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## Experimental responses to elevated water temperature in genotypes of the reef coral *Pocillopora damicornis* from upwelling and non-upwelling environments in Panama

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**Abstract** The authors investigated the response to experimentally elevated water temperature in genotypes of *Pocillopora damicornis* from three coral reefs in the upwelling Gulf of Panama and four coral reefs in the non-upwelling Gulf of Chiriquí, Panamanian Pacific. Sea-surface temperature in the Gulf of Panama declines below 20 °C during seasonal upwelling, while in the thermally stable Gulf of Chiriquí, the temperature ranges from 27 to 29 °C. Genotypes of *P. damicornis* from the seven locations were determined by allozyme electrophoresis. The most abundant genotype at each location was selected for a thermal tolerance experiment where corals were exposed to water temperature of 30 °C (1 °C above ambient) for 43 days. Four site coral genotypes can be uniquely differentiated by the GPI locus, two by the LGG-2 locus, and two by a combination of the MDH-1, LGG-2, and LTY-3 loci. A visual assessment of the coral condition after exposure to an elevated temperature showed that corals from localities in the non-upwelling environment retained a normal to slightly pale appearance, while corals from the upwelling environment bleached and their polyps were mostly retracted. A two-way ANOVA confirmed that corals were significantly affected by water temperature and locality. The zooxanthellae were also significantly affected by the interaction of elevated temperature and locality of the

corals. Mean zooxanthellae density decreased by 25 and 55%, respectively, in experimentally heated corals from the non-upwelling and upwelling environments. Low concentrations of photosynthetic pigments per live area of the corals were the norm in corals under elevated temperature. The mean concentration of chlorophyll *a* per live area of the corals was reduced by 17 and 49%, respectively, in heated corals from the non-upwelling and upwelling sites. Coral genotypes from the upwelling Gulf of Panama demonstrated higher vulnerability to thermal stress than coral genotypes from the non-upwelling Gulf of Chiriquí. However, the latter showed greater differences in their responses. Thus, even at small geographic scales, corals can display different levels of tolerance to thermal stress. The difference in thermal tolerance between corals from upwelling and non-upwelling environments is concomitant with greater genetic differences in experimental corals from the thermally stable Gulf of Chiriquí compared with corals from the upwelling Gulf of Panama.

**Keywords** Thermal tolerance · Coral genotypes · Upwelling · ENSO sea warming

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### Introduction

Patterns of bleaching on eastern Pacific reefs may be related to differences in coral species composition over spatial scales, duration of exposure to sea warming, response of the coral-zooxanthellae symbiosis, and species tolerances to elevated temperatures (Glynn et al. 2001). The interspecific variability of bleaching in eastern Pacific corals is influenced by the generally higher susceptibility to temperature-stress of branching corals, such as pocilloporid species with higher skeletal growth and metabolic rates, than in slow-growing massive forms of poritid and agariciid species (Maté 1997; Glynn et al. 2001; Hueerkamp et al. 2001). However, the biological mechanisms responsible for the

differential responses of bleaching in conspecific corals occupying the same habitat are still unknown. Likely mechanisms involved in the intraspecific response in temperature-stressed corals are phenotypic and genotypic adaptations of the symbiotic partners (Baker and Rowan 1997; Baker 2001). The history of thermal exposure (e.g., upwelling vs. non-upwelling areas; ENSO sea warming exposure) may also lead to differential responses to sea warming in coral species. Corals repeatedly exposed to warmer water temperatures may be subject to acclimation and selection for resistant genotypes, which survive better than genotypes of corals in cooler environments (Coles and Jokiel 1978; Jokiel and Coles 1990; Coles 1997; Marshall and Baird 2000; Brown et al. 2002).

Reef-building scleractinian species may contain members of more than one clade of endosymbiotic dinoflagellates [e.g., in the Caribbean coral complex *Montastraea annularis* (Rowan and Knowlton 1995) and in eastern Pacific *Pocillopora* spp. (Baker 1999; Baker and Rowan 1997; Glynn et al. 2001)]. The intraspecific patterns of photic zonation among colonies at different depths (Rowan and Knowlton 1995; Baker 2001), and between sunlit and shaded environments (Rowan et al. 1997) are closely tied to the dinoflagellate genotypes hosted by given colonies. Therefore, the respective types of zooxanthellae harbored by the coral may also influence bleaching patterns (Rowan and Power 1991; Fitt and Warner 1995; Rowan et al. 1997; Baker 1999). Furthermore, the loss of zooxanthellae may be followed by the acquisition of a new symbiotic consortium with zooxanthellae that are more tolerant to elevated temperatures (Buddemeier and Fautin 1993; Kinzie et al. 2001). *Pocillopora* species at Uva Island in the non-upwelling Gulf of Chiriquí, Panama, contain four *Symbiodinium* clades (C1, C2, C5, and D) of the six distinct algal symbiont taxa [five *Symbiodinium* clade C and one Clade D (sensu Baker 1999)] reported from scleractinian corals in the eastern Pacific (Glynn et al. 2001). This mixed community of algal symbionts in *Pocillopora* is unusual and may influence the coral tolerance to environmental stressors.

The existing knowledge of genotypic tolerance of zooxanthellae to environmental stressors contrasts with the meager equivalent genotypic data existing for the coral host. Information about host genotype may add to the understanding of conditions that cause the disruption of the coral-zooxanthellae symbiosis during bleaching (e.g., indirect evidence has suggested that genetically related corals exhibit similar tolerance to elevated temperatures; see Jokiel and Coles 1990; Hoeksema 1991; Edmunds 1994). Other potential mechanisms of protection to environmental disturbances, such as the production of heat stress proteins by both the zooxanthellae and the cnidarian host, have only been preliminarily studied (Sharp et al. 1994; Black et al. 1995).

