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Photoprotection from photoinhibition of symbiontic algae in corals by fluorescent pigments.

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Introduction

Hermatypic corals are sessile invertebrates, restricted to shallow-water tropical and subtropical environments, which receive high solar irradiances. Their distribution is linked to their dependence on the photosynthetic products derived from their dinoflagellate symbionts. However, under excessive sunlight these defences can be overwhelmed causing photoihibition and photodamage, leading to coral bleaching (eg. Coles & Jokiel 1978; Warner *et al.* 1996; Brown 1997; Jones *et al.* 1998). Many coral polyps are also fluorescently pigmented (e.g. Schlichter *et. al*. 1994; Mazel, 1995; Salih *et al.* 2000). Emission and absorption spectra of many of these pigments have been measured; some more than 50 years ago (Kawaguti 1944) but their function was largely unknown. Biochemical and molecular studies by Matz *et al.* (1999) recently revealed that anthozoan fluorescent pigments (FPs) show partial homology (typically > 30%) with GFP, the green fluorescent protein of *Aequoria victoria*, well known for its biotechnological applications. It is now evident, that anthozoan colours, including those of corals and anemones, are due to a large family of GFP-like fluorescent and non fluorescent pigment proteins, whose suncreening function is only beginning to be understood (Salih *et al.* 1998a, 2000; Matz *et al.* 1999; Wiedenmann *et al.* 1999; Lykaynov *et al.* 2000; Dove *et al.* 2001; Cox & Salih, submitted). Our research focuses on elucidating the photoprotective function of FPs in shallow water corals. We showed that FPs can act as sunscreens by dissipating excess energy via fluorescence wavelengths coupling and transformation, fluorescence at wavelengths of low photosynthetic activity and by scattering excess light (Salih *et al.* 1998, 2000; Cox & Salih, submitted). The finding that Great Barrier Reef corals commonly occur as differently pigmented fluorescent morphs (Salih *et al.* 1998a, Salih, 2000), presented an opportunity to compare photosynthetic responses of morphs with different concentrations of FPs to high sunlight and to further test the hypothesis that coral FPs in shallow water can reduce damaging effects of excessive irradiances.

Materials and methods

We compared the responses to light of dinoflagellates from three morphs: green highly fluorescent, F; brown medium fluorescent, M; and beige non-fluorescent, N, in a polymorphic *Acropora palifera.* All corals were collected from One Tree Island and Heron Island lagoons (0.5-1m depths), Great Barrier Reef, Australia. We investigated the reduction of Fv/Fm on exposure to high light in these morphs in the following experiments: (1) *in situ*, in F and N colonies (n=3 per morph) growing side by side,

first at 7:00 am and then at 15:00 pm; (2) at regular intervals throughout the day in replicate sub-colonies prepared from 3 *A. palifera* morphs at 06:00, 09:00; 12:00, 14:00, 18:00 hours in light-exposed upper and shaded lower branches (some of results previously presented in Salih *et al.* 2000); (3) on exposure to light and elevated temperature (32 ± 0.8^{o}C) in dinoflagellates in replicate sub-colonies of F and N morphs compared to controls at ambient temperature (27 ± 0.6^{o}C), for 6 h under full sunlight; (4) and after pre-treatment of F and N morphs with dithiothreitol (DTT), a xanthophyll cycle inhibitor. The latter DTT treatment allowed the evaluation of the photoprotective role of FPs without the confounding effect of dinoflagellates' own photoprotective defences. Maximal quantum yield of fluorescence (Fv/Fm) of dinoflagellates in dark-adapted corals was investigated as described previously (Jones *et al.* 1998; Salih et al. 1998b) using the DIVING-PAM. Dinoflagellate densities, normalised to coral surface area, were determined in frozen samples after extraction as described in Salih et al. (1998b). The effect of light and temperature on the morphology of dinoflagellate chloroplasts was examined by serial optical sectioning with confocal laser scanning microscopy and by subsequent 3-D image reconstruction as described in Salih et al. (1998b). All analyses used the significance level of 0.05 and statistical software JMP 3.2.2, Version 3 (SAS Institute Inc, Cary, NS, USA).

Results

In the experiment analysing intra-specific differences in algal photosynthetic efficiency *in situ* in *A. palifera*, by 15:00 h the Fv/Fm ratio of dinoflagellates in sun-exposed branches of both F and N morphs was decreased compared to values taken at 09:00 h and from shaded branches ($p < 0.0001$). Moreover, there was a pronounced difference in Fv/Fm of F and N morphs: reduction by 27.3% in N and only 12.2% in F morphs ($p = 0.012$; Fig.1 A). Laboratory measurements taken throughout the day, revealed a decreasing Fv/Fm of dinoflagellates in all three morphs (N, M, F) with increasing daylight irradiances (Fig. 1 B-C) as was shown previously (Salih et al. 2000). However, F colonies were the least susceptible to high irradiances and there were significant differences in the responses to light between F and N morphs ($p < 0.0001$), but not between F and M or M and N morphs. During the peak irradiances at 12:00-14:00 h, Fv/Fm was significantly reduced in N compared to M morphs ($p = 0.0137$) and N compared to F morphs ($p < 0.0001$). Moreover, the recovery of Fv/Fm levels was more rapid in F morphs; by 18:00 h Fv/Fm of F morphs was significantly higher that M ($p = 0.0334$) or N morphs ($p = 0.0146$) (Fig. 1C). Pre-treatment of corals with the xanthophyll cycle inhibitor DTT caused a massive reduction of Fv/Fm. However, there was a significant difference in Fv/Fm of dinoflagellates of F and N morphs, indicating that even when the photoprotective xanthophyll cycle was inhibited by DTT, symbionts of F morphs were less severely photoinhibited by light than those in N morphs. Mean Fv/Fm of F & N morphs decreased by 72.5% due to 33^{o}C under high light ($p< 0.0001$, compared to 28^{o}C) but dinoflagellates of F morphs were slightly less impacted than in N morphs by high sunlight ($p = 0.048$) and significantly less impacted by the interactive effects of sunlight and 33^{o}C temperature ($p = 0.006$) (Fig. 1D).

