Bleaching condition varies among *Acropora millepora* color morphs

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Abstract. Understanding the mechanisms that underpinvariation in bleaching susceptibility in corals is central tothe conservation of reefs in the face of climate change.Fluorescent proteins (FPs) are pigments associated with the coral animal that are expressed by many coral species and govern color polymorphisms in the wild. FPs can play photoprotective and anti-oxidant roles in corals;however, it is unclear whether the abundance of FPs affects bleaching susceptibility in the wild.Here, we analyse the bleaching condition of three color morphs with different FP levels in *Acropora millepora* from the northern Great Barrier Reef (GBR). Colonies were surveyed at four sites during a cold winter and a warm summer. In winter, following unusually cold temperatures, proportionally fewer green (high FP) morphs paled (48%) than yellow(intermediate FP) and red (low FP) color morphs (66 and 60%, respectively). In the warm summer, proportionally fewer green colonies bleached (20%) than the other two color morphs (34 and 33% for yellow and red, respectively). This is the first demonstration of differential susceptibility to natural temperature stress among fluorescent color morphs from similar habitats and highlights the functional importance of subtle intraspecific differences in FP content.

Key words: Coral, Bleaching, Color Morph, Fluorescent Proteins.

Introduction

Coral reefs are under threat from climate change that is causing chronic and acute stress to coral photosymbiosis and health, primarily through bleaching (Hoegh-Guldberg 1999). Coral bleaching, i.e. the loss or expulsion of endosymbiotic zooxanthellae (Donner et al. 2005), occurs most often in response to increased sea water temperature (Berkelmans 2009). Bleaching susceptibility is not equal among and within coral species (Buddemeier and Fautin 1993, Marshall and Baird 2000, Jones et al. 2004). Within populations, bleaching susceptibility often correlates with the algal symbiont type hosted by corals (Rowan 2004, Ulstrup et al. 2006, Abrego et al. 2008,), and the ability to swap algal partners for more tolerant strains can be important for coping with acute temperature stress (e.g., Baker 2003, Berkelmans and van Oppen 2006, Jones et al. 2008). However, differences in bleaching susceptibility still occur among populations without variation in symbiont strain, highlighting the importance of coral-host mediated factors that can influence colony level susceptibility to thermal stress (Baird et al. 2009, Barshis et al. 2010).

Fluorescent proteins (FPs) are found in many reef-building corals (Matz et al. 1999) and are an abundant component of the protein complement in coral tissues (Oswald et al. 2007). FPs contribute to the visual color appearance of coral (Kawaguti 1944, Matz et al. 2002, Dove 2004) and underpin intraspecific color polymorphisms (Mazel and Fuchs 2003, Alieva et al. 2008, Paley et al. in review), which are common in many reef building coral species (Takabayashi and Hoegh-Guldberg 1995, Veron 2000). The functional role for FPs in coral tissues is not yet clear (see Leutenegger et al. 2007, Palmer et al. 2009 for review). A prominent theory suggests FPs act as an ultraviolet-A (UVA)-screening shield to the algal-symbionts, whereby FPs convert damaging excess UVA energy into longer, non-photosythentically active wavelengths (Salih et al. 2000). Conversely, FPs may also have anti-oxidant properties (Bou-Abdallah 2006) and can help the host cope with radical oxygen species associated with bleaching stress (Lesser 2006). Despite their potential important role in coral physiology and stress tolerance, it is poorly understood how FPs affect bleaching susceptibility in corals (but see Salih et al. 2000, Dove 2004).

Here we compare the bleaching condition of three *Acropora millepora* color morphs with different absolute FP levels among four sites in the Lizard Island Group on the northern Great Barrier Reef (GBR). Bleaching condition was compared among

color morphs during a colder and warmer than average winter and summer.

Material and Methods
Study Species and Sites
A. millepora is found on reefs throughout the Indo-Pacific and is common on inshore and mid-shelf reefs on the GBR. At least four color morphs have been described in this species (Veron 2000, Cox et al. 2007, Alieva et al. 2008) and they commonly co-occur in shallow reef habitats. The visual color appearance is explained by the concentration of three FPs (cyan, green and red, Paley et al. in review). Green, yellow and red morphs contain the highest, intermediate, and lowest concentration of all three FPs corresponding to a 200% and 70% greater total FP concentration in green morphs compared with red and yellow morphs, respectively (Paley et al. in review).

