



## Responses of massive and branching coral species to the combined effects of water temperature and nitrate enrichment

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### Abstract

The branching coral species *Pocillopora damicornis* (Linnaeus) and the massive coral species *Porites lobata* Dana were exposed for 30 days to different temperatures and nitrate concentrations to study the response of the coral–zooxanthella symbiosis. Results suggest that the effect of nitrate enrichment on the polyp–zooxanthella symbiosis varies according to the coral morphology. After the experimental period only 30% of *P. damicornis* colonies remained healthy, in contrast to 90% of *P. lobata*. The branching *P. damicornis* was significantly affected by the addition of nitrate, whereas *P. lobata* was significantly influenced by water temperature. The two species showed enhanced zooxanthella volume, and chlorophyll contents per cell under high nitrate concentrations. The reduced zooxanthellae density in both species indicated a detrimental influence of the interaction of high nitrate and high temperature. Tissue soluble proteins in *P. lobata* were significantly reduced by elevated temperature. Results showed that tissue soluble proteins and chlorophylls in *P. lobata* were from two- to three-fold higher than in *P. damicornis*. The number of zooxanthellae in *P. lobata* was double that of *P. damicornis*. Therefore, we suggest that the

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slow-growing species *P. lobata* is better able to cope with changing environmental conditions than the fast-growing coral *P. damicornis*.

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## 1. Introduction

Coral species in the eastern Pacific Ocean are exposed to a wide range of temperature–nutrient scenarios such as those derived from high river discharge, wind-driven upwellings, and episodic El Niño–Southern Oscillation related sea-warming. These conditions likely exert an important role in the shaping of diversity and distribution of coral reefs in this region.

Although elevated water temperatures are rarely concurrent with high nutrient concentrations in nature, this condition may occur as the result of anthropogenic nutrient enrichment, either by the extensive use of agricultural fertilizers or because of discharge of raw sewage (Pastorok and Bilyard, 1985; Cuet et al., 1988; Matson, 1993). Runoff from tropical rain storms can be another potential source of nitrogen (D'Elia et al., 1981). This variability in the supply of nitrogen is critical to corals since zooxanthellae *in hospite* are nutrient limited (Cook and D'Elia, 1987; Muscatine et al., 1989; Falkowski et al., 1993).

Most experimental studies have centered on the effects of ammonium on corals (Kinsey and Davies, 1979; Stambler et al., 1991; Stimson and Kinzie, 1991; Hoegh-Guldberg, 1994; Jokiel et al., 1994; Muller-Parker et al., 1994; Snidvongs and Kinzie, 1994; Hoegh-Guldberg et al., 1997; McGuire and Szmant, 1997). However, very little is known about the effect of nitrate on the coral–zooxanthella symbiosis, even though nitrate is often the major form of dissolved inorganic nitrogen in eutrophic coastal waters (Marubini and Davies, 1996).

In contrast to the scattered information concerning the role of dissolved inorganic nitrogen on corals, there is little doubt about the negative effects of elevated water temperature on zooxanthellate corals (see reviews by Glynn, 1996; Brown, 1997). Sea-surface temperature anomalies of just 2 °C above ambient are enough to induce mass bleaching and extensive mortality of Panamanian coral species (Glynn and D'Croz, 1990; Glynn and Maté, 1997). Faster growing corals such as pocilloporids are more susceptible to high temperature related bleaching than slow-growing coral species, such as poritids (Brown and Suharsono, 1990; Maté, 1997; D'Croz et al., 2001; Hueerkamp et al., 2001). However, corals are not limited only by high temperatures, since sporadic exposure of corals to low temperature may also lead to bleaching and mass mortality (Coles and Fadlallah, 1991; Gates et al., 1992).

Although much work has been done on high temperature stress and coral bleaching, only a few authors have described the influence of nutrients and high temperature together as a potential stressor for the coral–zooxanthella symbiosis (e.g. Maté, 1997; Nordemar et al., 2003). In our research, two morphologically different coral species were exposed to extreme conditions simulating upwelling (high nutrient levels and low temperature), ENSO (low nutrients and high water temperature), and tropical coastal nutrification (high

nutrients and high temperature) due to human activities. The hypotheses tested in this experiment are: (1) massive and branching coral species respond differently to nitrate enrichment and (2) coral tolerance to nutrient enrichment varies according to the water temperature.