Dinoflagellate density in tissues of the 3 morphs were significantly different ($p < 0.0001$) and were highest in F corals. The main differences were between F and N morphs; M morphs contained intermediate algal densities.

The chloroplast morphology of dinoflagellates from F and N corals was similar, all cells with an outer envelope of compact chloroplast lobes. After exposure to 33^{o}C under high light, algal chloroplasts showed a series of degradative changes: thinning

of lobes, vacuolation, holes and plastoglobuli, as described previously (Salih et al. 1998b). These changes, however, were significantly more pronounced in chloroplast of dinoflagellates from N than F corals.

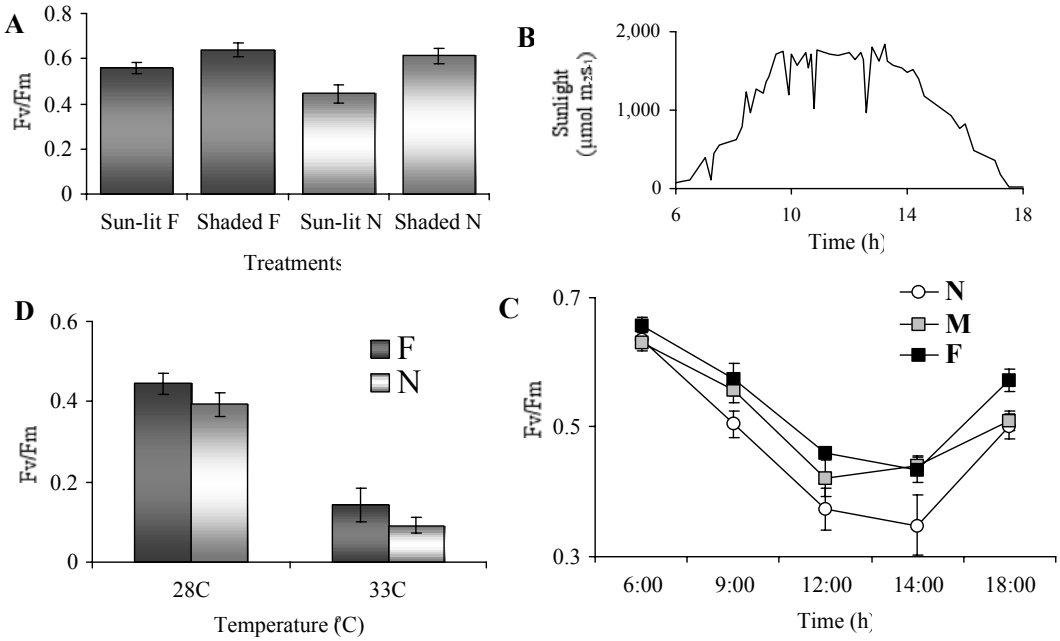

Fig. 1. Dark-adapted maximal quantum yield (Fv/Fm) of dinoflagellates in *Acropora palifera* morphs: highly fluorescent (F), medium (M) and non-fluorescent (N). **A.** In sun-lit and shaded branches of F and N morphs in the lagoon **B.** Irradiances measured throughout the day. **C**. Fv/Fm for F, M and N morphs exposed to sunlight in replicate tanks at different times of the day. **D**. Fv/Fm of F and N morphs exposed to 28°C and 33°C (error bars are means ± SD of replicate colonies, n=3, per morph).

Discussion

Chronic light exposure of corals in the field and in laboratory showed the superiority of F morphs compared to N morphs in withstanding high irradiances. Since dinoflagellates themselves have several photoprotective mechanisms (Brown et al. 2000), pre-treatment of corals with DTT, indicated that despite the inactivation of algal photoprotective xanthophyll cycle, photosynthetic quantum yield of F corals remained significantly higher than in N corals. This was, therefore, at least in part, due to the photoprotection by FPs. An experiment comparing photoinhibiting effects of elevated temperature similarly revealed intra-specific differences: F corals were found less susceptible, with a striking difference of 37% between F and N corals. Consequently, photoprotection by FPs is an important factor even at temperature extremes, a result of great significance in the light of present-day increases in ocean temperatures. Moreover, we found higher dinoflagellate densities in F morphs. One possible explanation of this difference may be linked to the reduced likelihood of photo-oxidative damage and consequent bleaching (Lesser 1997) in corals possessing effective fluorescent sunscreens. Such corals, therefore, are able to host higher symbiont populations. Our previous ecological studies of the abundance and the distribution of FPs in GBR corals revealed that most corals contain different concentrations of FPs and occur as differently pigmented morphs, often growing side by side, with either high or medium or low fluorescent pigmentation (Salih *et al.*

1998a, 2000). Since mass coral bleaching generally occurs under combined conditions of elevated temperature and high irradiance (eg. Brown, 1997), our discovery of the sun-screening properties of FPs, also indicate that FPs can reduce the susceptibility to bleaching of F compared to N coral species and morphs (Salih *et al.* 2000). The present results further confirm these findings. Thus, inter- and intra-specific variability in the presence of FPs in different corals, may provide clues into the observed inter- and intra-specific variability in bleaching (eg. Warner *et al.* 1996). This variability in susceptibility to stress would also explain the occurrence of both morphs on reefs. Following high stress bleaching events, fluorescent corals would outnumber non fluorescent ones, but during stable conditions, non fluorescent morphs will become more common.

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