE]) into the coral reef mesocosms during the experimental period.

Environmental variables	Control			Enriched		
	C1	C2	C3	E1	E2	E3
Area of mesocosm (m^2)	5.14			5.14		
Reef area of mesocosm (m^2)	1.54			1.54		
Volume of mesocosm (m^3)	3.6			3.6		
Temperature (°C) (n=120)	26.0±0.002	25.9±0.002	25.5±0.002	25.8±0.002	25.7±0.002	25.6±0.002
Salinity (n=120)	34.3±0.2	34.6±0.1	34.5±0.1	34.1±0.2	34.3±0.2	34.8±0.1
Dissolved oxygen (mg L^{-1}) (n=15)	9.02±0.66	9.08±1.24	9.08±0.95	9.62±0.84	9.35±1.65	9.72±1.30
pH (n=15)	8.47±0.03	8.37±0.03	8.38±0.02	8.53±0.07	8.53±0.07	8.56±0.10
Photosynthetically active radiation intensity (μmol photons s^{-1} m^{-2}) (n=15)	256±10.8	263±6.0	286±8.1	271±6.9	258±6.9	262±8.2
Chlorophyll *a* concentration (mg m^{-3}) (n=35)	0.09±0.01	0.10±0.01	0.09±0.01	0.49±0.06	0.40±0.03	0.47±0.06
NO_3^- addition (mmol m^{-2} d^{-1})	0			5.50		
PO_4^{-3} addition (mmol m^{-2} d^{-1})	0			0.48		
NO_3^- + NO_2^- background inflow (mmol m^{-2} d^{-1}) (n=35)	0.13±0.01			0.14±0.01		
PO_4^{-3} background inflow (mmol m^{-2} d^{-1}) (n=35)	0.007±0.002			0.007±0.002		
NO_3^- + NO_2^- inputs (mmol m^{-2} d^{-1})	0.13			5.64		
PO_4^{-3} inputs (mmol m^{-2} d^{-1})	0.01			0.49		
NO_3^- + NO_2^- uptake rate (mmol m^{-2} d^{-1}) (n=35)	0.15±0.06	0.14±0.06	0.10±0.07	1.68±0.10	1.65±0.10	1.60±0.10
PO_4^{-3} uptake rate (mmol m^{-2} d^{-1}) (n=35)	0.01±0.01	0.01±0.01	0.01±0.01	0.39±0.03	0.42±0.04	0.39±0.05
NO_3^- + NO_2^- concentration before the start of the experiment (μmol L^{-1}) (n=7)	1.70±0.10	1.86±0.11	1.88±0.14	1.67±0.11	1.77±0.07	1.87±0.11
PO_4^{-3} concentration before the start of the experiment (μmol L^{-1}) (n=7)	0.01±0.01	0.01±0.01	0.01±0.01	0.02±0.01	0.01±0.01	0.01±0.01
NO_3^- + NO_2^- concentration during the experimental period (μmol L^{-1}) (n=35)	2.11±0.10	2.13±0.10	2.24±0.11	4.12±0.20	4.14±0.21	4.21±0.20
PO_4^{-3} concentration during the experimental period (μmol L^{-1}) (n=35)	0.04±0.10	0.04±0.10	0.03±0.01	0.70±0.10	0.70±0.10	0.90±0.10

NO_2^-, $p < 0.001$ and $F_{1,4} = 3956$ for PO_4^{-3}, $p < 0.001$). There was also a significant interaction of time and treatment in NO_3^- + NO_2^- and PO_4^{-3} concentrations (repeated-measures ANOVA: $F_{31,124} = 18.83$ for NO_3^- + NO_2^-, $p < 0.001$ and $F_{31,124} = 207.54$ for PO_4^{-3}, $p < 0.001$). In the enriched tanks, the spike in NO_3^- + NO_2^- concentrations increased to 4.75 ± 0.44 μmol L^{-1} during the first 3 d, increased to 6.02 ± 0.07 μmol L^{-1} throughout the 4–54-d period, and then decreased to 3.62 ± 0.19 μmol L^{-1} during the 55–124-d period. Approximately 13-fold and 40-fold more NO_3^- + NO_2^- and PO_4^{-3} were taken up, respectively, in the enriched tanks than in the controls (Table 1). NH_4^+ concentrations were monitored before the start of the experiment and after 31 and 105 d of nutrient addition. The concentrations remained at low levels (<0.50 μmol L^{-1}) relative to those of NO_3^- + NO_2^- in both the enriched tanks and the controls. PO_4^{-3} concentrations steadily increased with time in the enriched tanks and reached 2.71 ± 0.11 μmol L^{-1} by the end of experiment.