## 2. Materials and methods

### 2.1. Sampling of corals

The branching coral *Pocillopora damicornis* (Linnaeus) and the massive coral *Porites lobata* Dana are two of the most common coral species and reef builders from the Gulf of Panama. Corals were collected in July 2001 using SCUBA. Colonies of *P. damicornis* of about 5–10 cm in diameter were collected from the bottom and pieces of *P. lobata* of about 10 cm in diameter were obtained with hammer and chisel from different colonies. *P. damicornis* was sampled from the coral reef at Uraba Island, whereas *P. lobata* was collected on the Saboga Island reef (Fig. 1). Water depth was

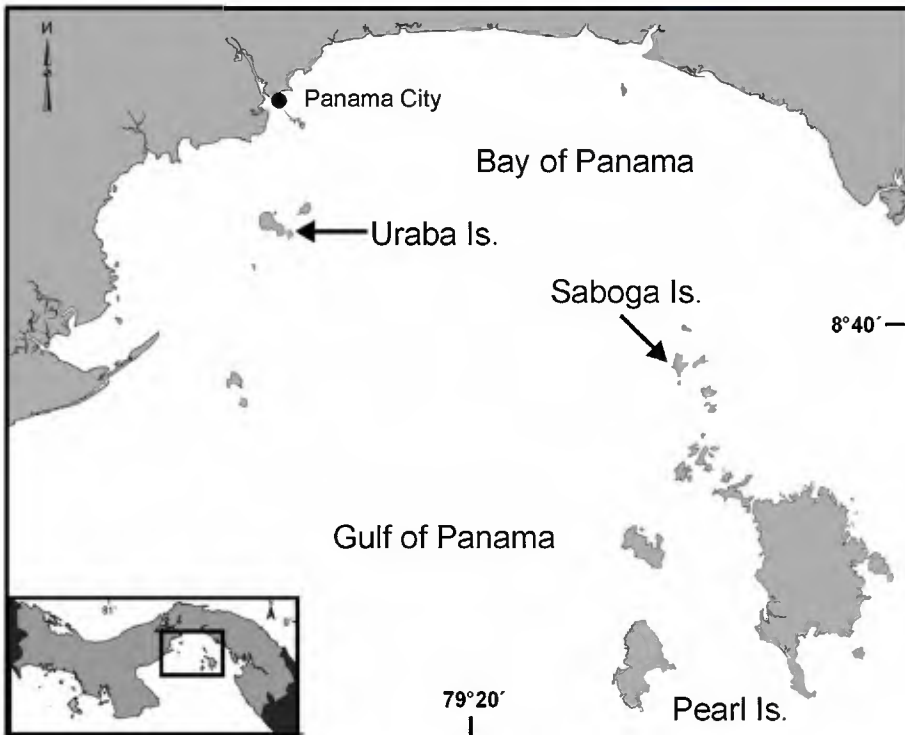


Fig. 1. Map of the sampling sites for corals in the Gulf of Panama. *P. damicornis* was collected from Uraba Island reefs, *P. lobata* from Saboga Island (Pearl Islands).

3–4 m below mean low water in both reefs. Immediately after the collection, corals were transported in insulated coolers to the Smithsonian Tropical Research Institute (STRI), Naos Marine Laboratory, Panama City. Upon arrival at the laboratory, corals were placed in large water tables in the STRI marine aquarium pavilion, shaded from direct sunlight with translucent roofing panels and supplied with a continuous flow of filtered seawater (Strainrite polyester felt bags, pore size 10  $\mu\text{m}$ ). Three days after collection, pieces of *P. lobata* were each cut into four smaller pieces of approximately 3 cm in diameter and allowed to recover from wound-healing stress. Before starting the experiments, corals were acclimated to these conditions for 14 days, until *P. lobata* tissue recovered at the cut edges. This experiment was conducted during the Panamanian rainy season, when nitrate concentrations are usually less than 0.5  $\mu\text{M}$ , phosphate concentrations below 0.2  $\mu\text{M}$ , and the water temperature is typically 28 °C (D'Croz and Robertson, 1997; Maté, 1997).

