Comparison of the photosynthetic bleaching response of four coral species common to the central GBR

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Abstract. The photosynthetic bleaching response of *Acropora microphthalma*, *Acropora formosa*, *Stylophora pistillata* and *Pocillopora damicornis* were compared under empirical conditions of environmental stress. Coral specimens were collected at Davies Reef located in the central region of the GBR from a depth of 10-15m and were exposed to natural and shaded light under temperature-controlled conditions for 48 h at a depth of 40 cm held within a shipboard, light-exposed tank. After 2 days of stress (high light/low temperature, high light/high temperature, and low light/high temperature), the acroporids were severely bleached whereas the *Stylophora* and *Pocillopora* specimens had retained some pigmentation. Light-adapted PSII Yield values declined to 0.1-0.2 units at midday in all coral species but had increased by partial recovery during the afternoon periods of exposure. From these 2-day experiments, the rate of PSII Yield recovery (*Yr*), calculated as the increment of light-adapted PSII Yield that had recovered from midday to dusk, were compared. Results of *Yr* determinations showed that *A. formosa* is a thermally sensitive species having reduced *Yr* values under both light conditions. *A. microphthalma* and *S. pistillata* were more thermally tolerant but light sensitive with greater *Yr* values under low light than under high light exposure. *Pocillopora* was least sensitive to bleaching and showed attributes of light and thermal tolerance with up to six times greater *Yr* values than the other coral specimens examined under identical conditions. The bleaching response of these corals under different stress conditions will be discussed in context with the photosynthetic response of their symbionts measured *in hospite*.

Key words:PSII recovery rate, *Symbiodinium*, Coral bleaching

Introduction

Coral reefs are a valuable asset; however, human activities that cause changes in global climate are putting this resource at risk(Hoegh-Guldberg 1999; Hughes et al. 2003). Reef-building corals are a symbiosis between photosynthetic dinoflagellates of the genus *Symbiodinium* (Dinophyceae), commonly known as zooxanthellae, and their host scleractinian coral(Muscatine et al. 1975). This symbiosis can be disrupted, often in mass bleaching events, when seawater temperatures are sustained at levels of1-2 degrees centigrade above the average summer maximum (Berkelmans and Willis, 1999), which in combination with light exposure, can trigger the coral bleaching response(Anthony et al. 2007). This failure is thought to be caused by increasing levels of reactive oxygen species (ROS) resulting from malfunction of the PSII reaction center to signal the disruption of symbiosis by inducing exocytosis of the dinoflagellate partner(Iglesias-Prieto et al. 1992; Lesser 1997; Smith et al. 2005; Lesser 2006; Starcevic et al., 2010; Weston et al., 2012).The coral holobiont, however, has evolved certain strategies to withstand such stressful conditions, including the biosynthesis of mycosporine-like amino acids(MAA) compounds for UV protection (Shick and Dunlap, 2002), secondary pigments to dissipate the excess of photosynthetic excitation via the xanthophyll cycle (Brown et al. 1999) and de-excitation by fluorescence (Salih et al. 2000), by up-regulation of antioxidant enzymes (Shick et al., 1995) and the induction of heat shock proteins and other stress-response proteins (Black et al. 1995; Lesser 2006)These strategies, in combination with diverse thermal and photophyscial attributes of coral symbiont genotypes, create different sensitivities of corals to stressful conditions. To examine such differences, we measured the effects of acute light and temperature on the photosynthetic efficiencies of four species of corals common to the central GBR, which are known to be sensitive to bleaching conditions (Baird and Marshall 1998; Marshall and Baird 2000). In this study we compared

the PSII recovery rate (*Yr*), a temporal measure of the photosynthetic performance of corals (4 species/3 genera) during a 48 h period of exposure to different combinations of light and temperature. Also, symbiont cultures were established from these coral specimens for future use.