Low-nutrient controls—In the low-nutrient controls, coverage of *C. edule* remained low, and it coexisted with live or dead *A. muricata* throughout the study period (Fig. 3). *Fv*:*Fm* values of *C. edule* gradually declined after 35 d (Fig. 4). Individual numbers of *M. genesis* also remained low throughout the study period (Fig. 5). However, *M. genesis* in contact with live *A. muricata* induced inflation of modified marginal aggressive organs known as acrorhagi tentacles (Williams 1991), which were observed for the first time to have injured or killed nearby *A. muricata* (Fig. 6). The linear extent of tissue damage to *A. muricata* attacked by *M. genesis* averaged 306 ± 28 mm after 58 d. Both *Fv*:*Fm* values of unattacked *A. muricata* and isolated *A. muricata* remained at the same levels (0.62 ± 0.01) as the initial values (Fig. 7).

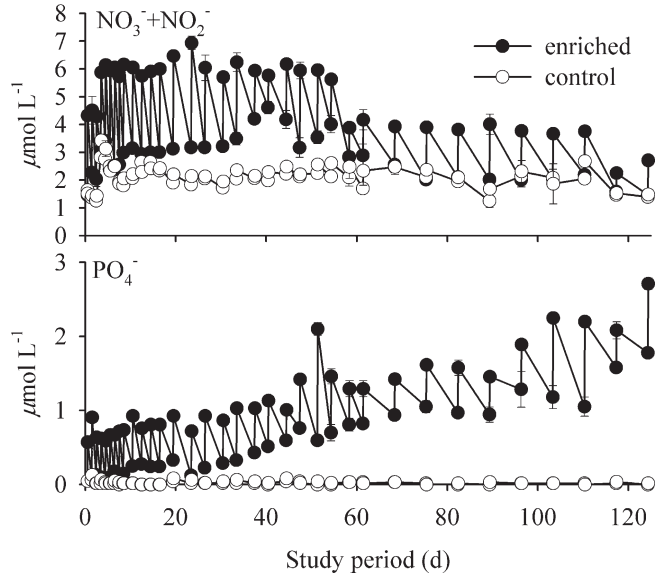

Fig. 2. Temporal variations in NO_3^- + NO_2^- and PO_4^{-3} concentrations (mean ± standard error [SE], $n = 3$) in enriched and control mesocosms during the experimental period.

Nutrient-enriched tanks—In response to elevated nutrient levels, *C. edule* cover significantly increased, especially when in contact with live *A. muricata*. Coverage of *C. edule* in nutrient-enriched tanks was 3.0-fold higher after 31 d of nutrient addition (Fig. 3), and it began to overgrow live *A. muricata* and increased to 6.6–9.3-fold higher levels after 101 d (Fig. 8). There was a significant interaction of nutrient enrichment and contact with live *A. muricata* in coverage of *C. edule* (repeated-measures ANOVA: $F_{1,8} = 9.95$, $p = 0.01$). Coverage of *C. edule* in contact with live *A. muricata* was remarkably greater than that growing with dead *A. muricata* after 21 d (Fig. 3; LSD, $p < 0.05$). The *Fv*:*Fm* value of *C. edule* in the enriched tanks was consistently high and significantly higher than that in the controls throughout the study period (Fig. 4; repeated-measures ANOVA: $F_{1,7} = 1918$, $p < 0.001$).