An increase of 1–4 °C in water temperature during exceptionally strong El Niño-Southern Oscillation

(ENSO) events has caused mass bleaching and mortality of eastern Pacific zooxanthellate corals (Podestá and Glynn 1997). Solar irradiance is another important factor in coral bleaching and may interact synergistically with elevated water temperature (Gleason and Wellington 1993; Brown et al. 2000). These two variables, acting independently or together, are thought to be the most important stressors responsible for extensive coral bleaching and mortality (see Glynn 1996 and Brown 1997 for review). During the 1997–1998 ENSO, Panamanian corals at offshore sites displayed higher mortality and bleaching than corals at nearshore locations, possibly because of the combination of elevated water temperature and water clarity, which allowed greater light penetration in oceanic settings (Glynn et al. 2001). However, experimental studies with the branching coral *P. damicornis* in Okinawa (Glynn et al. 1992) and the massive coral *Porites lobata* in Panama (D'Croz et al. 2001) concluded that although elevated water temperature invariably triggered the loss of zooxanthellae and photosynthetic pigments, no direct links were substantiated between irradiance and bleaching. On the other hand, experimental models have confirmed that solar irradiance acting together with water temperature hampered the recovery of temperature-stressed *P. damicornis* (D'Croz and Maté 2002).

In the present study the tolerance of *P. damicornis* to elevated water temperature was investigated for genotypes collected at seven reefs from upwelling and non-upwelling environments on Panama's Pacific coast. There are indications suggesting that eastern Pacific corals from upwelling environments are less tolerant to sea-warming than corals from warmer localities (see Glynn et al. 1988). Wind-induced upwelling in the Gulf of Panama during the dry season (January to March) exposes corals to cool and nutrient-rich waters (D'Croz et al. 1991; D'Croz and Robertson 1997). Sea-surface temperature decreases below 20 °C during upwelling and ranges over 10 °C annually. The warmer non-upwelling environment in the Gulf of Chiriquí experiences mean SSTs that fluctuate between 27–29 °C annually (Glynn and Maté 1997). Coral biomass attributes, including zooxanthellae densities, photosynthetic pigment concentrations, tissue total soluble protein, and the coral condition, are herein reported and examined vis-à-vis the response of the coral-zooxanthellae symbiosis to heat stress.

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## Materials and methods

This microcosm experiment was designed to test: (a) whether coral host genotypes of *Pocillopora damicornis* from Pacific Panama have a different tolerance to elevated water temperature and (b) if coral host genotypes of *P. damicornis* from a non-upwelling environment (Gulf of Chiriquí) have higher tolerance to elevated water temperature than coral host genotypes from an upwelling environment (Gulf of Panama).

## Coral collection and acclimation

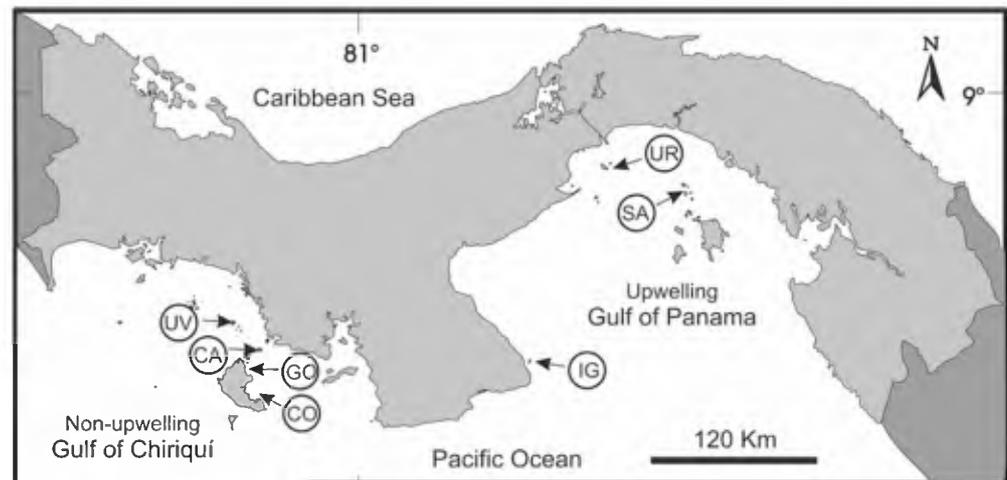
Colonies of *P. damicornis* (8–10 cm in diameter) were collected from three coral reefs in the upwelling Gulf of Panama (Urabá Island, Saboga Island, and Iguana Island), and from four reefs in the non-upwelling Gulf of Chiriquí (Uva Island, Granito de Oro Island, Canal de Afuera Island, and Coiba Island) Pacific Panama, from 4–22 August 2001 (Fig. 1). In all cases, collections were made at a depth of 4–6 m below mean low water (MLW). Sea-surface temperature ranged from 27–28 °C during this time of the year (non-upwelling condition). On average, 25 colonies were collected at each location and immediately transported in insulated coolers, with aeration from air pumps, to the Naos Marine Laboratory of the Smithsonian Tropical Research Institute (STRI) in Panama City. Corals were then placed in seven large water tables in the STRI marine aquarium pavilion with continuous flow of filtered seawater (Strainrite polyester felt bags, pore size 10 µm). The concentration of dissolved inorganic nutrients in the holding tanks was measured using the Flow Solution IV automated analyzer (OI Analytical, USA). The mean concentration of nitrate was  $0.75 \pm 0.10 \mu\text{M}$  (mean  $\pm$  SE,  $n = 42$ ) and phosphate  $0.38 \pm 0.02 \mu\text{M}$  (mean  $\pm$  SE,  $n = 42$ ). No food was offered to the experimental corals. Translucent fiberglass roof panels covered the aquarium

pavilion to prevent changes in salinity during rainstorms and to offer protection from direct sunlight. Over the course of this experiment, irradiance measurements were often performed between 10:00 and 14:00 h adjacent to the corals with an IL 1400A radiometer (International Light, Inc.) equipped with an underwater sensor for photosynthetically active radiation (PAR). PAR was  $142 \pm 40 \text{ W m}^{-2}$  (mean  $\pm$  SE,  $n = 321$ ). Corals were allowed to acclimate for 1 month before the commencement of the experiment.