Material and Methods

Sampling and experimental setup

Four healthy looking fragments from each coral, *Acropora microphthalma*, *Acropora formosa*, *Stylophora pistillata* and *Pocillopora damicornis* were collected (147° 37.778′ E : 18° 49.270′ S) at Davies Reef (AIMS trip 5164, 28 July – 04 August 2011)from a depth of 10-15m. These coral fragments were arranged on plastic-coated metal dish racks and distributed within a sun-exposed 600L tank under 40cm of constantly pumped and thermally adjusted water pumped on-site from the seawater supply of the AIMS ship, RV Cape Ferguson (Fig. 1A and B).The empirical design allowed measuring the photobiological response of these corals on exposure to three different combinations of stress conditions: (enhanced) High Light and Low Temperature (HL-LT) using ambient (26° C) seawater, (enhanced) High Light and (enhanced) High Temperature (HL-HT) by heating the tank seawater (31° C max,) and (ambient) Low-Light with (enhanced) High Temperature (LL-HT) by heating the tank seawater (31° C max.). In full sunlight, corals received up to 2000uE of photosynthetically active radiation (PAR) at noon, with an average PAR exposure of 466µE during the day for those corals exposed to HL conditions. For corals that were exposed to LL, a neutral 70% shade cloth filter placed above the tank gave an average exposure of 157 µE during the day to approximate light exposure at the coral's natural (10-15m) habitat. The seawater temperature was adjusted to 31° C during the day and 28° C at night for HT treatments using an inline temperature-controlled, heater/pump deviceproviding120L/min of water displacement.

To follow the stress-induced photosynthetic performance of the algal symbionts *in hospite* we used a Pulse Amplified Modulated Fluorometer (Diving PAM, Heinz Walz GmbH, Effeltrich, Germany) for measuring photophysiological parameters. Light-adapted PSII quantum yields were recorded three times a day (morning at8am; noon at 12pm; dusk at 5pm) at eight uniformly pigmented locations on branches of each of the coral fragment. From these 2-day experiments, the rate of PSII Yield recovery (*Yr*) for each specimen was calculated as the increment in light adapted PSII-Yield recovered from midday to dusk. Statistically significant differences in *Yr* were calculated using one-way ANOVA, and the post-hoc Tukey test (NCSS software).

Cell culture and genotyping

Symbionts were isolated from coral branches by air blasting followed by several sequential washes in 0.2µm filtered seawater using centrifugation (5 min at 1600g) for symbiont collection between procedures. Clean symbiont preparations were re-suspended in seawater, divided in half and each aliquot collected by centrifugation; one sample was preserved in liquid nitrogen for genotyping and the other sample was inoculated into 24-well plates with 1ml/well of sterile IMK medium (Wako Chemicals, Richmond, VA, USA) containing antibiotics (100µg/ml each of penicillin, neomycin, streptomycin and nystatin) and 50mM of the diatom growth inhibitor GeO_2(Santos et al. 2011). After one month growing in synthetic medium at 26°C, 60µE and a 14:10 light:dark photoperiod, visual inspections were made. Those cultures showing no apparent signs of contamination were expanded to a 25ml culture volume, from which a subsample was collected for additional genotyping. We used SSCP analysis of the PCR amplified ITS1 region(van Oppen et al. 2001) for preliminary symbiont genotyping.

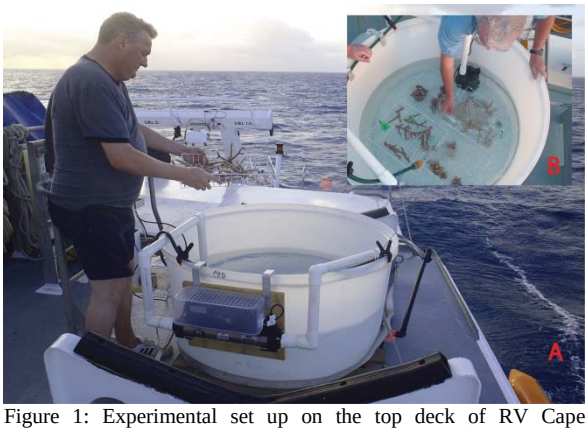

Figure 1: Experimental set up on the top deck of RV Cape Ferguson. A) Side view showing the relative size of the tank and the in-line temperature control unit. B) Top view (insert) of the tank showing the arrangement of coral fragments placed at the bottom.