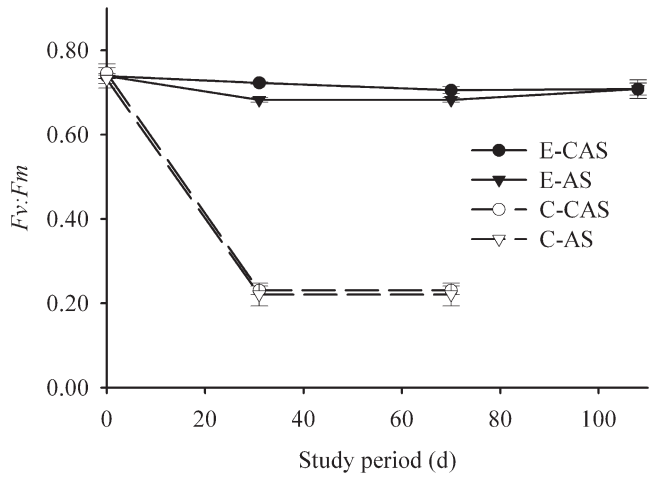

Fig. 4. Changes in the maximal quantum yield of photosystem II (*Fv*:*Fm*, mean ± standard error [SE], $n = 3$) of the green alga, *Codium edule*. E-CAS, *C. edule* grown with the scleractinian coral, *Acropora muricata*, and the sea anemone, *Mesactinia genesis*, in enriched tanks; C-CAS, *C. edule* grown with *A. muricata* and *M. genesis* in control tanks; E-AS, *C. edule* grown with dead coral and *M. genesis* in enriched tanks; C-AS, *C. edule* grown with dead coral and *M. genesis* in control tanks.

Nutrient enrichment stimulated asexual reproduction by lateral fission in *M. genesis* in contact with live *A. muricata*. After 89 d of nutrient addition, mean individual numbers of *M. genesis* in contact with live *A. muricata* had increased

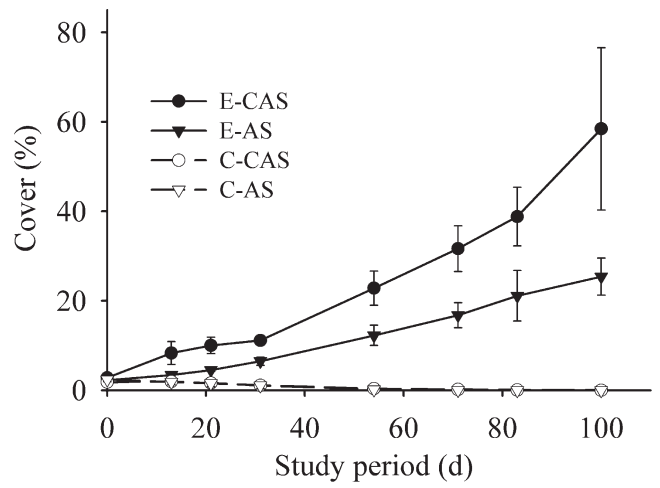

Fig. 3. Increase in coverage (relative to cover of the basket, mean ± standard error [SE], $n = 3$) of the green alga, *Codium edule*. E-CAS, *C. edule* grown with the scleractinian coral, *Acropora muricata*, and the sea anemone, *Mesactinia genesis*, in enriched tanks; C-CAS, *C. edule* grown with *A. muricata* and *M. genesis* in control tanks; E-AS, *C. edule* grown with dead coral and *M. genesis* in enriched tanks; C-AS, *C. edule* grown with dead coral and *M. genesis* in control tanks.

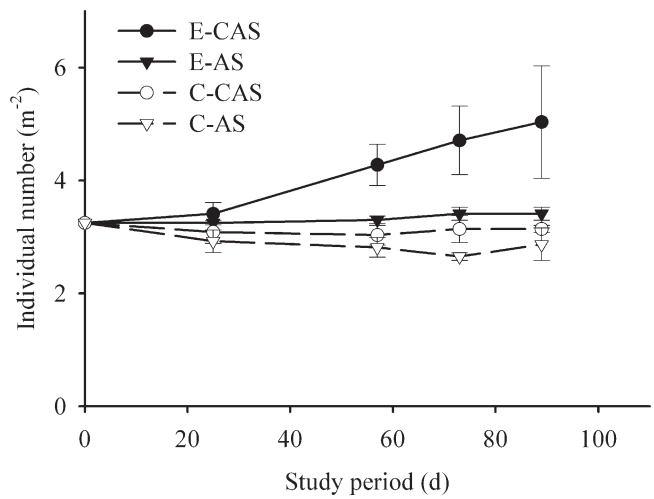

Fig. 5. Changes in individual numbers (relative to reef cover, mean ± standard error [SE], $n = 3$) of the sea anemone, *Mesactinia genesis*. E-CAS, *M. genesis* grown with the scleractinian coral, *Acropora muricata*, and the green alga, *Codium edule*, in enriched tanks; C-CAS, *M. genesis* grown with *A. muricata* and *C. edule* in control tanks; E-AS, *M. genesis* grown with dead coral and *C. edule* in enriched tanks; C-AS, *M. genesis* grown with dead coral and *C. edule* in control tanks.