Coral colony color and bleaching surveys were conducted on fourfringing reef flat sites (~3m depth) on the leeward side ofLizard, Palfrey and South Islands in the Lizard Island Group (14°40'S 145°28'E) (Fig 1). Here,three fluorescent color morphsoccupy the same habitats (Paley et al. in review), thereby allowing us to study bleaching responses independent of habitat effects. At Lizard Island, *A. millepora* are dominated bysymbiont *Symbiodinium* ITS-1 rDNA type C2, but up to 30%of corals also associate with type C2* in this region (Cooper et al. 2011). It is not yet clear whether C2 and C2* *Symbiodinium* have different levels of heat tolerance (Berkelmans and van Oppen 2006). We did not genotype the symbionts in the corals surveyed here and therefore assume that the distribution of dominant and background algal types was independent of coral color as was the case in *A. millepora* at Orpheus Island (Cooper et al. 2011).

Temperature Data
Water temperature data for the Lizard Island region was acquired from the Australian Institute of Marine Science (http://data.aims.gov.au/aimsrtds) from data loggers deployed at 2.7m at Lizard Island. Daily maximum temperatures were averaged over a period of a year, from April to March, for all available data (~7 years) spanning 2002-2010 and compared with daily maximum temperatures during sampling years (2008-2009).

Color morph surveys
To examine their bleaching condition we surveyed 434 and 494 colonies of*A. millepora* in winter and summer, respectively, among 3 replicate 50m x 10m haphazard belt transects at each site. Colony color and bleaching condition was recorded for all *A. millepora* colonies lying within transect dimensions. Red, yellow, and green morphs were recognized based on

the color categories described in Paley et al. (in review). Colony bleaching condition was determined using the CoralWatch Coral Health Chart (University of Queensland). We used a scale of 1-6 where 1= completely bleached and 6 = healthy/darkly pigmented using the chart's color fields as a reference. For analysis, number categories were pooled into three bleaching categories (1 - 2 = Bleached, 3 - 4 = Pale and, 5 - 6 = Healthy (darkly pigmented)).

Figure 1: Color morph survey locations in the Lizard Island Group, northern Great Barrier Reef.

Statistical Analysis
To test whether bleaching condition differed between color morphs and seasons we used a 3-way ANOVA implemented in STATISTICA V10. We observed large differences in color morph abundances, so we first tested whether morph distribution and abundance was equal among sites using a 2-way ANOVA. To identify variation, among color morphs independent of their relative abundance we undertook our 3-way ANOVA analysis on proportional data and transects were pooled among sites as the replicate unit. A Kolmogorov-Smirnov test for normality ($p < 0.1$) and a Cochran's test ($p = 0.7$) for equality of variances confirmed conformation to ANOVA assumptions when data were squareroot transformed. Planned, post-hoc multiple comparisons of bleaching condition among color morphs and sampling times were interpreted with a Benjamini and Hochberg (1995) type I error correction at the false discovery rate of 0.0245.

Results

The abundance of the three color morphs was unequal ($F_{2,60}$ = 7.5, p = 0.001) but the distribution of color morphs was consistent among sites ($F_{6,60}$ = 0.1, p = 0.999) where red colonies were always in highest abundance and green in lowest abundance. The mean abundance among sites of red, yellow and green morphs was 130.3 (± 27), 64.3 (± 14) and 6.3 (± 3.2), respectively. Of total colonies surveyed 521 were red, 257 yellow and 48 green.