Results

Photobiology analysis

To determine empirically the photosynthetic performance of coral symbionts *in hospite* during 48h of acute light and thermal stress treatments, we took time-lapse measurements of the light-adapted PSII quantum yield. Our results show that during HL-LT treatment (Fig. 2A) the symbionts from the two acroporids and *S. pistillata* followed a similar diurnal pattern of photosynthetic stress, whereas the symbionts of *P. damicornis* were significantly more resistant to stress under high irradiance on the second

day of exposure. Conversely, light-adapted yields taken during the HL-HT treatment (Fig. 2B) revealed that morning yields taken after 24 h of treatment showed significant accumulation of damage to PSII as compared to that measured at the morning of initial exposure. This diminished performance was further accentuated at midday when all four corals experienced a drastic reduction in PSII yields. In comparison, when corals were subjected to the LL-HT treatment (Fig. 2C) their symbionts all responded similarly at midday of the first day. However, on the second day, while *S. pistillata* and *A. formosa* maintained a similar reduction in their noon time PSII yields as on the first day, the *A. microphthalma* yield was significantly depressed compared to the preceding day. Of the four corals examined, *P. damicornis* appeared least sensitive to stress.

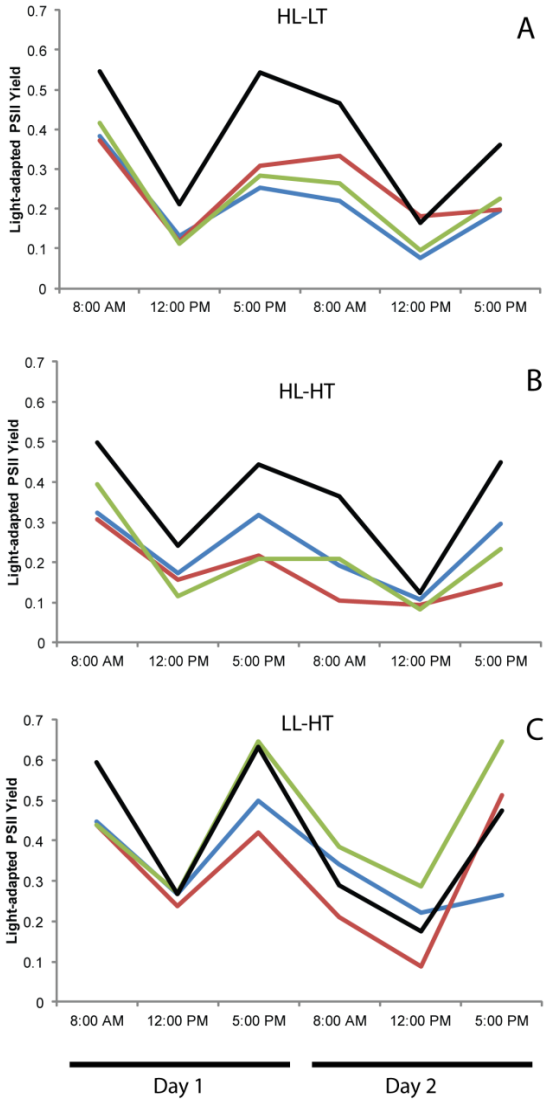

Figure 2: Temporal analysis of the light-adapted PSII yield of four corals species at three different conditions: A) HL-LT, B) HL-HT, C) LL-HT; blue, green, red, and black line codes for *A. formosa*, *S. pistillata*, *A. microphthalma*, and *P. damicornis*, respectively.

PSII recovery rate (*Yr*) *analysis*

Given that diverse coral species respond differently to stress, we wished to compare their recovery from acutely stressful conditions. To accomplish this we calculated the recovery rate from the slope of light-adapted PSII yields averaged from two consecutive days from midday to dusk. This measurement, known as the recovery rate (*Yr*), is an indication of the rate for specimens to recover photosynthetic performance that we test under conditions of identical stress. ANOVA analysis reveals that symbionts of *A. formosa* were significantly more sensitive *in hospite* to thermal stress than symbionts of the other coral specimens (Fig 3, HT-LL and HT-HT treatments). Conversely, the synergistic effect of temperature and light substantially reduced the *Yr* values of *A. microphthalma* compared to that of *P. damicornis* (Fig. 3, HT-HL treatment). Comparing *Yr* values for each specimen showed that *A. microphthalma* and *S. pistillata* recovered quicker from thermal stress under low light conditions (HT-LL) than under the combined effects of high temperature and high light (HT-HT). Our analysis revealed that *P. damicornis* recovered consistently best in all three stress treatments; differences in *Yr* values between treatments for the *P. damicornis* specimen were not significant.