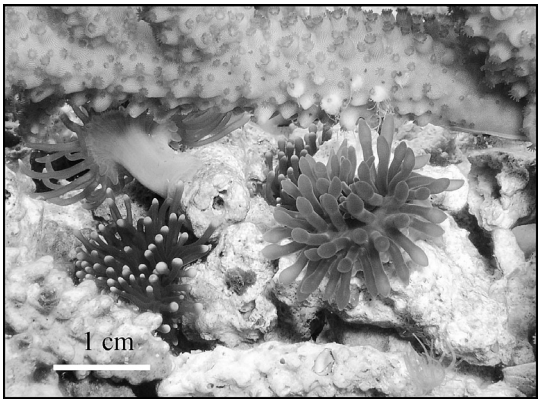

Fig. 6. Nutrient enrichment–induced inflation of modified marginal tentacles of *Mesactinia genesis*, known as acrorhagi tentacles, that are capable of injuring or killing nearby coral.

by 23% in the enriched tanks (Fig. 5). Individual numbers of sea anemones were significantly greater in the enriched tanks (repeated-measures ANOVA: $F_{1,8} = 13.97$, $p = 0.006$) and in those in contact with live *A. muricata* (repeated-measures ANOVA: $F_{1,8} = 7.08$, $p = 0.02$). Acrorhagi tentacles of *M. genesis* in contact with live *A. muricata* were also observed to have injured or killed nearby coral. The injured or killed length of *A. muricata* attacked by *M. genesis* averaged 631 ± 215 mm, which was about 2-fold greater than that length measured in the controls. However, *M. genesis* in contact with *C. edule* was not observed to develop any attacking acrorhagi tentacles; instead, it was observed to move away from the fast-growing *C. edule*.

There was a significant interaction of time and nutrient enrichment (repeated-measures ANOVA: $F_{12,96} = 12.19$, $p < 0.001$) and of time and growth with *C. edule* and *M. genesis* in *Fv*:*Fm* values of zooxanthellae in *A. muricata* (repeated-measures ANOVA: $F_{12,96} = 4.44$, $p = 0.006$). Mean *Fv*:*Fm* values of zooxanthellae in *A. muricata* increased up to 0.70 in the enriched tanks after 35 d of nutrient addition (Fig. 7), which was significantly higher than the increase measured in the controls (LSD, $p < 0.05$). During the 36–105-d period, mean *Fv*:*Fm* values of zooxanthellae in isolated *A. muricata* remained at high levels. However, mean *Fv*:*Fm* values declined to a low value of 0.66 ± 0.01 when *A. muricata* was in contact with and overgrown by *C. edule* after 49 d. There was also a significant effect of nutrient enrichment and growth with *C. edule* and *M. genesis* on *Fv*:*Fm* values of zooxanthellae in *A. muricata* (repeated-measures ANOVA: $F_{1,8} = 9.21$, $p = 0.01$). After 105 d, *A. muricata* in contact with *C. edule* had been totally overgrown and consequently died under nutrient enrichment (Fig. 8).

Discussion

Nutrient inputs to the enriched tanks were similar to the loadings to other enriched coral reefs or other marine systems. $NO_3^- + NO_2^-$ inputs (2.07 mol m^{-2} yr^{-1}) to the

