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Loss of *Symbiodinium* from bleached Australian scleractinian corals (*Acropora hyacinthus*, *Favites complanata* and *Porites solida*)

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Abstract. Coral bleaching results from the dissociation of *Symbiodinium* and is primarily related to sea surface temperatures above mean yearly maximums. The numbers of live, dead, and mitotic *Symbiodinium* cells lost from three scleractinian corals from three different families (*Acropora hyacinthus*, *Favites complanata*, and *Porites solida*), which have not been studied previously in central Queensland (Australia), were compared at 28, 30, 32, and 34°C. Specific expulsion rates, growth rates, and mitotic indices were compared for each host at each temperature. *Porites solida* was the most robust coral, *A. hyacinthus* bleached more readily at low temperatures and *F. complanata* showed levels of intermediate bleaching tolerance to elevated temperatures. However, the timing of *Symbiodinium* cell loss was similar between all corals tested. Mitotic indices and specific growth rates were found to be positively associated with increasing temperature; thus, symbiont reproduction increased despite elevated losses of *Symbiodinium* from the host. Because all corals in the present study were symbiotic with *Symbiodinium* from clade C, different levels of stress tolerance to temperature suggests that bleaching resistance is an attribute associated with the coral host and, to a lesser degree, the symbiont.

Extra keywords: bleaching, growth rates, mitotic indices, specific expulsion rates, zooxanthellae.

Introduction

Coral bleaching has become a major problem for coral reefs around the world. It is a process by which the endosymbiotic dinoflagellate alga, called *Symbiodinium* (a zooxanthella), and/or photosynthetic pigments residing within the host and/or symbiont are reduced within the coral tissue, exposing the white skeleton beneath. The symptoms of bleaching (turning pale white) are not confined to corals and have been observed in sea anemones, zoanthids, tridacnids, sponges, and foraminifera (Gomez and Mingoa-Licuanan 1998; Fromont and Garson 1999).

Studies indicate that numerous causes of bleaching exist, including disease, elevated oxygen, increased and decreased salinity, light intensity, and elevated temperature (Hoegh-Guldberg 1999; Fitt *et al.* 2001). Historically, elevated temperature appears to be the only factor that can be correlated with wide-scale regional bleaching in corals (see Hoegh-Guldberg 1999; Goreau *et al.* 2000). Many studies that have examined this phenomenon indicate that it occurs at temperatures >30°C or above a particular habitat's mean maximum seasonal temperature (Hoegh-Guldberg 1994; Jones *et al.* 1998).

In the present study, we examined the effects of heat stress on three types of scleractinian corals from three different families from the Keppel Islands in central Queensland, Australia. During bleaching episodes in the Keppel Bay area in 1992, some corals were either not affected or did not bleach completely (e.g. *Favites* spp. and *Porites* spp.; see Byron and O'Neill 1992), indicating a greater resistance to high temperatures in these species than reported for other corals that bleach more readily (e.g. *Acropora* spp.). The aims of this study were to: (*1*) determine the concentration and physiological state (live, dead, or mitotic) of *Symbiodinium* lost from several dominant corals in the Keppel Bay area; (*2*) compare and contrast the temporal and cumulative effects of live, dead, and mitotic *Symbiodinium* cells lost from the host; and (*3*) determine which corals were more tolerant of temperature-induced bleaching. Comparisons of cumulative *Symbiodinium* cell loss have been presented graphically to illustrate specifically how temperature affected

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the overall depletion of a coral's *Symbiodinium* complement over 48 h.

Materials and methods

Coral collection, storage, and acclimation

The scleractinian corals *Acropora hyacinthus* (Dana 1846), *Favites complanata* (Ehrenberg 1834), and *Porites solida* (Forskål 1775) were collected from the fringing reefs of Barren Island (23°10′S, 151°55′E) and Outer Rocks (23°4.3′S, 151°57.2′E) at depths of 7–10 m. All corals were maintained at the Central Queensland University laboratory in a 200-L holding tank containing 1 µm-filtered seawater (FSW) subjected to a 12 h : 12 h day–light regimen (150 µM quanta $m^{-2} s^{-1}$) for 96 h prior to being used in bleaching experiments.

Experimental treatments: flow-through apparatus

The apparatus used to evaluate the heat stress of corals consisted of seawater stored in a 4000-L storage tank pumped through 10- and 1-µm filters before being deposited into 4 250-L header tanks. In order to ensure uniform mixing during each experiment in each tank, a submersible pump and two air stones were used to promote water circulation and aeration. A peristaltic pump delivered a constant flow rate of 20 mL min^{-1} FSW to a series of 8 2-L plastic incubation containers; Millipore depth filters (1.2 µm) helped remove alga that may have accumulated in the header tanks. Each incubation chamber was insulated by a 'water jacket' consisting of a 10-L tank, a 125-W aquarium heater, and a small submersible pump. Each incubation container was elevated 1 cm off the bottom of the water jacket to help provide uniform water and temperature circulation. In addition, each water jacket enclosing an incubation chamber was positioned above a magnetic stirrer, within which a stir bar spun, mixing the FSW in each incubation chamber. Mixing also prevented settlement of any *Symbiodinium* lost from the corals. Flows through incurrent and excurrent pipes fitted to each incubation container were regulated so that volumes within each container were maintained at 1.5 L.

Salinities were monitored every 12 h. Temperatures within each incubation chamber were monitored every 15 min with submersible digital data-loggers and maintained within 0.5°C of the experimental temperature. If the seawater temperature fluctuated more than 0.5°C, the aquarium heaters were readjusted. Light levels were monitored every 6 h with a photometer.