## 2.2. Experimental design

Healthy corals, showing a normal coloration and expanded polyps, were selected for the experiment. Thirty coral pieces of each species were placed in growth position in single plastic beakers (1000 mL) on top of PVC rings (60 beakers in total). Five replicates of each species were subjected to the following treatments: (a) low temperature, ambient nitrate levels (LT/AN); (b) low temperature, high nitrate levels (LT/HN); (c) ambient temperature, ambient nitrate levels (AT/AN); (d) ambient temperature, high nitrate levels (AT/HN); (e) high temperature, ambient nitrate levels (HT/AN); (f) high temperature, high nitrate levels (HT/HN). The experimental set-up included a cascade system of seawater, pumped from the Bay of Panama at a rate of three liters per minute, forced through Strainrite polyester felt filter bags (10  $\mu\text{m}$ ) and first collected in three different 130 L reservoirs where water temperature was adjusted. For the simulation of upwelling temperatures, water was cooled down below 25 °C by flowing through custom made chillers. For ENSO sea-warming, water was heated up to 30 °C by means of three aquarium heaters (300 W each) attached to the reservoir. A third reservoir received ambient temperature water, which changed with day and night fluctuations between 25 and 30 °C during the time of the experiment. Each of these reservoirs served two smaller 20 L glass aquaria, one for ambient nitrate and the other one receiving a nitrate input through a peristaltic pump. The nitrate stock solution (3 mM) was kept in an additional constantly stone-aerated 80 L aquarium. Potassium nitrate ( $\text{KNO}_3$ ) diluted in seawater was used as nitrogen-source. Nitrate concentration in the mixing aquaria was estimated to be 20  $\mu\text{M}$  at an approximate water flow of 750 mL/min and at a nutrient input of 7.5 mL/min. Water was distributed from the small aquaria to the beakers holding the corals by means of individual Tygon tubing (5-mm internal diameter) wrapped in strips of black plastic to control algal growth. An Eppendorf pipette tip was inserted at the outflow end of each hose to guarantee a nearly equal water flow (90 mL/min). The position of the 60 plastic beakers either containing *P. damicornis* or *P. lobata* was assigned randomly in a large water table.

### 2.3. Monitoring

Water flow out of these reservoir tanks was measured three times per day and the average flow was calculated. The water temperature was checked randomly in three beakers of each treatment five times per day, and in all of the beakers at noon time using a calibrated mercury thermometer with a precision of  $\pm 0.1$  °C. In addition, temperature data loggers (HOBO, Onset Computer, USA) were placed in the three serving reservoirs. Water samples were taken twice a day in all of the treatments in order to determine nitrate and phosphate concentrations. To have a constant check on the concentration of phosphate in the seawater, water samples were analyzed by the ascorbic acid, potassium antimony-tartrate method (Murphy and Riley, 1962). Nitrate analysis followed the cadmium reduction column method (Morris and Riley, 1963).

After 30 days of exposure to experimental conditions, the physical appearance of the corals was checked visually and mortality was recorded. In addition, coral pieces were sampled, wrapped in aluminum foil, and frozen ( $-18$  °C). The following parameters, concerning the symbiosis, were selected to characterize the response of the two coral species to experimentally simulated environmental conditions: (a) zooxanthella density, (b) zooxanthella size, (c) chlorophyll concentrations, (d) total soluble proteins in the polyp tissue, and (e) C/N ratio in the zooxanthellae.

### 2.4. Sample analysis

The polyp tissue was removed from the coral skeletons with a jet of distilled water using an airbrush (50 psi) and the resulting suspension was homogenized by shaking. Zooxanthellae were counted in a Neubauer chamber. The surface areas of the zooxanthellae were measured using SigmaScan (SPSS Science, USA) and their volume calculated assuming the cells to be spherical. The suspension containing the zooxanthellae was split in two aliquots. Aliquots were centrifuged separately (2500 rpm, 10 min). The settled zooxanthella pellet of the first aliquot was lyophilized in an evaporator (DNA Speed Vac 110, Savant Instruments) for CHN analysis using an Elemental Analyzer (CNS Analyzer, Vario El, Elementar Analysensysteme, Hanau, Germany). Chlorophyll *a* and *c2* were extracted from the settled pellet of the second aliquot using 90% acetone in the dark for 24 h. The supernatant of the latter was used for the analysis of total soluble proteins. Chlorophyll was measured with a spectrophotometer (Milton Roy, Spectronic 1201) and concentrations calculated according to equations of Jeffrey and Humphrey (1975). The living surface area of *P. damicornis* was estimated using the paraffin method (Glynn and D'Croz, 1990), while the aluminum foil method (Marsh, 1970) was applied for *P. lobata*. The concentration of total soluble proteins in the polyp tissue was analyzed according to the method of Bradford (1976) with a BIO-RAD Protein Assay Kit using BSA as the protein standard.