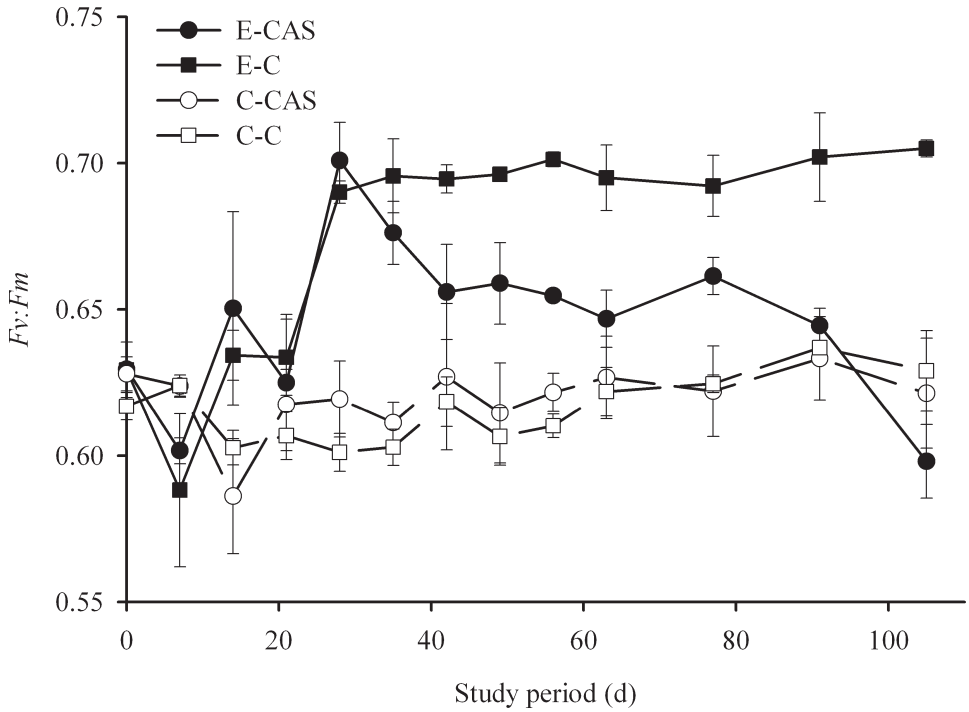

Fig. 7. Changes in the maximal quantum yield of photosystem II (*Fv*:*Fm*, mean ± standard error [SE], $n = 3$) of the scleractinian coral, *Acropora muricata*. E-CAS, *A. muricata* grown with the green alga, *Codium edule*, and the sea anemone, *Mesactinia genesis*, in enriched tanks; C-CAS, *A. muricata* grown with *C. edule* and *M. genesis* in control tanks; E-C, *A. muricata* grown alone in enriched tanks; C-C, *A. muricata* grown alone in control tanks.

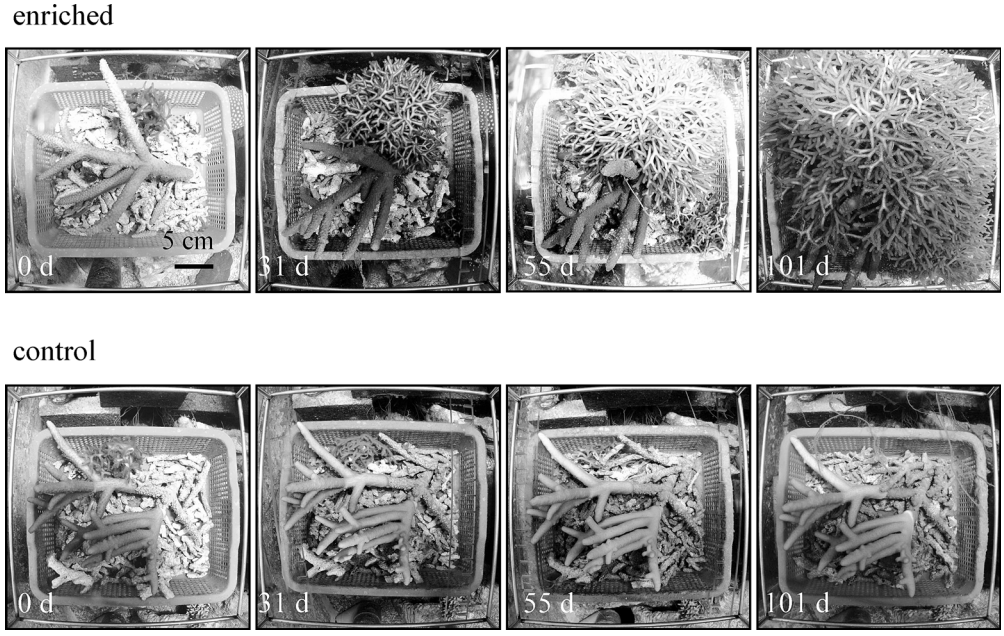

Fig. 8. Development of the green alga, *Codium edule*, living with the scleractinian coral, *Acropora muricata*, in enriched and control tanks. The cover remained low in the control tanks. However, *C. edule* totally overgrew *A. muricata* after 101 d of nutrient addition.