## Determination of coral genotypes

Collected corals were screened by allozyme electrophoresis and the most common genotype of *P. damicornis* from each location was selected for the temperature-tolerance experiment. Tissue samples for electrophoresis analysis were removed from the recently collected corals using flat-tip pliers, placed in cryovials and mixed with five drops of Stoddart grinding buffer (Stoddart 1983) before freezing in liquid nitrogen. Horizontal starch (SIGMA S-4501) gel electrophoresis was used to analyze seven enzymes coding for 12 loci under two buffer systems (Table 1). On the day of the electrophoresis run, a small portion of the tissue sample was placed on a grinding plate cell and macerated in three drops of

**Fig. 1** Map of Panama. Black arrows with encircled acronyms point to the seven collecting sites. Gulf of Panama: UR Urabá Island; SA Saboga Island; and IG Iguana Island. Gulf of Chiriquí: CO Coiba Island; GO Granito de Oro Island; CA Canal de Afuera Island; and UV Uva Island



**Table 1** Enzyme buffer systems employed in the electrophoretic analysis of 12 loci in *Pocillopora damicornis*

Enzyme	E.C. no.	No. loci	Buffer system
Glucose phosphate isomerase (GPI)	5.3.1.9	1	TC 8.0 <sup>a</sup>
Hexokinase (HK)	2.7.1.1	1	LiOH 8–1–8.4 <sup>b</sup>
Leucyl-glycine-glycine-peptidase (LGG)	3.4.11/13	2	LiOH 8–1–8.4
Leucyl-tyrosine peptidase (LTY)	3.4.11/13	3	LiOH 8–1–8.4
Malate dehydrogenase (MDH)	1.1.1.37	2	TC 8.0
Phosphogluconate dehydrogenase (PGDH)	1.1.1.44	1	TC 8.0
Triose phosphate isomerase (TPI)	5.3.1.1	2	LiOH 8–1–8.4

E.C. Enzyme commission numbers

<sup>a</sup>Tris citrate (TC 8.0), pH 8.0, 90 mAmp, 6–8 h (Selander et al. 1971)

<sup>b</sup>Tris citrate borate (LiOH), pH 8.4, 350 V, 4–6 h (Selander et al. 1971, modified by Harris and Hopkinson 1976)

Stoddart's buffer (Stoddart 1983). A piece of Miracloth filter (Calbiochem Inc.) was placed on top of the homogenate to reduce the amount of coral mucus that might adhere to the paper wicks (Whatman #3 filter paper) which were soaked in homogenate and loaded in starch gels and run at 4 °C. After the electrophoretic run, gels were sectioned with a custom-designed guitar-string slicer. Zymograms were visualized using stain recipes modified by Williams (1992) and Weil and Weigt (1996) from Harris and Hopkinson (1976). Alleles were labeled alphabetically according to their mobility. For those enzymes with two loci, the loci were labeled numerically starting with the fastest migrating one.

### Thermal tolerance experiment

Coral colonies of *P. damicornis* screened as the most common genotype from each location were used to test thermal tolerance (Table 2). Only corals showing normal coloration and active and expanded polyps were chosen for the experiment. We used eight coral colonies from each location, four of which were randomly assigned to the high temperature treatment and another four to the ambient temperature control. Each coral colony was placed inside a translucent 1-l plastic beaker and positioned on a PVC ring to minimize contact with sediments settling on the bottom of the beaker. This experiment was designed to mimic as closely as possible ambient conditions that corals encounter during El Niño events in terms of duration (1 to 6 months) and intensity of the warm water pulse (1–4 °C sea-surface temperature anomaly). The experimental setup consisted of two treatments that exposed the corals to ambient and elevated water temperature. An increase of 1 °C was selected since field and experimental studies have shown that this change is sufficient to cause thermal stress in Panamanian *P. damicornis* (Glynn and D'Croz 1990; Glynn et al. 2001; Hueerkamp et al. 2001; D'Croz and Maté 2002). Two 20-l glass aquaria supplied with aerated and filtered flow-through seawater were used as reservoirs. Aquarium heaters were placed in one of the reservoirs to elevate water temperature. Heated and ambient temperature water from these aquaria was dis-

tributed 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 supply a nearly equal water flow (150 ml min<sup>-1</sup>) to the beakers holding the corals. The beakers were arranged by strict random assignment in a large water table and rotated weekly to avoid possible position effects. The water temperature was monitored daily in approximately one-third of the randomly selected beakers at approximately 09:00, 12:00, and 15:00 using a mercury thermometer with a precision of 0.02 °C. The mean water temperature ( $\pm$ SE) for the heated treatment was 29.93 °C  $\pm$  0.02 and for the ambient treatment was 28.95 °C  $\pm$  0.01. There was a significant difference in water temperature between treatments (t-test,  $p < 0.001$ ,  $n = 2,709$ ).