### 2.5. Statistical tests

Statistical analysis was performed with SigmaStat (SPSS Science). Analysis of variance was applied to analyze parametric data in regard to temperature or nutrient treatments (one-way ANOVA), or their combined effect (two-way ANOVA). Tukey's-Test was

conducted post-hoc (given  $p$ -values in Table 1) to test the differences between the individual treatments. Differences between the species were also tested by one-way ANOVA. Nonparametric data were analyzed by a Kruskal–Wallis ANOVA.

Table 1

Results from two-way ANOVA (temperature\*nitrate) for coral and algal symbiont parameters

	<i>P. damicornis</i>			<i>P. lobata</i>		
	<i>df</i>	<i>F</i>	<i>p</i> -value	<i>df</i>	<i>F</i>	<i>p</i> -value
<i>Zooxanthella density</i> ( $10^6 \text{ cm}^{-2}$ )						
Temperature	2	0.672	0.520	2	4.278	0.024*
Nitrate	1	7.518	0.011*	1	0.041	0.841
Temperature*Nitrate	5	1.853	0.144	5	1.423	0.252
<i>Mitotic rate</i>						
Temperature	2	0.416	0.664	2	0.684	0.513
Nitrate	1	1.309	0.263	1	0.001	0.979
Temperature*Nitrate	5	0.512	0.764	5	0.674	0.647
<i>Zooxanthella volume</i> ( $\mu\text{m}^{-3}$ )						
Temperature	2	0.028	0.972	2	6.187	0.006**
Nitrate	1	0.619	0.438	1	7.441	0.011*
Temperature*Nitrate	5	0.161	0.974	5	5.484	0.002**
<i>Chlorophyll a per living coral area</i> ( $\mu\text{g cm}^{-2}$ )						
Temperature	2	2.230	0.128	2	2.856	0.075
Nitrate	1	4.9E-05	0.994	1	3.257	0.082
Temperature*Nitrate	5	0.807	0.557	5	1.181	0.348
<i>Chlorophyll c<sub>2</sub> per living coral area</i> ( $\mu\text{g cm}^{-2}$ )						
Temperature	2	2.229	0.129	2	3.850	0.034*
Nitrate	1	0.003	0.960	1	0.018	0.895
Temperature*Nitrate	5	0.902	0.497	5	1.602	0.198
<i>Chlorophyll a per zooxanthella</i> ( $\text{pg cell}^{-1}$ )						
Temperature	2	4.163	0.028*	2	2.508	0.100
Nitrate	1	6.409	0.018*	1	0.536	0.470
Temperature*Nitrate	5	6.480	<0.001***	5	1.481	0.233
<i>Chlorophyll c<sub>2</sub> per zooxanthella</i> ( $\text{pg cell}^{-1}$ )						
Temperature	2	6.113	0.007*	2	3.596	0.041*
Nitrate	1	8.395	0.008*	1	0.564	0.459
Temperature*Nitrate	5	19.839	<0.001***	5	2.137	0.096
<i>Total soluble proteins per living coral area</i> ( $\text{mg cm}^{-2}$ )						
Temperature	2	0.370	0.695	2	3.353	0.050
Nitrate	1	0.045	0.834	1	1.238	0.275
Temperature*Nitrate	5	0.884	0.508	5	2.180	0.090

Degrees of freedom (*df*), *F*-values and *P*-values (Tukey's post-hoc test) are presented,  $n=30$ .

\*  $p<0.05$ .

\*\*  $p<0.01$ .

\*\*\*  $p<0.001$ .



