Tolerance to thermal stress and dominant symbiont community.

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Introduction

Reef building corals are in a mutualistic symbiosis with single-celled dinoflaggelates of the genus *Symbiodinium.* Several different clades of *Symbiodinium* associate with coral (A. Baker, 2003), with each clade having unique physiological characteristics that enable the coral symbiosis to be successful in a wide range of environmental conditions (Berkelmans & Van Oppen, 2006; Rowan, 2004; van Oppen, Palstra, Piquet, & Miller, 2001). But this successful symbiosis is threatened by climate change. Elevated seawater temperatures can disrupt the symbiosis and cause coral bleaching this has led to mass coral mortality on reefs worldwide (Eakin et al., 2010; Hoegh-Guldberg, 1999).

By changing the symbionts corals might be able to increase their thermal threshold (A. C. Baker, Starger, Mcclanahan, & Glynn, 2004; Buddemeier & Fautin, 1993), or might be able to recover after a bleaching event with an increased tolerance (Donner, 2011). To establish tolerance and relate it to the ability to change symbiont community the dominant clade of symbionts in 9 species of coral prior, and post experimental bleaching was established.

To quantify tolerance, thermal stress was increased weekly and the photosynthetic efficiency of the symbionts in the corals was quantified using Pulse amplitude modulated (PAM) fluorometry (Warner, Fitt, & Schmidt, 1996).

We found that corals prior to bleaching contain an uniform symbiont community, dominated by one Symbiodinium type. After being severely bleached residual symbionts remaining were not the same as those that originally dominated the colony; and the residual symbionts that were left in bleached tissue were not always the same type, both within and between species.

Methods

Temperature treatment

For each species of coral (table 1) ten colonies were placed in a control tank and ten colonies were placed in a treatment tank. The tank setup was a flow through setup with each tank receiving 0.5l of filtered seas water per minute. The tanks were temperature controlled and placed in a greenhouse covered with 50% shade cloth and AT-FILMS Super 4 agricultural foil to block excess sunlight and UV radiation and simulate light conditions at depth.

Coral were allowed to acclimatize for 2 months at 25ºC, after that the temperature in the experimental tank was increased by 0.5ºC every week, until corals visibly started bleaching at 32ºC. Temperature was then reduced and held constant for a month before temperature was increased again (Fig. 1).

Assessment of bleaching
To monitor bleaching visual observations of the coral were complemented with pulse amplitude modulated (PAM) fluorometry (Manzello et al., 2009), as a quantitative measure of bleaching. Photoinactivation, a decrease in the maximum quantum yield of PSII fluorescence (Fv/Fm) is a more quantitative indication of physiological stress. Every week all corals were dark adapted for 30 minutes and the quantum yield of PS II (Fv/Fm) was measured.

Genetic identification of zooxanthellae
The dominant clade of symbiont was determined using standard Denaturing Gradient Gel Electrophoresis (DGGE) analysis of ITS-2 rDNA. This was done prior and post temperature stress.

Main Results
We found that, fairly consistently across the different species that were analyzed, corals of a particular species started off with a uniform symbiont community that was dominated by just one Symbiodinium type, with no other types detectable using standard methods (DGGE analysis of ITS-2 rDNA).

However, when severely bleached, the interesting findings are that: (1) in most cases, residual symbionts remaining in bleached tissues were not the same as those that originally dominated the colony; and (2) the residual symbionts that were left in bleached tissue were not always the same type, both within and between species (Table 1).

The tolerance to thermal stress of the species tested can be categorized in two groups. The more sensitive species were *Acropora cervicornis, Pocillopora damicornis, Porites astreoides* and Stylophora pistillata. One of the most tolerant species, *Siderastrea siderea* was the only coral with type D symbionts prior to the thermal stress.

Table 1, Species of corals and initial and final dominant *Symbiodinium* type.