Temperature regimes preceding winter and summer surveys exceeded the 7-year long-term average in this region (Fig. 2A). During 2008/2009 daily maximum temperatures were below 23°C for 11% of non-consecutive days compared with the long-term average where temperatures never reached below 23°C (Fig 2B). Similarly, daily maximum temperatures were above 32°C for ~1.4% of non-consecutive days compared with the long-term average where temperatures never exceeded this temperature (Fig. 2C).
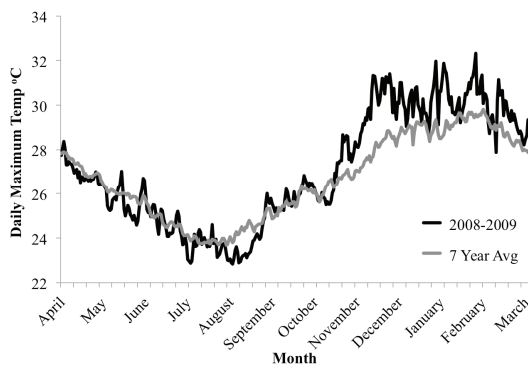

Figure 2: Daily maximum temperatures at Lizard Island from April to March. Temperature trends calculated from daily maximum values from 2002-2010.

The level of bleaching was ~40% greater in summer than winter and was dependent on colony color (3-way interaction, p = 0.033; Table 1). Green colonies were more likely to pale than bleach during seasonal temperature changes when compared with red and/or yellow colonies, which more commonly bleached in summer.In winter, when temperatures were below average, between 34 and 54% of colonies were pale and only 0.5% of colonies were bleached (Fig. 3 B-C). The proportion of pale red and yellow colonies was similar in winter (Fig 3B), but there was a trend of fewer pale green colonies compared to yellow (p = 0.048) but not red colonies (p = 0.236). In summer, between 22 and 50% of colonies bleached resulting in a further decline in healthy colonies of all colors from winter to summer (Fig. 3A). The proportion of pale red and yellow colonies was similar between winter and summer, but more green colonies became pale in summer compared to red (p = 0.024) but not yellow colonies (p = 0.14; Fig. 3B-C). A similar proportion of red and yellow colonies bleached in summer, however between 13.2 and 14.3% fewer green colonies bleached at this time compared with red and yellow colonies, respectively (p = 0.01 and p = 0.007, respectively; Fig. 3C).

Source of Variation	SS	df	F	p
Season	0.00542	1	0.6	0.457
Color	0.01362	2	0.7	0.498
Condition	3.77822	2	195.8	0.000*
Season*Colour	0.00399	2	0.2	0.814
Season*Condition	4.13851	2	214.5	0.000*
Colour*Condition	0.07054	4	1.8	0.137
Season*Colour*Condition	0.10988	4	2.8	0.033*
Error	0.52087	54	0.1	

Table 1: Three-way factorial ANOVA of bleaching condition between seasons and among colony colors of *A. millepora* at Lizard Island. *Denotes significance at α= 0.05.

Discussion

Our results confirm that colony color can influence the bleaching condition of corals (Gleason 1993, Salih et al. 2000, Dove 2004, Dove et al. 2006). Depth and light environment can affect FP abundance, and as a consequence coral color is typically brighter (highly fluorescent) in shallow, high-light habitats and more drab (non-fluorescent) in deeper, low-light habitats (Gleason 1993, Takabayashi and Hoegh-Guldberg 1995). In our study, colors with high FP levels bleached less in shallow water than corals with low to intermediate FP levels at the same depth. Our results demonstrate for the first time that bleaching condition differs amongst fluorescent color morphs within the same habitat. This finding highlights the value of considering fluorescent color variation in future investigations into host-mediated stress tolerance and adaptive responses to climate change.