### 3. Results

#### 3.1. Experimental conditions

Mean water temperature during the experiment was 23.8 °C in the cold treatment, 29.1 °C for ambient condition, and 29.9 °C for the elevated temperature treatment. Mean temperature between treatments was significantly different (Kruskal–Wallis ANOVA,  $p < 0.001$ ;  $n = 276$ ). While the elevated water temperature treatment remained stable around 30 °C over the whole experimental period, ambient and cold temperature treatments showed night/day variations. Ambient temperatures ranged from 25 to 30 °C, and cold temperatures from 19 to 25 °C. The mean ambient temperature was close to the upper thermal tolerance limit for corals in the eastern Pacific Ocean (Glynn and D'Croz, 1990). Ambient nitrate concentrations in the aquarium averaged 2 µM, which is up to four-fold higher than in the field, where concentrations range between 0.5 and 1.0 µM (D'Croz et al., 1991; Maté, 1997). High nitrate concentrations were calculated to be 20–30 µM, however, actual mean concentrations during the experiment were 16.6 µM in the cold treatment, 20.81 µM in the ambient and 26.5 µM in the hot water treatment. Difference between high and low nitrate concentrations was highly significant (Kruskal–Wallis ANOVA  $p < 0.001$ ;  $n = 300$ ). Phosphate concentrations in the aquarium were determined simultaneously and averaged 0.70 µM. This value is also higher than encountered in the Bay of Panama (D'Croz et al., 1991; D'Croz and Robertson, 1997). There was no significant difference in the concentration of phosphate between the treatments (one-way ANOVA,  $p < 0.442$ ;  $n = 300$ ). Mean water flow from the reservoirs for the cold and ambient water treatments was 661.47 and 681.41 mL/min for the elevated temperature treatment. These water flow rates were not found to be significantly different (Kruskal–Wallis ANOVA,  $p < 0.0531$ ;  $n = 276$ ).

#### 3.2. Visual condition of the corals

All colonies were classified as normal-looking at the beginning of the experiment, with the typical brownish color and active, expanded polyps of healthy corals. In general, *P. lobata* hardly changed its physical appearance during this experiment. After 30 days of exposure to elevated nitrate concentrations 90% of *P. lobata* colonies remained unchanged, while only 30% of *P. damicornis* appeared normal. *P. damicornis* showed a pale color mainly at ambient and high temperatures. At the time of the experiment, ambient water temperatures were particularly high, which explains the sensitivity of the corals to this condition. In addition, two of the *P. damicornis* colonies treated with nitrate enrichment and elevated water temperature died in the course of the experiment.

#### 3.3. *Zooxanthella* density

The detrimental effects of the combination of high temperature and nitrate enrichment were reflected in the reduction of the zooxanthella population in *P. damicornis* and *P. lobata* (Fig. 2). This decline was significant in *P. damicornis* when subjected to the nitrate enriched condition (Table 1), but not to the water temperature treatments. On the contrary,

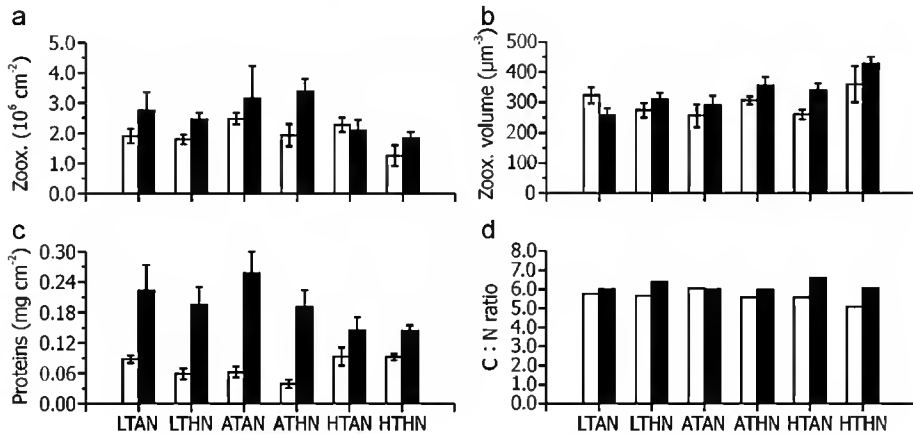


Fig. 2. Mean values ( $\pm$ S.E.) of (a) zooxanthellae density, (b) zooxanthella volume, (c) soluble protein concentrations and (d) C/N ratio for the branching coral species *P. damicornis* (open bars) and the massive coral species *P. lobata* (solid bars) exposed to different water temperatures and nitrate concentrations. LT/AN=low temperature, ambient nitrate (23.8 °C/2  $\mu$ M); LT/HN=low temperature, high nitrate (23.8 °C/16.6  $\mu$ M); AT/AN=ambient temperature, ambient nitrate (29.1 °C/2  $\mu$ M); AT/HN=ambient temperature, high nitrate (29.1 °C/20.81  $\mu$ M); HT/AN=high temperature, ambient nitrate (29.9 °C/2  $\mu$ M); HT/HN=high temperature, high nitrate (29.9 °C/26.5  $\mu$ M).

the density of zooxanthellae in *P. lobata* decreased significantly when exposed to high and low temperature, but no significant effect was derived from the nitrate-enriched condition (Table 1). Zooxanthella density was higher in *P. lobata* than in *P. damicornis* in all treatments (Table 2).