Species	Initials (sample size)	Finals (sample size)
Caribbean species		
Montastraea cavernosa	C3 (N=8)	Unidentified C-type, but not C3 (N=4) B1 (N=2)
Acropora cervicornis	A3 (N=7)	Unidentified, but not A3 (N=5) Unidentified, but not A3 (N=2)
Montastraea faveolata	B1 (N=8)	C3 (N=7) Unidentified, but not B1 or C3 (N=1)
Montastraea annularis	C3 (N=8)	Nothing detectable (N=6) Trace unidentified, not C3 (N=2)
Siderastrea siderea	C1+D1a (N=8)	D1a (N=5) C1+D1a (N=2) (same as initials)
Porites furcata	Did not amplify (N=8) Faint unidentified B-type (N=1)	C-type (N=7) A3 (N=2)
Meandina meandrites	Unidentified B-type (N=7)	Gel needs to be re-done
Pacific species		
Pocillopora damicornis	C1 (N=6)	Nothing detectable (N=3) Unidentified B-type (N=2) A3 (N=1)
Stylophora pistillata	C1 (N=6)	C1 (N=3) (same as initials) Unidentified (N=2) Unidentified (N=1)

Table 2, Light conditions at the top of the water tanks.

	305 nm	330 nm	380 nm	PAR
No film	1.99	17.8	30.75	970
With film	0.0245	0.19	6.8	360.46

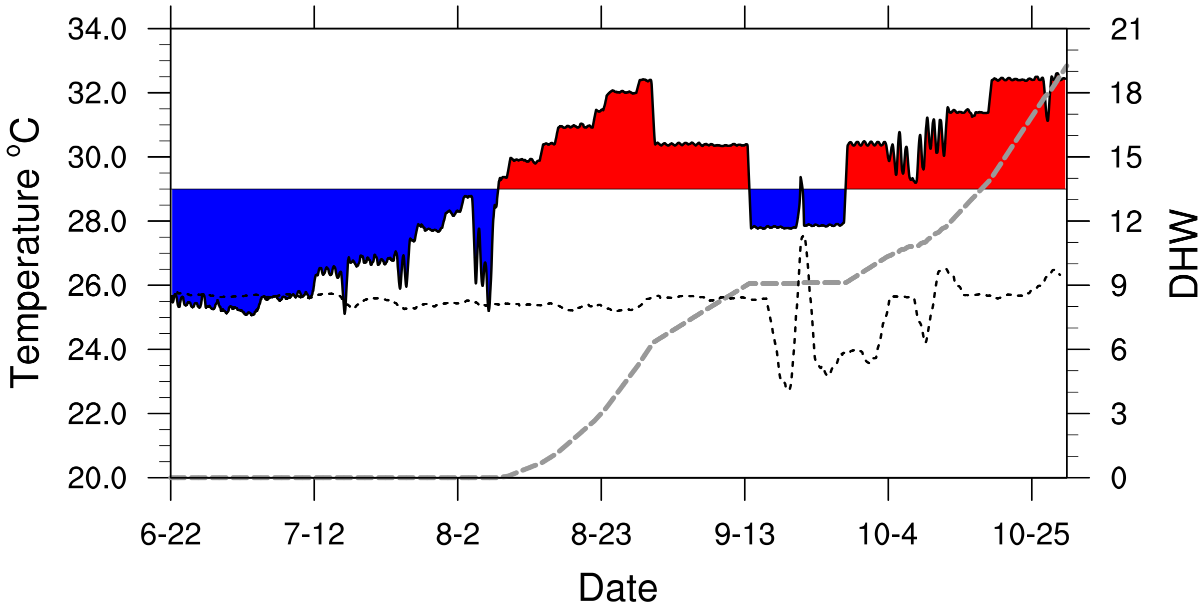

Figure 1, Temperature in treatment tank (solid) and control tank (finely dashed). Degree heating weeks for the treatment tank are plotted with the axis on the right (grey line).