Corals were exposed to experimental conditions for 43 days, from September 26 to November 7, 2001. Coral appearance was monitored weekly until the end of the experiment, when a branch tip of approximately 2–3 cm was clipped from each coral colony. The coral fragments were wrapped in aluminum foil, frozen (–20 °C), and processed within the next 24 h. Coral tissues were removed from the skeleton with a jet of distilled water from an airbrush. Two aliquots of the resulting suspension were examined with a Nikon Labophot microscope using a hemacytometer to determine the density of zooxanthellae (three replicate counts). Digital images of zooxanthellae were imported into SigmaScanPro (SPSS, Inc.) image analysis package using a Hitachi VK-C370 color video camera attached to the microscope. Cell volume was calculated assuming a spherical shape. The suspension was then centrifuged at 2,500 g for 10 min and the supernatant used for tissue total soluble protein analysis. A solution of 90% acetone in distilled water was added to the settled zooxanthellae pellets which were then ground with a Teflon pestle and the solution stored refrigerated in the dark for 24 h. The extract was analyzed spectrophotometrically and the concentration of chlorophylls *a*, *c*<sub>2</sub>, and carotenes calculated according to Jeffrey and Haxo (1968). Coral tissue total soluble proteins were analyzed according to the method of Bradford (1976) using the SIGMA Bradford reagent (No. B6916).

**Table 2** Summary chart of the most common genotype of *Pocillopora damicornis* from seven coral reefs on the Pacific coast of Panama. These genotypes were selected for a thermal tolerance experiment. HK, PGDH, MDH-2, LGG-1, LTY-1, LTY-2, TPI-1, TPI-2 are monomorphic. Letters indicate alleles

Allozyme				
Site	GPI	MDH-1	LGG-2	LTY-3
Non-upwelling environment				
Uva Island	CE	AA	BC	AA
Granito de Oro Island	EE	AB	AA	AA
Coiba Island	DD	AA	AA	AA
Canal de Afuera Island	CD	AB	AB	BB
Moderate upwelling environment				
Iguana Island	DD	BB	AB	BB
Intense upwelling environment				
Uraba Island	DD	BB	BB	BB
Saboga Island	AD	BB	AB	BB

Branch tip surface area was estimated using the paraffin method of Glynn and D'Croze (1990).

Parametric statistics were applied to the data, and when necessary, logarithmic transformations were used to comply with the requirements of the analysis. Visual coral condition was assessed qualitatively for all colonies at the end of the experiment, following a modified description of Glynn et al. (2001). Coral tissue pigmentation was scored as normal (full color characteristics in healthy state), pale (slight loss of coloration), bleached (evident loss of coloration), and dead.

## Results

There were differences in up to 4 of 12 allozymes screened in *P. damicornis* (Table 2). We found no genotypes in common between localities (Table 2). The GPI locus uniquely characterized corals from Uva, Granito de Oro, Canal de Afuera, and Saboga (Table 2). LGG-2 locus uniquely differentiated the corals from Urabá and Uva. The two remaining localities not uniquely differentiated (corals from Coiba and Iguana) were separated by the MDH-1, LGG-2, and LTY-3 loci. Further differences between some coral genotypes were observed at the MDH-1, LGG-2, and LTY-3 loci

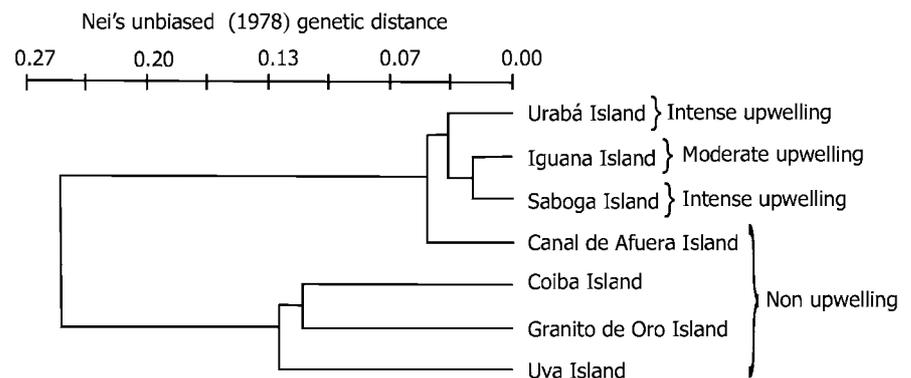
(Table 2). Loci differentiating between corals from the upwelling environment (Iguana, Urabá, and Saboga) were basically restricted to only one allozyme. Corals from the non-upwelling environment (Uva, Granito de Oro, Canal de Afuera, and Coiba) differed in up to four allozymes (see Table 3). An unweighted pair group method average (UPGMA) cluster analysis (Fig. 2) was constructed for the localities analyzed by the BIOSYS-1 software package (Swofford and Selander 1989) using Nei's (1978) unbiased genetic distances (D) between samples. All upwelling localities clustered together before joining the non-upwelling localities (Fig. 2). The non-upwelling Canal de Afuera locality clustered with the upwelling sites before joining the other non-upwelling localities.