Experimental design

A randomised statistical block design (Zar 2000) was used to test corals subjected to the different experimental temperatures over 48 h. Eight incubation containers were used for each run and the order of temperatures within chambers was randomised for each run. Corals were incubated at 30, 32, or 34°C and compared with a control at 28°C, with two incubation containers at each temperature. The temperature of 28°C was considered as the control because observations of these corals held in aquarium tanks at this temperature have shown no symptoms of bleaching over a 4-month period. Temperatures >30°C were chosen because many authors have suggested that massive bleaching events will occur above this temperature (Warner *et al.* 1996). At the end of each run, the incubation containers were washed in phosphate-free detergent and then rinsed, first with 70% (v/v) ethanol and then FSW. This rinsing regimen was used between runs to help remove any alga and/or metabolites that may have attached to the inside of the incubation container. A sample size of ten replicates ($n = 10$) for each coral at each temperature was used. A replicate consisted of a single colony of coral from the same species whose wet weight was approximately 100 g.

Sampling to collect Symbiodinium *cells*

Sampling of *Symbiodinium* lost from the corals was performed every 3 h over 48 h. At each sampling period, 250 mL water was collected from the excurrent pipe of each incubation container and centrifuged at 700*g* for 5 min at room temperature. Following centrifugation, 1 mL supernatant was removed and the remaining supernatant discarded. The pellet containing *Symbiodinium* cells was then resuspended in the 1 mL supernatant and centrifuged at 700*g* for 5 min at room temperature. Finally, 800 µL supernatant was removed and, to the remaining 200 µL supernatant, 400 µL of 0.2% Trypan blue was added and the tubes were vortexed gently for 10 s. The microcentrifuge tubes were then incubated at room temperature for 5 min to allow absorption of Trypan blue into the dead cells. Replicate counts ($n = 10$) of live, dead, and mitotic *Symbiodinium* cells were made using a hemocytometer and a light microscope. Cells were characterised as three types based on morphology and Trypan blue staining as follows: (*1*) live cells not mitotic (did not stain with Trypan blue); (*2*) dead cells not mitotic (stained blue with Trypan blue); and (*3*) mitotic cells. Mitotic cells, recognised by the presence of paired nuclei or two mitotic figures per cell (Brown and Zamani 1992), included both live and dead cells because it was not known whether these cells died *in situ* or upon exposure to the environment outside the host.

Indices of live, dead, and mitotic Symbiodinium *cells*

At the beginning of each experimental period, the number of *in situ* live, dead, and mitotic *Symbiodinium* cells at 28°C was determined for each replicate coral. Standing stock was measured as the total number (live + dead + mitotic) of *Symbiodinium* per unit volume of extracted coral tissue (1 g in 1 mL FSW).

Cell state indices were graphed through time using both standard and cumulative data. The former depict the time-course of *Symbiodinium* dissociation from a particular host; the latter reveal patterns of temperature susceptibility for each host and differentially between hosts.

Specific expulsion rate

Rates of loss of *Symbiodinium* standing stock cells (live, dead, and mitotic) were measured. In order to compare these rates among corals over a 48 h period, the specific expulsion rate (S_{ER}) was determined for each host at each temperature. The S_{ER} (eqn 1) is based on the equation described by Hoegh-Guldberg *et al.* (1987):

$$S_{ER} = \frac{1}{N} \times \frac{\Delta E}{\Delta t} \tag{1}$$

where N is the standing stock of *Symbiodinium* at 28°C and ΔE is the total number of *Symbiodinium* lost over 2 days (Δt) at each temperature.

In situ *specific growth rate*

The specific growth rate (S_{GR}) of *Symbiodinium* was calculated to determine which symbionts from each host could maintain high *in situ* growth rates to compensate for increased expulsion rates associated with elevated temperatures. Calculations of S_{GR} (eqn 2) *in situ* were determined based on the fraction of cells in mitosis and the duration of the division (Hoegh-Guldberg *et al.* 1987) as follows:

$$S_{GR} = \frac{1}{td} \times \ln(1 + f) \tag{2}$$

where td is the duration of *Symbiodinium* cytokinesis per day and f is the fraction of mitotic cells (f = mitotic index (MI%); see below). The td function is a calculated constant (McDuff and Chisholm 1982) based on the estimated cytokinesis period of *Symbiodinium* (Falkowski and Raven 1997). A cell division frequency of 0.46 days was used to determine S_{GR} (Falkowski *et al.* 1993).

Mitotic index of Symbiodinium *cells* in situ

The term mitotic index (MI%; eqn 3) is used to describe the percentage of cells observed undergoing mitosis (Wilkerson *et al.* 1983) and is calculated as follows:

$$MI\% = \frac{MN}{total} \times 100 \quad (3)$$

where MI% is the percentage of mitotic *Symbiodinium* cells. The number of mitotic (MN) *Symbiodinium* cells (live and dead) *in situ* after 48 h at each temperature provides an estimate of the effect of temperature on symbiont cell division. 'Total' is the total number of *Symbiodinium* cells (live and dead) counted at 28°C (immediately before increasing the temperature to 30, 32, or 34°C). The total number of *Symbiodinium* cells at 28°C was used as a best estimate of the maximum number of cells immediately before elevated heat stresses. Thus, the rate of expulsion in eqn 1 and the rate of growth in eqn 2 are both relative to the total number of cells present at the beginning of the experiment.

Statistical analyses

Data were checked for normality and heteroscedasticity of sample variances (Zar 2000). If heteroscedasticity was found, the data were log transformed before analysis to normalise the data. Data were analysed by standard parametric tests using model I ANOVAs, Tukey's *a posteriori*, multiple comparisons tests, and linear regression analyses. Only significant results will be discussed.

Results

Statistical analysis

A three-way model I repeated-measures ANOVA of live, dead, and mitotic *Symbiodinium* cell loss (dependent variables) was performed for each coral species with run, time, and temperature as the main factors. Replicate runs (represented by 'Run' in Table 1) between subjects were not significantly different for any species for any cell type. The effect of temperature on numbers of lost *Symbiodinium* of each cell type was highly significant for each coral species (Table 1). In general, there were significant higher-order (three-way) interactions between time and temperature (Table 1). The significant interaction between time and temperature occurred because, as temperature increased, less time passed before the host began to lose *Symbiodinium* cells. The significance of the time factors (Table 1) also indicated that *Symbiodinium* cells were lost in bursts during the course of the experiments, rather than at a constant rate. The lack of significant interaction between time and run indicated that the replicated experiments were relatively consistent to the time at which *Symbiodinium* cells were lost (Table 1).

