membership in species groups to assign speciation events to islands. Previous researchers assigned all Caribbean species to species groups (series); recent phylogenetic work[9] confirms that the species groups are almost invariably monophyletic. Therefore, if all species within a species group occur on a single island, then all speciation events within that species group were assumed to have occurred on that island, even those involving species not included in the phylogeny. In a few cases all members of a species group occur on a single island, with the exception of obviously recent dispersers to other islands (for example, all 15 species of the *sagrei* series occur on Cuba, with the exception of some populations of *A. sagrei* that occur on other islands); in these cases, we considered all species on the primary island to have arisen by within-island speciation. The phylogenetic affinities of two rare Hispaniolan species are unknown and these species were not included in our analysis.

On all landbridge islands near the Greater Antilles and the Bahamas, co-occurring species are always more closely related to species on other islands and thus do not provide evidence for within-island speciation. The one equivocal case involves two members of the *equestris* series that occur on Santa Maria, off the northern coast of Cuba. Whether these species are sister taxa remains to be determined. Again with one exception, all species on small nonlandbridge (oceanic) islands also belong to different species groups. The one exception is the two species on the Lesser Antillean island of St Vincent, whose sister taxa status is controversial[28]. In addition, sympatric species on islands in the northern Lesser Antilles belong to the same species group (the *bimaculatus* series), but each island is occupied by one species from each of the two distinct subclades within the series[29]. Because the *bimaculatus* series is monophyletic, it is conceivable that these two subclades initially arose by within-island speciation on one island, but we consider the alternative of allopatric differentiation on different islands to be more plausible. In summary, within-island speciation occurs very rarely or not at all on small islands. We do not consider any of the three possible exceptions as representing strong cases for within-island speciation. However, even if we had included them in our analyses, they would not have altered our conclusions.

Estimation of rate of speciation and extinction

We used computer simulation to test our prediction that number of recorded speciation events on large islands should correlate with area. To carry out the simulation, we used parsimony to infer inter-island immigration events and to determine the relative date on which new lineages immigrated to islands. In the most parsimonious reconstruction, Hispaniola was the ancestral locality for much of the anole radiation from which lineages on Cuba were derived independently several times. To examine the robustness of our analyses, we included the slightly less parsimonious (14 versus 15 steps) alternative possibility that Cuba was ancestral and that Hispaniola had been occupied independently by seven different lineages[30]. Each simulation run began at the time, as indicated by the phylogeny, that the first species to a given island was recorded. In each time interval, all species present on an island had a probability, r, of speciating, thus increasing the number of species on the island by one. Additional species were added to the island at the times at which new lineages appeared (presumably by immigration or possibly by vicariance as island blocks collided[21,22]), again as indicated by the phylogeny. In this way, the effect of the addition of new lineages to an island was incorporated into speciation rate estimates. Five hundred simulation runs were conducted and the mean number of species produced was calculated. Simulation trials were conducted iteratively, changing the value of r, until the mean number of species produced converged on the actual number of species that occurs on that island.

We used a modification of the likelihood method of ref. 12 to fit the *Anolis* phylogeny to a birth and death process. Our likelihood for birth and death parameters was the product of two parts. The first part is equation (17) of ref. 12 and is the probability density of the 32 observed waiting times between successive branching events of the phylogeny from the first branching event near the root to the 33rd branching event near the half-way point. The second part of the likelihood is based on equation (11) of ref. 12 and is the product of the probabilities that each lineage i living after the 33rd branching event has exactly k_i species at the present time, where k_i is its observed number of descendants, $k_i > 0$, and $i = 1, 2, \ldots, 34$. For each lineage i this probability is $(1 - \eta_t)\eta_t^{k_i - 1}$ where t is the time between the 33rd branching event and the present time, $\eta_t = (\exp(rt) - 1)/(\exp(rt) - a)$, $r =$ (speciation − extinction) and $a =$ extinction/speciation.