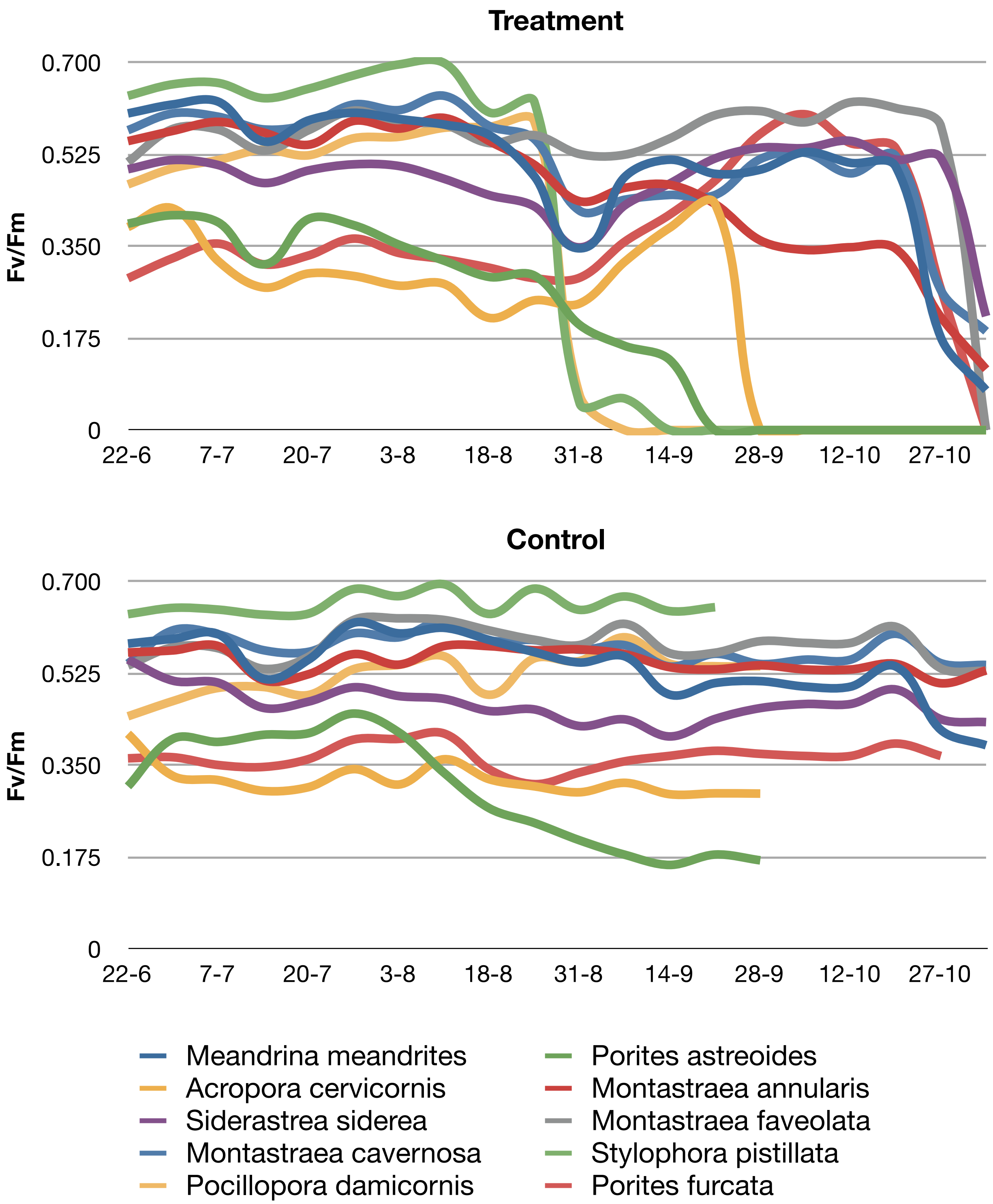

Figure 2, The maximal dark acclimated quantum yield of PS II (Fv/Fm) for corals exposed to temperature stress (treatment) and coral in the control tank.

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