Table 1. Repeated-measures ANOVA (three-way model I) of live, dead, and mitotic (reproducing) *Symbiodinium* cells lost from scleractinian coral hosts *Acropora hyacinthus*, *Porites solida*, and *Favites complanata* over 48 h

	MS			d.f.			F			Significance		
	Live ($\times 10^{14}$)	Dead ($\times 10^{16}$)	Mitotic ($\times 10^{13}$)	Live	Dead	Mitotic	Live	Dead	Mitotic	Live	Dead	Mitotic
Acropora hyacinthus												
BS												
Run	55	1.6	6.4	9	9	9	1.0	1.5	1.2	NS	NS	NS
Temp	12	29	64	3	3	3	21.6	27.6	12.2	***	***	***
WS												
Time	18	0.32	0.46	15	15	15	47.4	18.2	18.2	***	***	***
Time × run	32	0.018	0.026	135	135	135	0.8	1.0	1.0	NS	NS	NS
Time × temp	5.6	0.15	0.23	45	45	45	14.8	8.4	9.2	***	***	***
Porites solida												
BS												
Run	0.6	0.00038	0.0072	9	9	9	1.8	1.4	2.5	NS	NS	***
Temp	2.7	0.0018	0.016	3	3	3	8.1	6.4	5.4	***	***	***
WS												
Time	0.13	0.00014	0.0016	15	15	15	26.9	16.6	21.8	***	***	***
Time × run	0.0089	0.00001	0.00014	135	135	135	1.9	1.3	2.0	NS	NS	*
Time × temp	0.033	0.00005	0.00032	45	45	45	6.9	6.3	4.5	***	***	**
Favites complanata												
BS												
Run	16.7	0.59	0.29	9	9	9	1.3	0.8	0.7	NS	NS	NS
Temp	142	4.6	1.4	3	3	3	10.8	6.4	3.2	***	**	**
WS												
Time	5.4	12.9	0.037	15	15	15	31.6	15.3	8.9	***	***	***
Time × run	0.22	0.94	0.0031	135	135	135	1.3	1.1	0.7	NS	NS	NS
Time × temp	1.5	4.7	0.011	45	45	45	8.9	5.6	2.7	***	***	*

Significance: *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$; NS, not significant; $n_i = 10$ in all cases.
BS, Between subjects; WS, within subjects; MS, mean square; d.f., degrees of freedom; F, Fisher statistic; Temp, temperature.

Loss of live, dead, and mitotic Symbiodinium *cells through time*

At 28°C, *A. hyacinthus* had the highest number of live, dead, and mitotic *Symbiodinium* cells *in situ* compared with *P. solida* or *F. complanata* (Table 2).

The number of live *Symbiodinium* cells lost from corals at 28 or 30°C over 48 h was low (Fig. $1a_1$–c_1). An increase in temperature from 30 to 32°C resulted in a unimodal loss of live symbiont cells from *A. hyacinthus*, peaking at the 9 h mark (Fig. $1a_1$) and a bimodal loss from *P. solida* and

Table 2. Fraction of *in situ* coral *Symbiodinium* cells (live, dead, mitotic-reproducing and standing stock) at 28°C

Coral species	Coral *Symbiodinium* cell states *in situ* at 28°C (cells mL^{-1} × 10^7)			
	Live	Dead	Mitotic	Total (=standing stock)
Acropora hyacinthus	7.48 ± 0.89	6.64 ± 0.97	0.27 ± 0.05	14.39 ± 1.65
Porites solida	4.40 ± 0.22	0.58 ± 0.02	0.02 ± 0.01	5.01 ± 0.27
Favites complanata	4.40 ± 0.25	1.08 ± 0.16	0.01 ± 0.002	5.49 ± 0.38

Data are the mean ± s.e.m. ($n_i = 10$).