### 3.4. Zooxanthella volume

The largest zooxanthellae occurred when both coral species were exposed to the combination of high temperature and nitrate enrichment (Fig. 2). The zooxanthella

Table 2

Results of a one-way ANOVA comparing the symbiosis attributes between the coral species *P. damicornis* and *P. lobata*

Parameter	df	F	p-value
Zooxanthella density (10 <sup>6</sup> cm <sup>-2</sup> )	1	6.234	0.016*
Mitotic index	1	3.990	0.052
Zooxanthella volume (μm <sup>-3</sup> )	1	1.988	0.165
Chlorophyll <i>a</i> per living coral area (μg cm <sup>-2</sup> )	1	164.840	<0.001***
Chlorophyll <i>c</i> <sub>2</sub> per living coral area (μg cm <sup>-2</sup> )	1	208.447	<0.001***
Chlorophyll <i>a</i> per zooxanthella (pg cell <sup>-1</sup> )	1	234.631	<0.001***
Chlorophyll <i>c</i> <sub>2</sub> per zooxanthella (pg cell <sup>-1</sup> )	1	422.946	<0.001***
Total soluble proteins per living coral area (mg cm <sup>-2</sup> )	1	35.579	<0.001***

p-values, degrees of freedom (df), and F-values are presented, n=30.

\*  $p < 0.05$ .

\*\*\*  $p < 0.001$ .



volume in *P. lobata* was significantly affected by water temperature, nitrate enrichment, and by its interaction, whereas this was not the case for *P. damicornis* (Table 1). The size of zooxanthellae did not vary significantly between the two coral species (Table 2).

### 3.5. Chlorophyll concentrations

The concentration of chlorophylls per living surface area in *P. lobata* was three-fold higher than in *P. damicornis*, and was significantly different between the species (Table 2). The concentration of chlorophylls in *P. damicornis* remained fairly similar regardless of the treatments (Fig. 3) and there was no significant effect of either temperature or nitrate enrichment (Table 1). Chlorophyll *a* in *P. lobata* was not affected by temperature or nitrate level, but chlorophyll *c*<sub>2</sub> decreased significantly when exposed to high or low temperature, regardless of the nutrient concentration (Table 1).

### 3.6. Chlorophyll *a* and *c*<sub>2</sub> in the zooxanthellae

The cellular concentration of chlorophylls in both coral species remained stable at low nitrate concentrations, regardless of water temperature. But when corals were exposed to the combination of elevated water temperature and nitrate enrichment, cellular chlorophyll increased (Fig. 3). Statistical analysis supports the effects of water temperature, nitrate enrichment, and its interaction on zooxanthella chlorophyll *a* and *c*<sub>2</sub> in *P. damicornis* (see Table 1). However, in *P. lobata* zooxanthella, chlorophyll *c*<sub>2</sub> was only significantly affected by water temperature. Although trends were fairly similar for both coral species,