Corals from upwelling and non-upwelling environments in Panama varied significantly in their response to elevated water temperature, with the exception of the coral tissue total soluble protein (Table 4, Fig. 3). The density of zooxanthellae and the concentration of chlorophylls *a*, *c*<sub>2</sub>, and carotenenes per live tissue area exhibited significant declines when corals were exposed to an increase of 1 °C in water temperature (Table 4). Low concentrations of photosynthetic pigments were the norm in corals under the heated treatment from the non-upwelling sites of Coiba and Granito de Oro, and from

**Table 3** Summary chart of loci differentiating between *Pocillopora damicornis* from seven coral reefs on the Pacific coast of Panama. Corals from the non-upwelling Gulf of Chiriquí: *UV* Uva Island; *GO* Granito de Oro Island; *CO* Coiba Island; and *CA* Canal de Afuera Island. Corals from the upwelling Gulf of Panama: *IG* Iguana Island; and *SA* Saboga Island

	GO	CO	CA	IG	UR	SA
UV	GPI MDH-1 LGG-2	GPI LGG-2	GPI MDH-1 LGG-2 LTY-3	GPI MDH-1 LGG-2 LTY-3	GPI MDH-1 LGG-2 LTY-3	GPI MDH-1 LGG-2 LTY-3
GO		GPI MDH-1	GPI LGG-2 LTY-3	GPI MDH-1 LGG-2 LTY-3	GPI MDH-1 LGG-2 LTY-3	GPI MDH-1 LGG-2 LTY-3
CO			GPI MDH-1 LGG-2 LTY-3	MDH-1 LTY-3	MDH-1 LGG-2 LTY-3	GPI MDH-1 LGG-2 LTY-3
CA				GPI MDH-1	GPI MDH-1 LGG-2	GPI MDH-1
IG					LGG-2	GPI
UR						GPI LGG-2

**Fig. 2** Unweighted pair group method average (UPGMA) phenogram using Nei's unbiased genetic distances (Nei 1978) summarizing the relationships between colonies of *Pocillopora damicornis* collected from three localities in the upwelling Gulf of Panama and four localities in the non-upwelling Gulf of Chiriquí



**Table 4** Significance values from the two-way ANOVA of the factors temperature and site of coral collection on symbiosis attributes in *Pocillopora damicornis*. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; ns, non significant

Source of variation	df	Mean square	F-ratio	P
<b>Zooxanthellae (<math>10^6</math> cells <math>\text{cm}^{-2}</math>)</b>				
Sites	6	5.961	24.717	***
Temperature	1	4.691	19.451	***
Sites* temperature	6	0.131	0.544	ns
Error	37	0.241		
<b>Chlorophyll a (<math>\mu\text{g cm}^{-2}</math>)</b>				
Sites	6	59.125	23.274	***
Temperature	1	66.400	26.138	***
Sites* temperature	6	5.764	2.269	ns
Error	37	2.540		
<b>Chlorophyll <math>c_2</math> (<math>\mu\text{g cm}^{-2}</math>)</b>				
Sites	6	19.135	24.059	***
Temperature	1	13.477	16.945	***
Sites* temperature	6	1.214	1.526	ns
Error	37	0.795		
<b>Carotenenes (<math>\mu\text{g cm}^{-2}</math>)</b>				
Sites	6	103.744	17.101	***
Temperature	1	112.327	18.516	***
Sites* temperature	6	13.150	2.168	ns
Error	37	6.066		
<b>Chlorophyll a (pg zooxanthella<math>^{-1}</math>)</b>				
Sites	6	63.425	8.758	***
Temperature	1	94.416	13.038	***
Sites* temperature	6	24.242	3.348	**
Error	37	7.242		
<b>Chlorophyll <math>c_2</math> (pg zooxanthella<math>^{-1}</math>)</b>				
Sites	6	29.313	10.297	***
Temperature	1	59.381	18.400	***
Sites* temperature	6	13.944	4.898	**
Error	37	2.847		
<b>Total protein (mg <math>\text{cm}^{-2}</math>)</b>				
Sites	6	28100.699	10.599	***
Temperature	1	63.084	0.024	ns
Sites* temperature	6	2986.881	1.127	ns
Error	37	2651.246		
<b>Zooxanthellae volume (<math>\mu\text{m}^3</math>)</b>				
Sites	6	5441715.089	231.924	***
Temperature	1	5532330.046	235.786	***
Sites* temperature	6	331261.388	14.118	***
Error	2342	23463.377		

the upwelling sites of Iguana, Urabá, and Saboga. In contrast, corals from Uva and Canal de Afuera, in the non-upwelling environment, barely showed changes in symbiotic algae density and concentration of photosynthetic pigments when exposed to warm water (Fig. 3).

The two-way ANOVA confirmed that coral responses were significantly affected by water temperature and the site of collection of the specimens, but not by the interaction of these factors (Table 4). Biomass attributes of the zooxanthellae such as the cellular concentration of photosynthetic pigments and cell volume were affected by the water temperature, the site of collection of the corals, and by the interaction between these (Fig. 3, Table 4). Zooxanthellae in heated corals increased their cellular volume and the concentration of chlorophyll *a* and carotenenes (Fig. 3). This was particularly evident for the colonies from Iguana, in the upwelling environment. In addition, these corals exhibited the lowest density of symbiotic algae and small concentrations of chlorophyll *a*

and carotene per live tissue area. Zooxanthellae in corals from Iguana had the largest cellular volume and highest concentrations of photosynthetic pigments per cell (Fig. 3). Differences in the concentration of tissue total soluble protein in experimental corals were related to the sites of collection but not to thermal exposure (Table 4).