Local temperature regimes and bleaching condition
Corals live close to their thermal thresholds and commonly bleach when temperatures exceed long-term averages by only a few degrees (Jokiel and Coles 1990, Berkelmans 2002). Temperatures deviated substantially from those recorded between 2002 and 2010 in both our summer and winter sample and we recorded moderate levels of coral paling and bleaching in *A. millepora* (Fig. 3) and other branching pocilloporid and acroporid species (personal observations). These genera and growth forms arewidely recognised for their bleaching sensitivity (e.g.,
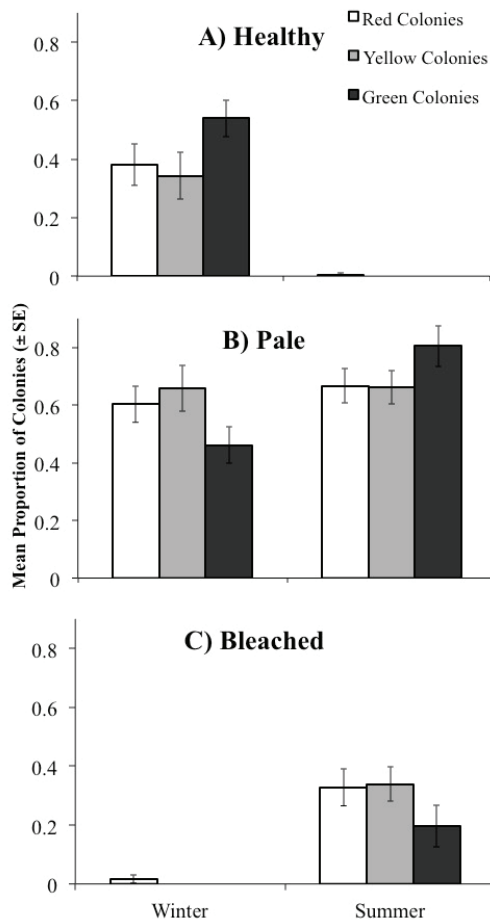Marshalland Baird 2000). The timing of bleaching was consistent with a temperature-driven response

Figure 3: Mean proportion (±SE) of healthy, pale and bleached *A. millepora* colonies, by color, in August 2008 (winter) and March 2009(summer) in the Lizard Island Group.

increased their susceptibility to bleaching during the warmer than average summer.

Different bleaching susceptibility among color morphs

Red and yellow colonies displayed similar bleaching condition in summer and winter despite having low and intermediate FP levels (Paley et al. in review). In contrast, green morphs, with high FP levels had a lower proportion of bleached colonies in summer and lower incidence of paling in winter. This demonstrates different bleaching susceptibilities among fluorescent coral color morphs that occupy similar habitats in the field. Our results therefore extend the laboratory findings of Salih et al. (2000) that coral morphs with high FP levels have higher bleaching tolerance compared to weakly or non-fluorescent morphs. This contrasts with experimental evidence that high FP levels confer a hypersensitivity to thermal stress (Dove 2004). Our field surveys suggest that green colonies are less likely to bleach during thermal stress possibly due to enhanced photoprotection (Salih et al. 2000) or antioxidant properties (Bou-Abdallah 2000) resulting from a greater abundance of FPs.The symbiont type and/or background symbiont levels were not quantified here (nor in Salih et al. 2000, Dove 2004) and remain potentially important sources of variation in bleaching condition (e.g., Berkelmans and van Oppen 2006). At present no information is available on associations between symbiont type and host color and FP content, however, this must be considered in future studies.

Testing the importance of FP content in response to climate change

Our results suggest that the lower bleaching susceptibility of green colonies was a result of their higher FP concentration. Understanding of the costs and benefits of FPs in coral-host tissues will be strengthened by long term studies of temporal FP variation in tagged colonies combined with the response of coral color morphs under experimentally controlled heat stress. The ability to express FPs in high levels is likely to be important in determining the response of corals to environmental stress. An improved understanding of coral-host mediated processes in bleaching tolerance will enhance our knowledge of potential mechanisms of adaptation to climate change.

although other environmental parameters presumably varied between sampling times due to the field setting of our study.

Coral bleaching is a general stress response and can occur in response to colder, as well as warmer, than normal conditions (Coles and Jokiel 1997, Hoegh-Guldberg et al. 2005). For example, Saxby et al. (2003) observed similar physiological symptoms in cold and heat stressed coral including reduction of algal cell densities and loss of efficiency at photosystem II. Temperatures in August were lower than the long term averageby 0.8°C, therefore, cold stress may explain why a substantial proportion of colonies (46 to 66%) were pale during the winter survey period. Then, from November to February temperatures routinely exceeded the long term average by 2-3°C. Thermal historycan be an important predictorof the health of corals, including the temperature regimes during winter months (e.g. Heron et al. 2010,Bruno et al. 2007). A colder-than-average winter may have pre-stressed corals and

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