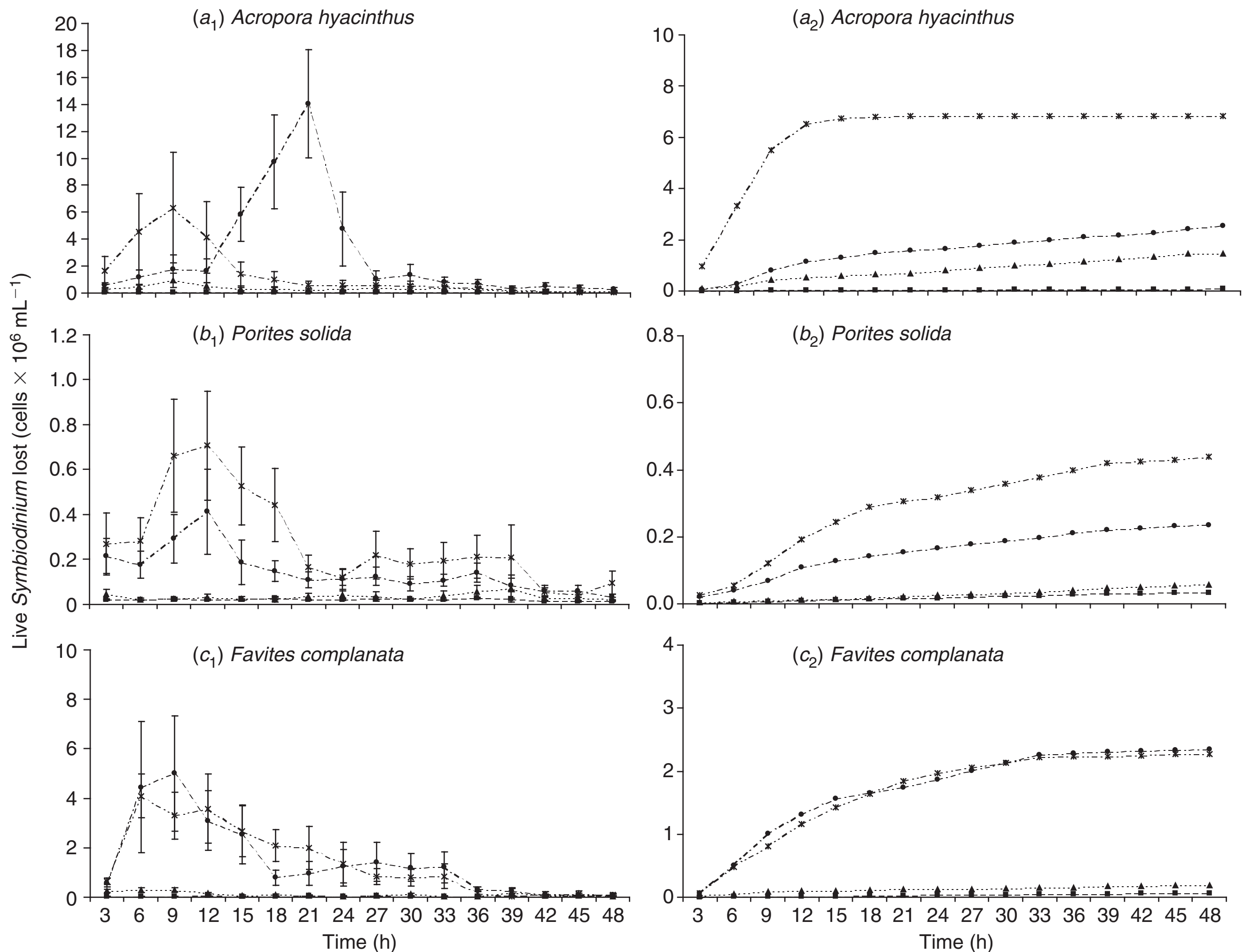

Fig. 1. Live *Symbiodinium* cells lost from *Acropora hyacinthus* (a_1, a_2), *Porites solida* (b_1, b_2) and *Favites complanata* (c_1, c_2) at 30°C (▲), 32°C (●) and 34°C (∗) sampled every 3 h over a 48 h time period. Control temperature = 28°C (■). Mean ± s.e.m. shown; $n_i = 10$. Comparisons of temporal *Symbiodinium* cells lost (×10^6 mL^{-1}; a_1, b_1 and c_1) *v.* cumulative *Symbiodinium* cells lost (×10^7 mL^{-1}; a_2, b_2 and c_2) are shown. Note how cumulative graphs (a_2, b_2 and c_2) illustrate the effect of temperature on overall depletion of a coral's *Symbiodinium* complement over 48 h.

F. complanata, peaking at approximately 12 and 30 h (Fig. 1b_1, c_1). At 34°C, the loss of live symbiont cells appeared unimodal, with maximal loss occurring between 6 and 12 h from each coral host (Fig. 1a_1–b_1).

No clear peaks in the loss of dead symbiont cells were observed at temperatures <30°C in any of the corals (Fig. 2a_1–c_1). However, at 32°C, the loss of dead *Symbiodinium* cells from *A. hyacinthus* occurred with bimodal peaks at 9 and 45 h, whereas at 34°C only one peak was observed (at 9 h; Fig. 2a_1). *Favites complanata* (Fig. 2c_1) exhibited a unimodal loss of dead symbiont cells at 32 and 34°C, peaking at 12–18 h. *Porites solida* was the only coral that exhibited a uniform release rate of dead cells at 32°C (Fig. 2b_1) and a trimodal loss peaking at 15, 27, and 42 h at 34°C.

Low levels of heat stress (<30°C) did not cause peaks in the loss of mitotic cells through time in any of the corals tested (Fig. 3a_1–c_1). However, at 32 and 34°C, *F. complanata* exhibited a unimodal loss of mitotic cells, with a peak at 9 h (Fig. 3c_1). *Porites solida* was the only coral that had a different pattern of mitotic *Symbiodinium* loss (sinusoidal) at each temperature (Fig. 3b_1).

Comparisons of cumulative and total cell loss of Symbiodinium *after 48 h*

Live Symbiodinium *cell loss*

Comparing all three coral species, significantly different numbers of *Symbiodinium* were lost at 28°C over a period of 48 h ($P < 0.001$, ANOVA). At 30°C, *A. hyacinthus* (approximately 1.8×10^6 cells mL^{-1}; Fig. 1a_2; $P < 0.001$, ANOVA) lost significantly more live symbiont cells than *P. solida* (0.04×10^6 cells mL^{-1}; Fig. 1b_2; $P < 0.001$, ANOVA) and *F. complanata* (0.3×10^6 cells mL^{-1}; Fig. 1c_2; $P < 0.01$, ANOVA). Similar trends of loss, where *A. hyacinthus* lost more live symbiont cells than the other corals, were observed at temperatures >30°C. At 32°C, *P. solida* lost the smallest number of dead *Symbiodinium* cells (Fig. 2b_2; $P < 0.01$)