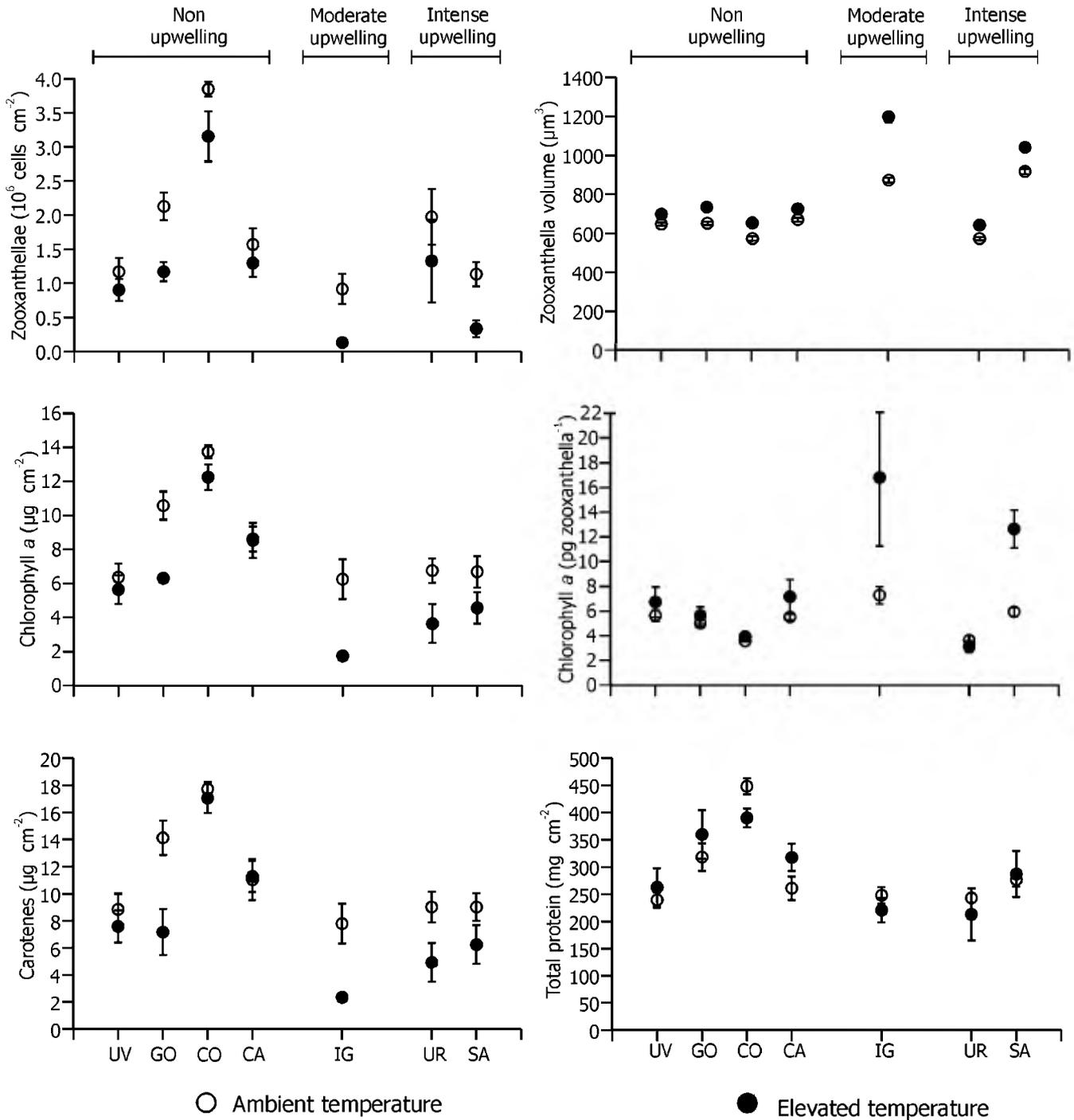
Bonferroni multi-comparison tests showed that exposure of corals to an increase of 1 °C in water temperature caused different responses among coral genotypes from the upwelling and non-upwelling environments (Table 5). Highest contrasts in measured parameters resulted from the comparison of coral genotypes from the upwelling and non-upwelling environments. The latter is particularly clear when corals from Coiba, in the non-upwelling environment, are compared to corals from Iguana and Saboga in the upwelling environment (see Table 5). However, exception to this pattern is the comparison between corals from Uva (non-upwelling environment) with corals from Urabá and Saboga in the upwelling Gulf of Panama, which had almost identical responses to elevated water temperature.

Thermal tolerance of corals from Coiba differed greatly from that of corals from all other sites in the Gulf of Chiriquí (see Fig. 3). Zooxanthellae densities and the concentration of photosynthetic pigments in coral genotypes from Uva and Canal de Afuera from the Gulf of Chiriquí barely suffered any consequence after being exposed to elevated temperatures, whereas coral genotypes from Granito de Oro and Coiba, all in the same vicinity, exhibited great decline in zooxanthellae and pigments (Fig. 3). We observed less difference in the thermal response of the three coral genotypes from the upwelling Gulf of Panama which were mostly related to changes in cellular volume and concentration of photosynthetic pigments of the symbiotic algae. The thermal response of corals from Urabá showed significant differences compared to those corals of Iguana and Saboga, but no difference was detected between the responses of the latter two genotypes (Table 5).

Visual inspection of corals during this experiment showed that colonies from the non-upwelling Gulf of Chiriquí retained a normal to slightly pale appearance under both temperatures, except for corals from Uva, most of which became pale (Fig. 4). None of the corals from the upwelling Gulf of Panama kept normal coloration and their polyps were mostly retracted after exposure to elevated temperature. Furthermore, two corals from Iguana Island completely bleached under heated conditions. During the experimental period, three dead corals were recorded, two from Coiba (one from each experimental condition), and one from Urabá under the heated treatment.