enriched tanks were very similar to the loadings to moderately enriched Shiraho coral reefs in the Ryukyu Islands (1.76–2.51 mol m^{-2} yr^{-1}; Umezawa et al. 2002*a*) and temperate lagoons such as Moriches Bay (2.4 mol m^{-2} yr^{-1}; Ryther 1989). PO_4^{-3} inputs (0.05 mol m^{-2} yr^{-1}) to the enriched tanks were higher than those of most Mexican tropical lagoons (Smith et al. 1997). However, nutrient inputs to the controls were low when compared with those of unenriched coral reefs or other marine systems. NO_3^- + NO_2^- inputs (0.17 mol m^{-2} yr^{-1}) to the controls were lower than the loadings to Kabira coral reefs in the Ryukyu Islands (0.17–0.90 mol m^{-2} yr^{-1}; Umezawa et al. 2002*a*) and Kaneohe Bay (0.21 mol m^{-2} yr^{-1}; Ryther 1989) in the Hawaiian Islands after 1978, when the loading of Kaneohe Bay was reduced sharply by sewage diversion (Nixon et al. 1986). PO_4^{-3} inputs (0.003 mol m^{-2} yr^{-1}) to the controls were also lower than those of most Mexican tropical lagoons (Smith et al. 1997).

Mean NO_3^- + NO_2^- concentrations in the mesocosms before nutrient addition (1.47 ± 0.02 μmol L^{-1}) and those in the controls during the experimental period (2.16 ± 0.10 μmol L^{-1}) were slightly higher than the NO_3^- concentrations in the offshore waters of Nanwan Bay (0.83–1.33 μmol L^{-1}; Chen et al. 2005) but were comparable to the NO_3^- concentrations in the outer reef seawater of the Ryukyu Islands (1.5–2.1 μmol L^{-1}; Umezawa et al. 2002*b*). The mean PO_4^{-3} concentrations (0.02 ± 0.01 μmol L^{-1}) were lower than those in the offshore waters of Nanwan Bay (0.10–0.14 μmol L^{-1}; Chen et al. 2005), likely resulting in a P-limited environment prior to nutrient addition. However, both added N and P were rapidly consumed in the evening after daily nutrient addition at noon in the enriched tanks, indicating a combined N- and P-limited environment. Nevertheless, PO_4^{-3} concentrations in the enriched tanks accumulated after 30 d of daily nutrient addition, while added N was continually consumed (Fig. 2). It follows that the enriched tanks gradually shifted to an N-limited environment after nutrient enrichment at an N : P molar ratio of 12, which is the ratio of sewage effluents on coral reefs in Nanwan Bay (Lin et al. 2007).

This study provides direct system-scale experimental evidence that the branching scleractinian coral *A. muricata*, the green alga *C. edule*, and the sea anemone *M. genesis* coexist under low nutrient conditions, although the relationship shifts to that of a competitive nature after enrichment with N and P. The mesocosm experiment clearly demonstrated the process of competitive interactions among the coral, macroalga, and sea anemone. Nutrient addition to the water column stimulated the photosynthetic efficiency of zooxanthellae living symbiotically in *A. muricata* tissue, the growth of *C. edule*, and asexual reproduction by *M. genesis*. However, the ultimate result was an increased intensity of macroalgal and sea anemone competition with the coral. Our results further indicate that the process was rapid: it took only about 1 month for macroalgae to overgrow corals and only about 3 months for macroalgae to completely replace corals.