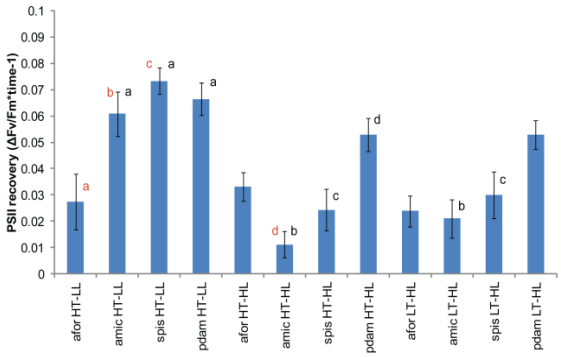

Figure 3.PSII recovery rates (*Yr*) and ANOVA analysis; afor, amic, spis, and pdam are abbreviations for *Acropora formosa*, *Acropora microphthalma*, *Stylophora pistillata*, and *Pocillopora damicornis*, respectively. Significant differences ($F=8.3$, $p<0.0001$) are depicted at the top of each measurement bar with red colored letters against its counterpart in black coloration to denote significant difference.

Determination of symbiont genotypes

To genotype the endosymbionts of our coral specimens we used SSCP analysis of the PCR amplifiedintragenomicITS1 region of rDNA obtained

from fresh field isolates and of symbionts growing in IMK-antibiotics medium. Symbionts freshly isolated from our four coral specimens were identified to belong predominately to clades C and D (Table 1).SSCP analysis of the symbionts of *S. pistillata* showed that they belong to the highly diverse clade C that are known to be prevalent amongst populations of *S. pistillata* (Sampayo et al., 2007), which are similar, but clearly different (Shick et al., 2011), to that of the ITS1 reference sequence for subclade C1 (*A. tenuis*). Furthermore, unlike clade C1 symbionts, the phenotype of *S. pistillata* is highly refractory to culture. Cultures of *Symbiodinium* spp. from three of the four coral specimens were established, however, only symbionts of *P. damicornis* retained the same genotype in culture as that which predominates in its original isolate (Table 1). Isolates from both *A microphthalma* and *S. pistillata* gave clade A symbionts on culture, which proves that *Symbiodinium* populations are not a monophyletic group within these specimens.

Species	Genotype (Fresh isolate)	Genotype (Culture)
afor	C3	-
amic	D1	A
spis	C1-like	A
pdam	C1	C1

Table 1: ITS1 genotypic analysis of symbionts from fresh isolates and cultures obtained from each coral species; afor, amic, spis, and pdam are abbreviations for *Acroporaformosa*, *Acropora microphthalma*, *Stylophora pistillata*, and *Pocillopora damicornis*, respectively.

Discussion

Bleaching dynamics measured by PSII fluorescence

Coral bleaching occurs by a complex process that is dependent upon the physiological traits of both symbiotic partners. The photobionts of corals comprise a diverse assemblage

Of *Symbiodinium* spp. clades and subclades (LaJeunesse 2001; Santos et al. 2002; LaJeunesse et al. 2004), each having distinct traits evolved to accommodate various environmental regimes. Such adaptations allow important niche diversification of coral communities within the photic zone (Iglesias-Prieto et al. 2004).The Scleractinia comprise a wide diversity of coral species, each also having particular adaptations to metabolic stress. Another attribute of symbiosis is that coral photobionts may be acquired (or exchanged) with conspecifics from the environment, or they may be maternally inherited. Accordingly, symbiont populations within a coral may be species specific with only one symbiont type, or the symbiosis may be flexible to accommodate several symbiont clades within a coral species, in which the composition may change with environmental circumstance (Rowan et al. 1997; Berkelmans and van Oppen, 2006; Baker and Romanski 2007).