## Discussion

The differentiation of *Pocillopora damicornis* from Panamanian localities by molecular tools represents a step



**Fig. 3** Biomass attributes of *Pocillopora damicornis* from three upwelling and four non-upwelling localities after 42 days of exposure to experimentally elevated water temperature. Error bars denote the SE. Zooxanthella densities and volumes, chlorophyll *a* and carotene pigment concentrations, and tissue total soluble protein. UV Uva Island; GO Granito de Oro Island; CO Coiba Island; CA Canal de Afuera Island; IG Iguana Island; UR Urabá Island; and SA Saboga Island

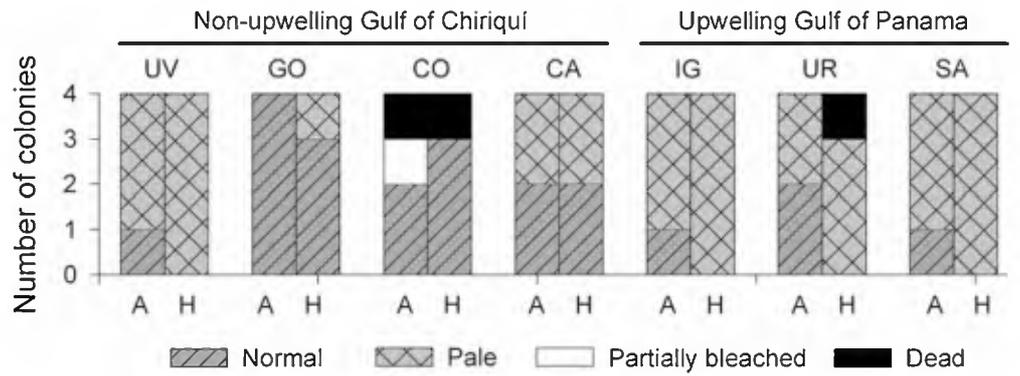
further in understanding how the coral host responds to thermal stress. So far, the explanation of different responses to sea warming in eastern Pacific corals has centered on the comparison between corals from

upwelling and non-upwelling environments, and coral species with different growth morphologies (see overview by Glynn 1996). An additional explanation of the pattern of bleaching observed in Panamanian coral reefs have been related to the thermal tolerance of genotypes of zooxanthellae harbored by the corals (Glynn et al. 2001). During our experimental study, genotypes of the coral *P. damicornis* collected from the upwelling Gulf of Panama and the non-upwelling Gulf of Chiriquí environments in Panama showed different responses when exposed to experimentally elevated water of

**Table 5** Matrix of multiple pairwise comparison probabilities with Bonferroni adjustment applied to indicate genotypic differences of *Pocillopora damicornis* from seven coral reefs in the Pacific coast of Panama. Corals from the non-upwelling Gulf of Chiriqui: *UV* Uva Island; *GO* Granito de Oro Island; *CO* Coiba Island; and *CA* Canal de Afuera Island. Corals from the upwelling Gulf of Panama: *IG* Iguana Island; and *SA* Saboga Island. *Zoox* zooxanthellae cm<sup>-2</sup>; *Chlaa* chlorophyll *a* cm<sup>-2</sup>; *Chlca* chlorophyll *c*<sub>2</sub> cm<sup>-2</sup>; *Chlaz* chlorophyll *a* zooxanthella<sup>-1</sup>; *Chlcz* chlorophyll *c*<sub>2</sub> zooxanthella<sup>-1</sup>; *Prota* total protein cm<sup>-2</sup>; *Carota* carotenes cm<sup>-2</sup>; *Vol* zooxanthellae volume

	GO	CO	CA	IG	UR	SA
UV	Zoox Chlaa Chlca Prota Carota Vol	Zoox Chlaa Chlca Prota Carota Vol	Zoox Chlaa Chlca Prota Carota Vol	Chlaz Chlcz Vol	Vol	Vol
GO		Zoox Chlaa Chlca Carota Vol		Zoox Chlaa Chlca Chlaz Chlcz Prota Carota Vol	Chlaa Chlca Prota Vol	Zoox Chlaa Vol
CO			Zoox Chlaa Chlca Prota Carota Vol	Zoox Chlaa Chlca Chlaz Chlcz Prota Carot Vol	Zoox Chlaa Chlca Prota Carota Vol	Zoox Chlaa Chlca Chlaz Chlcz Prota Carota Vol
CA				Zoox Chlaa Chlca Chlaz Chlcz Carota Vol	Chlaa Chlca Vol	Chlaa Vol
IG					Zoox Chlaz Chlcz Vol	
UR						Chlaz Chlcz Vol

**Fig. 4** Visual condition of *Pocillopora damicornis* from upwelling and non-upwelling localities after 1 month of exposure to experimentally elevated water temperature. *UV* Uva Island; *GO* Granito de Oro Island; *CO* Coiba Island; *CA* Canal de Afuera Island; *IG* Iguana Island; *UR* Urabá Island; and *SA* Saboga Island. *A* Corals under ambient water temperature (28.95 °C ± 0.01); *H* Corals under elevated water temperature (29.93 °C ± 0.02)



30°C. Genotypes of *P. damicornis* from the upwelling sites manifested higher vulnerability to thermal stress than those from the non-upwelling sites. Heat-resistant coral genotypes from the non-upwelling environment displayed greater differences in responses (Table 5). Reports of extensive bleaching and mortality elsewhere have been connected to coral clones belonging to probable temperature-sensitive genotypes (Jokiel and Coles 1990; Edmunds 1994; Brown 1997). However, none of these studies identified the genotypes of the coral

hosts limiting the conclusions regarding the reported differences in thermal tolerance.