Macroalgae are widely considered to compete with corals for space (or light), and interactions between the two groups are frequently interpreted simply in terms of macroalgal competitive superiority. However, there is surprisingly little direct, experimental evidence demonstrating competition between the two groups (McCook et al. 2001). Our results clearly demonstrated the interference competition between the macroalga and coral. It was evident that in the enriched tanks, *C. edule* grew faster when in contact with live *A. muricata* and had aggressively wrapped around live *A. muricata*, but not dead *A. muricata*,

by the end of experiment. The experiment also showed for the first time the interference competition between this sea anemone and coral. *M. genesis* specimens remarkably increased their rate of asexual reproduction, in which an identical animal buds out of the anemone's side, when in contact with live *A. muricata* under nutrient enrichment. Moreover, *M. genesis* was observed to have developed acrorhagi tentacles and to have aggressively attacked nearby live, but not dead, *A. muricata*. The photosynthetic performance of nearby *A. muricata* was found to be inhibited, and the coral was ultimately killed by the presence of the two competitors. Because our study did not experimentally manipulate only the presence of *C. edule* and *M. genesis*, we were unable to discern their interactions without the presence of the coral. Nevertheless, *M. genesis* was not observed to attack *C. edule*, but moved away from the fast-growing *C. edule* in the enriched tanks. It is clear that the response of *C. edule* to nutrient enrichment was more rapid and the scale of effects on *A. muricata* was broader than those of *M. genesis*. Consequently, when *A. muricata* was completely overgrown by *C. edule* and died after 105 d of nutrient addition, the portion of *A. muricata* that was injured or killed by *M. genesis* was limited only to areas where there was direct contact. The experimental results indicate that the hierarchy of competitive superiority under nutrient enrichment was in the order of *C. edule* > *M. genesis* > *A. muricata*.

In Nanwan Bay, however, *M. genesis* is more frequently observed in summer and fall in the coral reefs (Chen and Dai 2004). It appears that *M. genesis* outcompetes *C. edule* for space at these times, although *M. genesis* was observed to move away from the fast-growing *C. edule* in the enriched tanks. In this study, the water temperature in the mesocosms was maintained at about 25°C, which is the water temperature in spring when blooms of *C. edule* occur in Nanwan Bay (Dai 1997). A seasonal pattern of macroalgae with greater biomass in winter and spring and lower biomass in summer and fall has been documented in southwestern Taiwan (Lin and Hung 2004) and Hong Kong (Kaehler and Williams 1996). This seasonal pattern may be typical of macroalgae in tropical and subtropical waters, which can be attributed to limitations due to high summer temperatures (Lin and Hung 2004). *M. genesis* is expected to respond to nutrient enrichment and potentially replace the dominance of *C. edule* in summer when water temperatures increase to over 28°C or with decreased nutrients, at which time *C. edule* abundance declines.

The consistently high *Fv*:*Fm* values of zooxanthellae in isolated *A. muricata* for over 100 d in response to nutrient enrichment (Fig. 7) indicate that the decline of *A. muricata*, when in contact with *C. edule* and *M. genesis* in the enriched tanks, was more complex than simply nutrient stress to the coral (Koop et al. 2001), and the coral was not killed by bleaching, corallivory, or other damage. It appears that *C. edule* killed *A. muricata* by overgrowth, resulting in both smothering and specifically preventing effective photosynthesis. *A. muricata* was likely injured or killed by *M. genesis* through peptide toxins, which have been isolated from acrorhagi tentacles of the sea anemone *Actinia equina* (Honma et al. 2005).

Because eutrophication is recognized as a problem stemming from pollution in coastal waters worldwide, there is particular interest in understanding how anthropogenic nutrient loadings regulate the structure and functioning of coastal ecosystems. Our results indicate that nutrient enrichment might be a major cause of the decline of coral reefs in Nanwan Bay as a result of its effect of shifting the hierarchical dominance of the dominant taxa: green algae, corals, and sea anemones. Disease, bleaching, and sedimentation (Harvell et al. 1999; Hughes et al. 2003; Fabricius 2005), which are known to have caused coral reef declines in other locations and which would leave more substrates open for macroalgal or sea anemone colonization, might not be prerequisites for the decline if these factors proceed and follow nutrient-stimulated ecological disruption in this system.

Our results also reveal that reduced levels of herbivory are insufficient to consume excess growth of macroalgae that result from nutrient enrichment. Thus, overfishing of herbivores in particular makes corals even more vulnerable. As reefs worldwide have frequently been reported to be experiencing overfishing (McManus et al. 2000), these ecosystems are at particular risk from groundwater and non–point source nutrient inputs, as well as from sewage treatment plant discharges. Nutrient enrichment, therefore, must be considered in attempts to understand and manage the degradation of coral reefs.

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