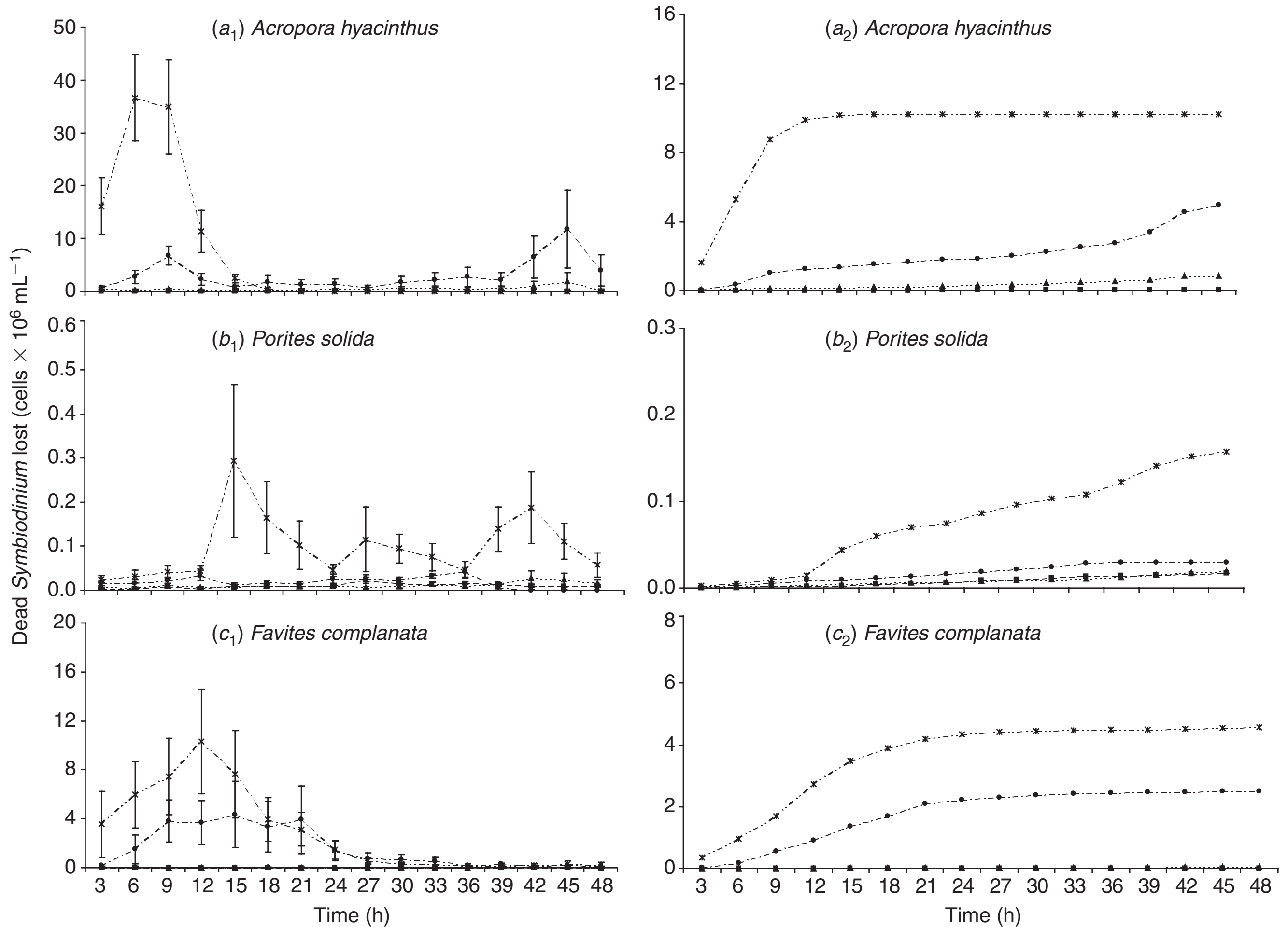

Fig. 2. Dead *Symbiodinium* cells lost from *Acropora hyacinthus* (a_1, a_2), *Porites solida* (b_1, b_2) and *Favites complanata* (c_1, c_2) at 30°C (▲), 32°C (●) and 34°C (✳) sampled every 3 h over a 48 h time period. Control temperature = 28°C (■). Mean ± s.e.m. shown; $n_i = 10$. Comparisons of temporal *Symbiodinium* cells lost ($\times 10^6$ mL^{-1}; a_1, b_1 and c_1) *v.* cumulative *Symbiodinium* cells lost ($\times 10^7$ mL^{-1}; a_2, b_2 and c_2) are shown. Note how cumulative graphs (a_2, b_2 and c_2) illustrate the effect of temperature on overall depletion of a coral's *Symbiodinium* complement over 48 h.