The observed coral responses during our study are in agreement with previous experiments with eastern Pacific corals (Glynn and D’Croz 1990; Maté 1997; D’Croz et al. 2001; Hueerkamp et al. 2001) and mostly related to the decline in the abundance of zooxanthellae and photosynthetic pigments, which characterize the pale/bleached condition in thermally stressed corals. Zooxanthellae responses usually involved an increase in the

concentration of photosynthetic pigments and in algal volume (Fig. 3). High content of cellular chlorophyll *a* has been the norm in algal symbionts in corals exposed to high temperature (Fitt et al. 1993; Jones 1997; Brown et al. 2000, 2002; D'Croz et al. 2001), and this is probably due to the larger cellular volume observed in remaining zooxanthellae. The biological meaning of this condition is not completely clear. However, the increase in zooxanthellar chlorophyll *a* concentration may be due to higher availability of nutrients to the remaining algal symbionts, as suggested by Jones and Yellowlees (1997). We noticed in our experiment that despite the fact that the concentration of coral tissue total soluble protein (an indication of tissue nitrogen) was not affected after 6 weeks of exposure to high temperature, zooxanthellar volume was significantly increased (see Table 4). Prolonged exposure to elevated temperature may contribute to the decline of tissue proteins in *P. damicornis*, as was observed when corals were subjected to 10 weeks of experimental exposure to warm water (Glynn and D'Croz 1990).

The thermal susceptibility of zooxanthellae belonging to different clades harbored by corals may contribute to the extreme variability in bleaching patterns of Panamanian corals. Algal symbionts may exhibit different tolerances to environmental stressors. We did not collect information on the genetics of the zooxanthellae in the coral genotypes tested for thermal tolerance. However, existing data suggest that zooxanthellae clade *C* was highly replaced by clade *D* in recovered *P. damicornis* from the Gulf of Chiriquí after the 1997–1998 ENSO sea-warming (Glynn et al. 2001). Zooxanthellae clade *C* persisted in massive coral species. Clade *C* was identified as the only algal complement of *P. damicornis* in the Gulf of Panama (Baker and Rowan 1997), and this situation possibly continues because the Gulf of Panama was not affected by the 1997–1998 ENSO sea-warming.

The history of exposure of corals to high temperature might also define the thermal tolerance of corals either by acclimation and/or adaptation of resistant genotypes of zooxanthellae, coral hosts, or both (Coles and Jokiel 1978; Jokiel and Coles 1990; Coles 1997; Marshall and Baird 2000; Brown et al. 2002; Hoegh-Guldberg et al. 2002). Field studies during ENSO episodes in the eastern tropical Pacific confirmed that bleaching and mortality are higher in corals growing in upwelling environments (Glynn 1990). During the intense 1982–1983 ENSO sea-warming, coral mortality in the upwelling Gulf of Panama reached 85%, whereas in the non-upwelling Gulf of Chiriquí it was 75% (Glynn et al. 1988). The 1997–1998 ENSO sea-warming affected the Gulf of Chiriquí but not the Gulf of Panama, and 4 weeks with elevated temperatures this time caused only 17% of bleaching and mortality in reef-building corals (Glynn et al. 2001).

Our experimental results suggest that even in small geographic areas, where environmental conditions are highly alike, corals can display different levels of tolerance to thermal stress. The observed responses between

corals from upwelling and non-upwelling environments were concomitant with the genetic differences between these corals (Table 5). Coral genotypes from the warm and thermally stable Gulf of Chiriquí environment suggest better adaptation and/or acclimation to cope with temperature stress, than coral genotypes from the colder upwelling Gulf of Panama. However, since no single genotype was common to both the upwelling and non-upwelling environments, it is not possible to evaluate the role that adaptation might have in these responses. There are indications suggesting that *P. damicornis* from the Gulf of Chiriquí has higher genotypic diversity than in the upwelling Gulf of Panama. The preliminary survey of *P. damicornis* (D'Croz et al. 2003; Maté 2003) identified 19 genotypes in approximately 14.3 ha of coral reefs in the Gulf of Chiriquí (Uva Island, Canal de Afuera Island, Secas Island, and Granito de Oro Island) and 11 genotypes in 31.3 ha of coral reefs in the Gulf of Panama (Iguana Island, Saboga Island, and Urabá Island). The maximum number of genotypes was recorded at Uva Island (nine genotypes) in the non-upwelling environment, whereas the minimum at Urabá Island (two genotypes) in the upwelling environment.

The possibility of combination of the algal symbiont clade *C* and *D* in *P. damicornis* from the non-upwelling environment (Glynn et al. 2001), together with the high number of host genotypes reported, may cause not only their higher tolerance to elevated temperatures, but also their high differences in thermal response. On the contrary, the combination of fewer host genotypes and only one zooxanthellae genotype (clade *C*) in corals from the upwelling environment may cause the high, and rather uniform, susceptibility to warm water observed during our experiment. The higher recurrence of ENSO-related sea-warmings in the Gulf of Chiriquí than in the Gulf of Panama (as observed during the 1997–98 event) possibly resulted in the selection of heat-resistant genotypes of zooxanthellae and coral hosts which partially explain the different responses observed in *P. damicornis*. This may also be the explanation for the reduced coral mortality in the Gulf of Chiriquí during the intense 1997–1998 ENSO sea-warming as compared with the 1982–1983 event. In contrast, coral host genotypes from the upwelling Gulf of Panama, which did not experience sea-warming during the 1997–1998 ENSO, and therefore have not been exposed to the effects of elevated water temperature in almost 20 years, and presumably harbored heat sensitive zooxanthellae clade *C* (Baker and Rowan 1997), expressed higher susceptibility to elevated water temperature during this experiment.

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