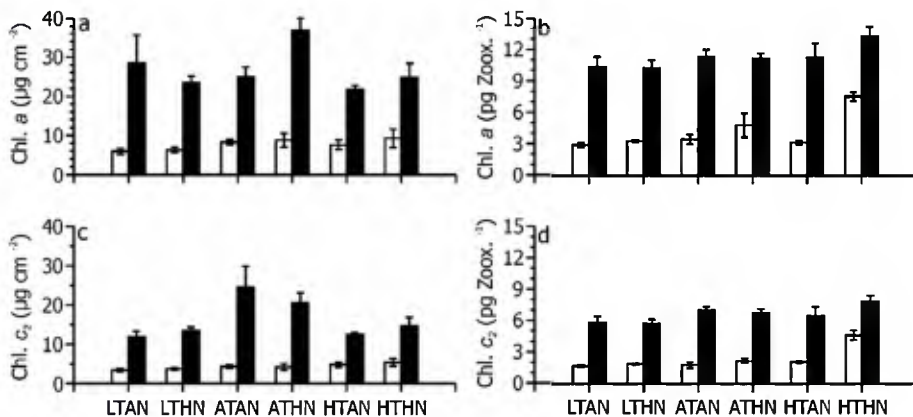


Fig. 3. Mean values ( $\pm$ S.E.) of (a,c) chlorophyll *a* and *c*<sub>2</sub> concentrations per living coral area and (b,d) per zooxanthellae for the branching coral species *P. damicornis* (open bars) and the massive coral species *P. lobata* (solid bars) exposed to different water temperatures and nitrate concentrations. LT/AN=low temperature, ambient nitrate (23.8 °C/2 µM); LT/HN=low temperature, high nitrate (23.8 °C/16.6 µM); AT/AN=ambient temperature, ambient nitrate (29.1 °C/2 µM); AT/HN=ambient temperature, high nitrate (29.1 °C/20.81 µM); HT/AN=high temperature, ambient nitrate (29.9 °C/2 µM); HT/HN=high temperature, high nitrate (29.9 °C/26.5 µM).

concentrations of chlorophylls per zooxanthella were significantly higher in *P. lobata* (two to three times) than in *P. damicornis* (Table 2).

### 3.7. Soluble protein concentrations in the coral host

The concentration of total soluble proteins in the tissue is an indicator of the health condition of the coral host. Decline of total soluble proteins in polyp tissues of *P. lobata* was correlated with the elevated water temperature treatment (Fig. 2). The exposure of this coral species to high nitrate levels did not have significant effects on tissue proteins (Table 1). Protein values in *P. damicornis* were slightly higher under ambient nitrate conditions (Fig. 2), but in general, values did not change much under the different treatments. Temperature and nitrate addition had no significant effect on *P. damicornis* tissue proteins (Table 1). A highly significant difference was determined between the protein contents of the two species (Table 2), as concentrations were two to three times higher in *P. lobata*.

### 3.8. C/N ratio

The C/N ratio of the zooxanthellae from both coral species ranged from 5 to 6.5 and no major differences were observed between the treatments or the species (Fig. 2). Due to the small amount of material from each sample, replicates from each treatment were combined for the C/N analysis. Therefore, it was not possible to apply statistical tests.

## 4. Discussion

Results from this experiment suggest that the effect of nitrate enrichment on the polyp–zooxanthella symbiosis varies according to the coral morphology. *P. damicornis* was significantly affected by the addition of nitrate, whereas *P. lobata* was significantly influenced by water temperature. As the coral–zooxanthella symbiosis is an adaptation to oligotrophic environments, nutrient enrichment may represent a disturbing agent for the stability of the mutualistic partnership. Reports suggest nitrogen limitation of the zooxanthellae in corals thriving in nutrient poor environments (Muscatine and Porter, 1977; Cook and D'Elia, 1987; Kinsey, 1991; Rees, 1991). However, this generalization may not be necessarily applicable to all eastern Pacific coral species. Given the potential for high nutrient concentrations within this region due to upwelling and terrestrial inputs corals might not be nutrient limited. In addition, our results from the analysis of the C/N ratio in zooxanthellae pellets from *P. damicornis* and *P. lobata* from the upwelling Gulf of Panama did not exhibit major variations among the treatments, suggesting nitrogen sufficiency.

Although coral growth in the eastern Pacific may not be limited by low nutrient supply, an extreme exposure may be detrimental. In our experiment, the extended exposure to nitrate enrichment caused paling and bleaching in *P. damicornis* due to the significant loss of zooxanthellae. When corals were exposed to the combination of high temperature and nitrate addition, the remaining zooxanthellae increased in size and supported a higher concentration of chlorophyll, suggesting a compensatory mechanism. The lowered

competition for nutrients due to the loss of zooxanthellae in stressed corals, as well as the reduced self-shading, possibly enables the high chlorophyll content (Jones and Yellowlees, 1997).

We also observed polyp retraction when the corals were exposed to heat stress. In the field, this likely limits coral planktivory and the flow of nutrients to the zooxanthellae. Zooxanthella nutrient limitation was also observed in starved specimens of the sea-anemone *Aiptasia pallida* (Verrill) (Cook et al., 1988). We propose that during periods of elevated water temperatures and limited planktivory, nitrate enrichment might sustain the remaining zooxanthellae for a short period of time. We believe this is the reason why *P. damicornis* exhibited a more intensive brownish color when exposed to the combined effect of high temperature and nitrate addition, as opposed to the paler colored corals observed in the high temperature, ambient nitrate treatment. A similar response has been reported in previous coral experiments, as ammonium increased the number of zooxanthellae and cellular chlorophyll when added to *Stylophora pistillata* Esper (Muscatine et al., 1989) and *P. damicornis* (Snidvongs and Kinzie, 1994).