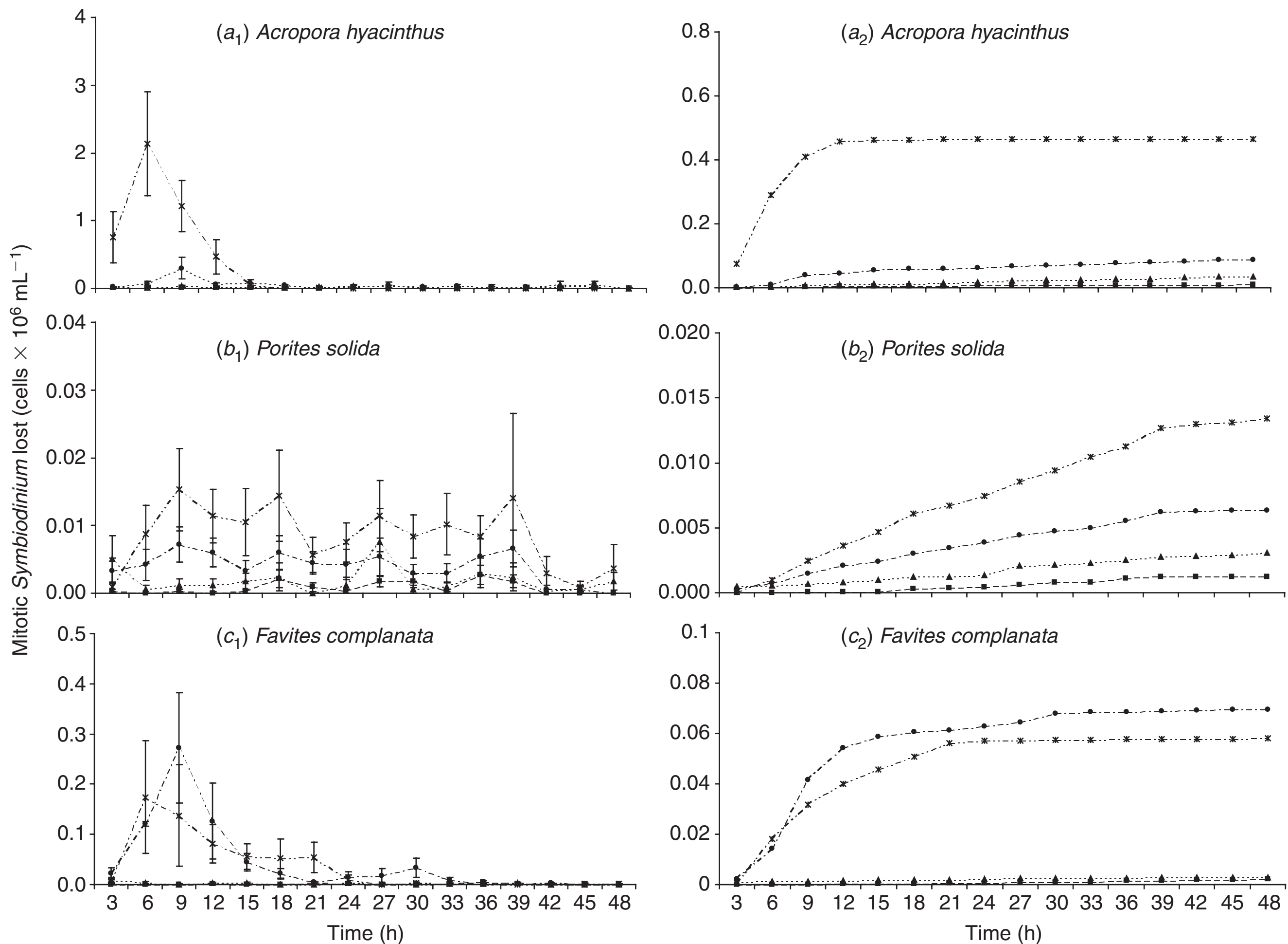

Fig. 3. Mitotic *Symbiodinium* cells lost from *Acropora hyacinthus* (a_1, a_2), *Porites solida* (b_1, b_2) and *Favites complanata* (c_1, c_2) at 30°C (▲), 32°C (●) and 34°C (∗) sampled every 3 h over a 48 h time period. Control temperature = 28°C (■). Mean ± s.e.m. shown; $n_i = 10$. Comparisons of temporal *Symbiodinium* cells lost ($\times 10^6$ mL^{-1}; a_1, b_1 and c_1) *v.* cumulative *Symbiodinium* cells lost ($\times 10^7$ mL^{-1}; a_2, b_2 and c_2) are shown. Note how cumulative graphs (a_2, b_2 and c_2) illustrate the effect of temperature on overall depletion of a coral's *Symbiodinium* complement over 48 h.

and did not show symptoms of bleaching (K. B. Strychar, M. Coates and P. W. Sammarco, unpublished observations).

Loss of dead Symbiodinium *cells*

The loss of dead symbiont cells at 30°C was not significantly different to that at 28°C for all coral species (Fig. 2a_2–c_2). Only *A. hyacinthus* (Fig. 2a_2; $P < 0.001$, ANOVA) and *F. complanata* (Fig. 2c_2; $P < 0.01$, ANOVA) exhibited significantly higher losses of dead symbiont cells at 32 compared with 30°C. At 34°C, all corals had significantly higher losses of dead *Symbiodinium* cells (Fig. 2a_2–c_2; $P < 0.01$).

Loss of mitotic Symbiodinium *cells*

There were no significant differences in the rate of loss of mitotic *Symbiodinium* cells between 28 and 30°C in any of the corals (Fig. 3a_2–c_2; $P < 0.05$, ANOVA). However, at 30 and 32°C, *P. solida* (Fig. 3b_2; $P < 0.001$) and *F. complanata* (Fig. 3c_2; $P < 0.05$) lost a significantly greater number of mitotic cells than at 28°C. There were no significant differences in the number of these cells lost from *F. complanata* at 32 compared with 34°C (Fig. 3c_2; $P < 0.05$).

Specific expulsion rate of Symbiodinium *cells lost per 48 h period*

Specific expulsion rates were significantly related to temperature in all corals ($P < 0.001$, linear regression analyses; Fig. 4*a*). At 28 and 30°C, *A. hyacinthus* had a significantly higher S_{ER} than the other corals ($P < 0.001$) and this result was repeated at 34°C ($P < 0.05$). At 32°C, *P. solida* exhibited a significantly lower S_{ER} than the other two corals ($P < 0.001$).