In this investigation we have compared the photosynthetic response of four common bleaching-sensitive corals (Marshall and Baird 2000) exposed to combinations of stress. Our results show clearly that our coral specimens responded differently to thermal and light-induced stress. For example, *A. formosa* recovered at a significantly reduced rate than that of the other coral specimens when exposed to thermal stress alone (Fig. 3C, LL-HT). This would make *A. formosa* a sensitive candidate to monitor as an early-warning bioindicator of thermal stress in coral reef communities. *A. microphthalma* is thermally more resistant but more sensitive to the synergy of light-enhanced stress (Fig. 3, HL-LT *vs* LL-HT). This specimen was the only one to harbour clade D1symbionts (Table 1), and the sensitivity of this symbiosis to light is a typical trait of corals living in conditions of reduced irradiance(Glynn et al. 2001; Fabricius et al. 2004).Nevertheless, the photosynthetic response of *S. pistillata* was similar to that of *A. microphthalma*, although it harbours a clade C symbiont, yet this coral is not known to be light sensitive given that this species inhabits the clear shallow waters of the GBR. Such tolerance to high light environments may be a product of regional adaptation as observed across latitudinal gradients of *Symbiodinium* diversity (Macdonald et al 2008; Howells et al. 2012) and may contribute to the bathymetric distribution of this coral along an environmental light gradient.

Pocillopora damicornis, which harbours clade C1 symbionts, recovered quicker in all treatments than *A. Formosa* harboring clade C3 symbionts (Fig. 3). In this comparison, the host scleractinians have evolved specific morphological, cellular and metabolic traits that may moderate the response of their endosymbionts to environmental stress (Enriquez et al. 2005; Baird et al. 2009). Although it is tempting to speculate from our *Yr* data upon the relative sensitivities of these photobiont genotypes *per se* to conditions of stress, this would be imprudent given that "the host does matter" in affecting the photosynthetic performance of its endosymbionts *in hospite*. (Baird et al. 2009; Fitt et al. 2009). Nonetheless, *Yr* has proved to be adequately sensitive to discriminate between differences in the recovery response of bleaching-sensitive corals.

Symbiont clade identification

Our results for the assignment of symbiont clades for *A. microphthalma* and *S. pistillata* specimens (Table 1; clade D1 and C1-like, respectively)are consistent with those published previously for corals of the GBR and Pacific region (Starcevic et al. 2010; Wicks et al. 2010; Shick et al., 2011). The SSCP patterns of migration of the ITS1 region for cultured symbionts obtained from these specimens, however, do not match the freshly isolated genotype (Table 1).This suggests that clade A symbionts exist in the native population of these corals at low density, which by natural selection became predominate on culturing. Symbionts isolated from *A. formosa* were identified to be predominately clade C3*Symbiodinium* sp., which agrees with the assignment for this coral to harbour clades C3 and A *Symbiodinium* spp., in *A. formosa* collected from distant regions (Darius et al. 2000; LaJeunesse et al. 2003). Repeated attempts to culture this clade in various media by inoculation with symbionts freshly isolated from our specimen of *A. formosa* had failed. Our ITS1 assignment of clade C1 *Symbiodinium* sp. from *P. damicornis* (Table 1) is consistent with that reported previously for this coral from the GBR (LaJeunesse et al. 2003), and it retained clade C1 assignment as the predominant cultured genotype. Given the relative tolerance of *P. damicornis* in our treatments, evaluation of other coral species by infection with this symbiont phylotype may augment our understanding of the symbiotic processes that mediate the stress tolerance of corals to bleaching.

Utilization of the data

Additional samples were collected at T=0, T=24h and T=48h during each treatment for qualitative and quantitative high-throughput proteomic analyses of the intact coral holobiome, as published for the endosymbiont-enriched fraction of *Stylophora pistillata* (Weston et al. 2012). It is anticipated that the photophysical data presented in this manuscript will provide a vital backdrop to discussing the effects of heat and light on coral proteomes under exact conditions of bleaching stress.

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