During upwelling in the Gulf of Panama, corals retract their polyps due to the exposure to pulses of cold water (15–18 °C). Although this may limit the nitrogen supply to the zooxanthellae, this condition is possibly offset by the elevated water column nutrients which typically occur during upwelling. For example, the mean nitrate concentration during upwelling in the Gulf of Panama is rather high, slightly above 1  $\mu\text{M}$ , yet maximum concentrations might surpass 2  $\mu\text{M}$  (D'Croz et al., 1991; D'Croz and Robertson, 1997). Upwelling as a source of nutrients for potentially nutrient limited corals has also been suggested for the Indo-Pacific Ocean (Marsh, 1977; Andrews and Gentian, 1982). It is important to keep in mind that extreme upwelling events can also lead to coral bleaching (Glynn and Stewart, 1973). In addition, the coral host can be stressed further if nutrient concentrations stay high over a longer time scale, as this condition conducted to the significant loss of zooxanthellae and reduction of total soluble proteins in the polyp tissue during our study (see Table 1).

Our results indicated that *P. damicornis* and *P. lobata* react in different ways to the treatments. Low amounts of proteins were found in *P. damicornis* exposed to the different treatments, which is not surprising as this coral species becomes rapidly stressed by drastic variations of temperature and dissolved nutrients (Maté, 1997). Previous experiments have reported an increase of tissue proteins at elevated ammonium levels in *P. damicornis* (Muller-Parker et al., 1994), but unchanged host cell protein content has also been indicated (Hoegh-Guldberg and Smith, 1989; Stambler et al., 1991; Marubini and Davies, 1996). *P. lobata* was more affected by temperature than by nitrate input. The zooxanthella density and the concentration of chlorophyll  $c_2$  per living coral area declined significantly under heat stress. Although these are clear signs of coral bleaching (Glynn, 1996; Brown, 1997), *P. lobata* showed almost no signs of stress and maintained active polyps and a brownish color over the course of this experiment. The synergistic effect of elevated temperature and high nitrate caused an increase in cellular volume of the zooxanthellae and in their intracellular chlorophyll  $c_2$ . Our data confirm that *P. lobata* is more resistant to heat stress than *P. damicornis*, as documented in a variety of laboratory and field studies (Glynn et al., 2001; Hueerkamp et al., 2001).

Different responses of coral species to heat stress and nitrification might be closely related to the growth strategies and metabolic rates in branching and massive coral species (Szmant, 1986; Gates and Edmunds, 1999). Branching corals are fast-growing, whereas massive coral species are slow-growing, but long-living. Thin tissue layers of branching corals provide less energy-rich compounds and photosynthetic products, resulting in lower adaptability to drastic environmental changes (Jiménez et al., 2001; Mc Clanahan et al., 2001). Protein metabolism, for example, is a key factor for the stabilization of the physiological condition in corals and ample variation might result from environmental disturbances. This was observed in the Caribbean corals *Acropora cervicornis* and *Montastraea franksi*, which showed up to 43% variation in protein concentration within a day period, when exposed to elevated water temperature (Kendall et al., 1983). Corals with high resistance to bleaching have high concentrations of symbionts, chlorophyll, and soluble proteins (Gates and Edmunds, 1999). This is the case for *P. lobata* as tissue proteins and chlorophylls were from two- to three-fold higher and the number of zooxanthellae was double that of *P. damicornis* (Figs. 2 and 3).

The results of our study suggest that coral symbiosis can tolerate limited nitrification over short periods of time (see also Koop et al., 2001), as during upwelling episodes in the Gulf of Panama, but not long standing nitrification when corals are also exposed to warm temperature. The documented detrimental effect from nitrate enrichment in *P. damicornis* might be of great relevance for coral conservation, as this is the major reef-building coral species in shallow reef areas in the eastern Pacific Ocean. Coral reef development might be severely impacted if corals are exposed to the runoff of nutrients from agricultural fertilizers or from raw sewage disposal.

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