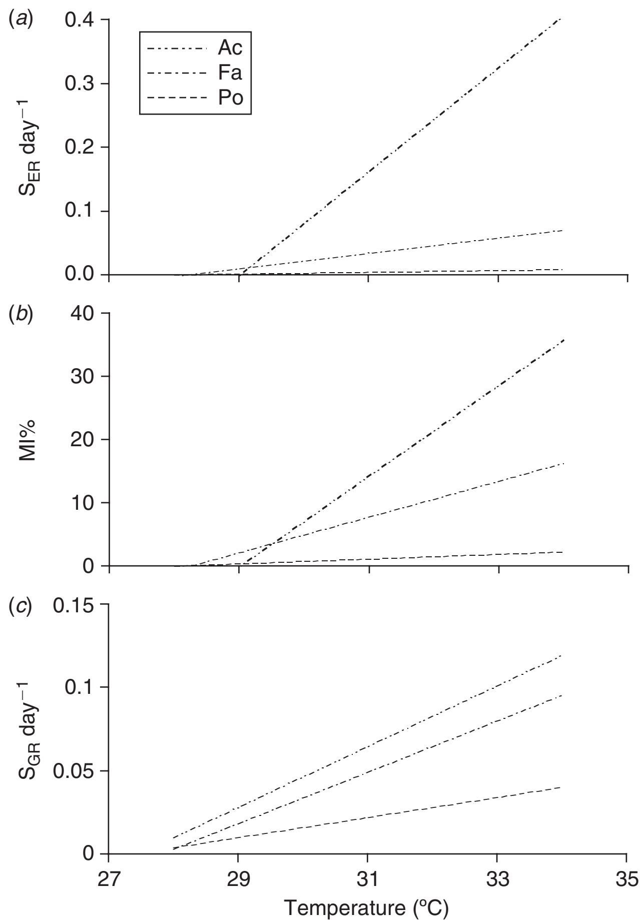

Fig. 4. Total number of *Symbiodinium* cells lost from scleractinian corals at 28°C (control), 30°C, 32°C and 34°C measured over a 48 h time period. (*a*) Specific expulsion rates per day (S_{ER} day^{-1}) for 3 coral species: *Acropora hyacinthus* (Ac), ($R^2 = 0.523$; $P < 0.001$, least squares linear regression analysis, $y = 0.0738x - 2.1404$); *Favites complanata* (Fa), ($R^2 = 0.32$, $P < 0.001$, $y = 0.0315x - 0.9083$, regression); *Porites solida* (Po) ($R^2 = 0.232$, $P < 0.001$, $y = 0.0005x - 0.0156$, regression). (*b*) *In situ* mitotic indices (MI%) is the percentage of mitotic *Symbiodinium* cells for the three corals: *A. hyacinthus* (Ac) ($R^2 = 0.705$, $P < 0.001$, $y = 7.2105x - 209.35$, regression); *F. complanata* (Fa) ($R^2 = 0.731$, $P < 0.01$, $y = 2.831x - 79.961$, regression); *P. solida* (Po) ($R^2 = 0.936$, $P < 0.01$, $y = 0.378x - 10.603$, regression). (*c*) Specific growth rates per day (S_{GR} day^{-1}) for the three coral species: *A. hyacinthus* (Ac) ($R^2 = 0.946$, $P < 0.001$, $y = 0.0183x - 0.5028$, regression); *F. complanata* (Fa) ($R^2 = 0.898$, $P < 0.001$, $y = 0.0154x - 0.4284$, regression); *P. solida* (Po) ($R^2 = 0.968$, $P < 0.001$, $y = 0.006x - 0.164$, regression).

In situ *MI% of* Symbiodinium *cells after 48 h*

There were no significant differences in MI% in any of the corals between 28 and 30°C or between 30 and 32°C (Fig. 4*b*; $P < 0.05$, ANOVA). *Acropora hyacinthus* was the only coral to exhibit significant increases of MI% between 32 and 34°C ($P < 0.001$).

Specific growth rates of Symbiodinium *cells* in situ

There was a general increase in the S_{GR} of *Symbiodinium* in all corals as the temperature increased. Linear regression analyses of *Symbiodinium* S_{GR} were significant in all corals, indicating that temperature influenced the rate of symbiont growth (Fig. 4*c*; $P < 0.001$).

Discussion

Temporal and cumulative patterns of heat stress

Patterns in the loss of *Symbiodinium* from these corals suggest that the corals are not stressed at temperatures <30°C. Fitt and Warner (1995) obtained similar results in examining effects of heat stress on *Montastrea annularis* and *M. cavernosa*. Warner *et al.* (1996) found the same in *M. annularis*, *Agaricia lamarcki*, *A. agaricites*, and *Siderastrea radians*, provided that the duration of the heat stress is short. However, these authors emphasise that prolonged exposures over 2–3 weeks, for example, may reduce coral health.

In the present study, *Symbiodinium* from *A. hyacinthus* were the most sensitive to slight temperature elevations. These results are consistent with those of others, who have reported that bleaching of *Acropora* spp. results after slight elevations in temperature (Williams and Bunkley-Williams 1990; Marshall and Baird 2000). Hoegh-Guldberg (1994) studied a variety of bleached scleractinian corals from French Polynesia (mean summer temperatures 27–28°C) and showed that low temperatures caused bleaching in numerous species, but that *Acropora* spp. showed the greatest susceptibility (89–100% completely bleached). Marshall and Baird (2000) also recorded similar observations of bleaching sensitivity in *Acropora* spp. on the Great Barrier Reef at 30–31°C. *Acropora palifera*, *A. hyacinthus*, and *A. cytherea* suffered high mortality, with <8% of some populations remaining alive (Marshall and Baird 2000).

All corals exposed to a temperature of 32°C lost more *Symbiodinium* cells, both living and dead, than at 28 or 30°C. The fact that *P. solida* lost the least number of *Symbiodinium* at 32°C agrees with the results of similar studies on *Porites* spp. (Goreau *et al.* 2000; Lough and Barnes 2000). The upper thermal limit for *A. hyacinthus* appears to be <32°C. This is consistent with the results of Coles *et al.* (1976), who reported that the upper thermal tolerance for *A. formosa* and *A. hyacinthus* at Enewetak, Marshall Islands, is approximately 31°C. A similar pattern of sensitivity to a heat stress of 32°C has been reported for other *Acropora* spp. (Wilkinson 1998; Marshall and Baird 2000). Marshall and Baird (2000), who reported bleaching susceptibilities of 4160 coral colonies, observed patterns that support our results: severe bleaching of *Acropora* spp., moderate bleaching of *Favites* spp., and moderate to limited bleaching of *Porites* spp.

It has been suggested that mean sea surface temperatures may increase by 3°C over the next 30–50 years (Hoegh-Guldberg 1999; Goreau *et al.* 2000; McClanahan *et al.*, in

press). This should be of major concern because ocean temperatures of 33–34°C may result in the demise of many coral reefs (Goreau *et al.* 2000). In the present study, most corals stressed at 34°C completely lost their live *Symbiodinium* complement within 24 h. *Acropora hyacinthus* lost most of its live *Symbiodinium* within 9 h and had completely bleached 12–15 h later. Because the other two species tolerated 34°C for longer periods of time, it is clear that the upper thermal tolerance, particularly for *P. solida*, may be ≥34°C. This is in agreement with results from other studies (Goreau *et al.* 2000; Lough and Barnes 2000). Some authors have suggested that *Porites* spp. bleach at temperatures <32°C (Goreau 1992; Marshall and Baird 2000). It is possible that these differences in tolerance may be related to variation in inter- and intraspecific bleaching mechanisms, but this has yet to be investigated. Association with genetically different *Symbiodinium* may also contribute to this variation (Baker and Rowan 1997; Fitt *et al.* 2000); however, all corals in the present study possessed clade C *Symbiodinium* (Strychar 2002), indicating that the observed differences in stress tolerance may be attributed to the coral host.

Differences in mitotic cell loss by *A. hyacinthus* compared with the other corals at 28°C may be due to different *in situ* standing stocks. The standing stock of *A. hyacinthus* (17.1×10^7 cells mL^{-1}) was much higher than that of the other corals and may have contributed to the elevated rates of *Symbiodinium* population turnover there (Falkowski *et al.* 1993; Leletkin 2000). We propose that the high standing stock of symbionts from *A. hyacinthus* enables this host to lose significantly higher numbers of *Symbiodinium* without detrimental effects in the short term under adverse environmental conditions.

Specific expulsion rate

Values of S_{ER} of *Symbiodinium* lost from corals at 28°C in the present study ($0.89–1.7 \times 10^{-4} day^{-1}$) were similar to the values reported by Hoegh-Guldberg *et al.* (1987), who reported values of 0.49×10^{-4} and 1.67×10^{-4} day^{-1} for *Millipora dichotoma* and *S. pistillata*, respectively, at 25°C. Hoegh-Guldberg *et al.* (1987) suggested that an S_{ER} value of 9.6×10^{-4} day^{-1} indicates that less than 1 per 1000 alga of the symbiont standing stock are lost from the host per day. Thus, the number of *Symbiodinium* lost by corals in the present study at 28°C was comparatively low. Linear regression analyses indicated that the S_{ER} of *Symbiodinium* cells was directly proportional to temperature. The S_{ER} of symbiont cells from *P. solida* indicated that, although the S_{ER} of *Symbiodinium* did not increase at lower temperatures (30 and 32°C), the rates of cell loss were affected by heat stress. The lowest S_{ER} observed in any of the corals studied as temperature increased occurred in *P. solida* (88.4×10^{-4} day^{-1} at 34°C). This provides additional evidence that this coral (and/or its symbionts) have mechanisms that help reduce the rate of loss of *Symbiodinium* cells (yet to be described).

The *in situ* MI% data suggest that *Symbiodinium* associated with different hosts may possess mechanisms that help the symbionts to adapt physiologically to temperature anomalies. Otherwise, continuing reproduction may lead to overpopulation within a host and a subsequent greater loss of mitotic cells. This concurs with the results of Fitt *et al.* (2000), who observed higher *Symbiodinium* growth in scleractinian corals as temperature was increased. However, the growth of *Symbiodinium* cells reported by Fitt *et al.* (2000; at temperatures up to 32°C) was *in vitro*. This is opposed to the *in situ* results reported in the present study; thus, the *in vitro* data of Fitt *et al.* (2000) may not be directly comparable with the data reported here.

Increasing temperature appears to elevate S_{GR} in the symbionts investigated in the present study. The S_{GR}s reported by Falkowski *et al.* (1984) for *S. pistillata* from the Gulf of Eilat (0.013–0.0094 day^{-1}) are similar to those reported for *P. solida* (0.006 day^{-1}) and *F. complanata* (0.008 day^{-1}) at 28°C. Hoegh-Guldberg *et al.* (1987) reported S_{GR}s of 0.040–0.082 day^{-1} and 0.028–0.032 day^{-1} for *Seriatopora hystrix* and *Stylophora pistillata*, respectively, also similar to the levels observed in the present study. Wilkerson *et al.* (1983) and Muscatine *et al.* (1985) reported similar values, ranging from 0.013 to 0.094 day^{-1}, for *S. pistillata* in the Red Sea. However, some authors have reported much higher S_{GR} values. Patton and Burris (1983) reported that *S. pistillata* had an S_{GR} of 0.182 day^{-1}, whereas Chang *et al.* (1983) reported that *Symbiodinium microadriaticum* from *Tridacna maxima* had S_{GR} values between 0.22 and 0.32 day^{-1}, 0.24–0.30 day^{-1} for *Aiptasia pulchella* and 0.22–0.43 day^{-1} for *Montipora verrucosa*. The S_{GR} values reported in these last two studies may be considered exceptionally high in light of other published data (Muscatine *et al.* 1985; Hoegh-Guldberg *et al.* 1987). They demonstrate that S_{GR} is highly variable between species, may be related to ambient seawater temperatures (although such data were not provided), or may have been induced by elevated temperatures.

The consistency of low S_{GR}s in each host at low temperatures may indicate that some mechanism is present that limits the rate of symbiont cell growth. Although the mechanism for this is not currently known, *in situ* molecular cell studies indicate that cell development, reproduction, growth, and death are not solely influenced by extrinsic factors, like temperature (Pennell and Lamb 1997); they may be influenced by a multitude of synergistic factors. The phenomenon of increased growth rates in the presence of increasing temperature is a molecular process that has not yet been addressed and requires further study.

In summary, the present study examined the effects of heat stress on three scleractinian corals from three different families. Despite reports of some corals depicting greater resistance to bleaching (e.g. *Favites* spp. and *Porites* spp.; see Byron and O'Neill 1992), it is evident that the time interval including maximal *Symbiodinium* cell loss decreases

as temperature increases. This indicates that higher ambient seawater temperatures equate to lower time thresholds for coral exposure to heat stress. Furthermore, linear regressions of MI% and S_{GR} showed that despite elevated losses of *Symbiodinium* from corals, symbiont reproduction increased. This factor may delay the onset of bleaching for some corals (e.g. *Porities* spp.). Observed differences in heat stress tolerance may also be an attribute associated with the coral host and, perhaps, to a lesser degree, the symbiont because all the corals studied here were associated with clade C *Symbiodinium*. Because the degree of dependence of the coral on *Symbiondium* for food differs between hosts, we propose that the loss of symbiont cells may be slightly advantageous for corals whose feeding rates increase to compensate for loss of nutrients translocated by the symbionts. Several questions arise from this study as follows. If *Symbiodinium* do not contribute significantly to the diet of all scleractinian corals equally, what other roles are these alga playing within their coral hosts? How does bleaching, the associated symbiont, and host feeding affect survival? And, could future bleaching experiments be better represented by using more than one type of coral from each family?

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