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Fluorescent pigments in corals are photoprotective

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All reef-forming corals depend on the photosynthesis performed by their algal symbiont, and such corals are therefore restricted to the photic zone. The intensity of light in this zone declines over several orders of magnitude—from high and damaging levels at the surface to extreme shade conditions at the lower limit[1]. The ability of corals to tolerate this range implies effective mechanisms for light acclimation and adaptation[2]. Here we show that the fluorescent pigments[3–9] (FPs) of corals provide a photobiological system for regulating the light environment of coral host tissue. Previous studies have suggested that under low light, FPs may enhance light availability[4,5]. We now report that in excessive sunlight FPs are photoprotective; they achieve this by dissipating excess energy at wavelengths of low photosynthetic activity, as well as by reflecting of visible and infrared light by FP-containing

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chromatophores. We also show that FPs enhance the resistance to mass bleaching of corals during periods of heat stress, which has implications for the effect of environmental stress on the diversity of reef-building corals, such as enhanced survival of a broad range of corals allowing maintenance of habitat diversity.

The bright colours of corals (Scleractinia) and other Anthozoa are due to pigments of animal-host origin[3], many of which are intensely fluorescent under ultraviolet-A (UVA) and blue light, with emission maxima at 420–620 nm(refs 4–9; Fig. 1). In many corals, distinct morphs are found that differ greatly in their concentration of FPs. Fluorescent proteins are part of a group of coral pigments for which the generic term "pocilloporins" has been proposed[9,10]: this group includes both brightly coloured, low-fluorescence forms and the highly fluorescent forms[9] described here. Both types of pigments are partially homologous to green fluorescent protein (GFP)[8,9], first found in the luminescent jellyfish *Aequorea*[11] and widely used in cell biology. Although the function of GFP in luminescent systems is known, the role of similar FPs in non-luminescent anthozoans has hitherto been unclear[6,10,12].

We surveyed the distribution of fluorescent corals on the Great Barrier Reef (GBR; see Methods) and found that 124 species of 56 genera in 16 sampled families contained fluorescent morphs, often found growing side-by-side with non-fluorescent morphs. Colour polymorphism is typical of corals[12,13], but many FPs are invisible in daylight, so this widespread abundance has not been previously reported. The highest numbers of fluorescent morphs were recorded at the shallowest sites; thus, 97% of sampled reef-flat corals at Heron Island, southern GBR, contained medium or high FP concentrations. Moreover, the relative concentration of FPs was significantly higher in parts of the colonies that were exposed to sunlight as compared to those parts that were shaded ($P < 0.001$, *t*-test). We therefore explored an early suggestion[3] that fluorescent pigments in shallow water corals might function in photoprotection, by comparing fluorescent and non-fluorescent morphs.

We found that the emission maxima of FPs ranged from blue to green to red (Fig. 1), which is consistent with studies on the isolated FP proteins of corals[9]. The shorter wavelength FPs were more abundant. Microscopically, we identified two broad groups: FPs bound within fluorescent pigment granules (FPGs; 0.2–8 μm in size) as reported previously[3,5,7]; and inter- or intracellular FPs that were not enclosed in granules (CFPs). Most corals contained multiple FPG and CFP types. Correspondences between emission and excitation maxima of FPs that occur in close association (Fig. 1e, f) suggest that energy transformation to longer, non-photosynthetically active, wavelengths might in some cases be a sequential process, with the fluorescence of one pigment exciting another, as expected from spectra of isolated proteins[8,9]. We showed that this process could occur by comparing fluorescence of green FPGs (excitation maximum, 482.5 nm) alone and mixed with blue FPGs (excitation maximum, 382.5 nm). Only weak green fluorescence is seen under 330–380 nm excitation; addition of blue FPGs emitting at 480 nm enhanced green fluorescence intensity by 4–17 times. (The effect was strongly dependent on distance, with maximal enhancement when blue and green granules were less than 10 μm apart.) The final energy spill (the wavelength of the energy at the end of the pigment coupling process) would then, depending on the pigments involved, lie between the two main peaks of the coral photosynthetic action spectrum and hence be relatively inactive in photosynthesis (Fig. 1f). This would be the inverse counterpart of the process of light transfer to photosynthesis that others have proposed for light-limited habitats[4,5] (Fig. 1g).

High levels of light cause photodamage and photoinhibition[14] in coral symbionts[15–19]. We considered that FPs may reduce the susceptibility to photoinhibition of fluorescent corals by filtering out damaging UVA and excessive photosynthetically active radiation (PAR). We compared the degree of daytime photoinhibition in a polymorphic intertidal species, *Acropora palifera,* by exposing replicate sub-colonies made from green fluorescent, brown medium fluorescent and beige non-fluorescent mother colonies to full sunlight, and monitored photosynthesis by chlorophyll fluorescence

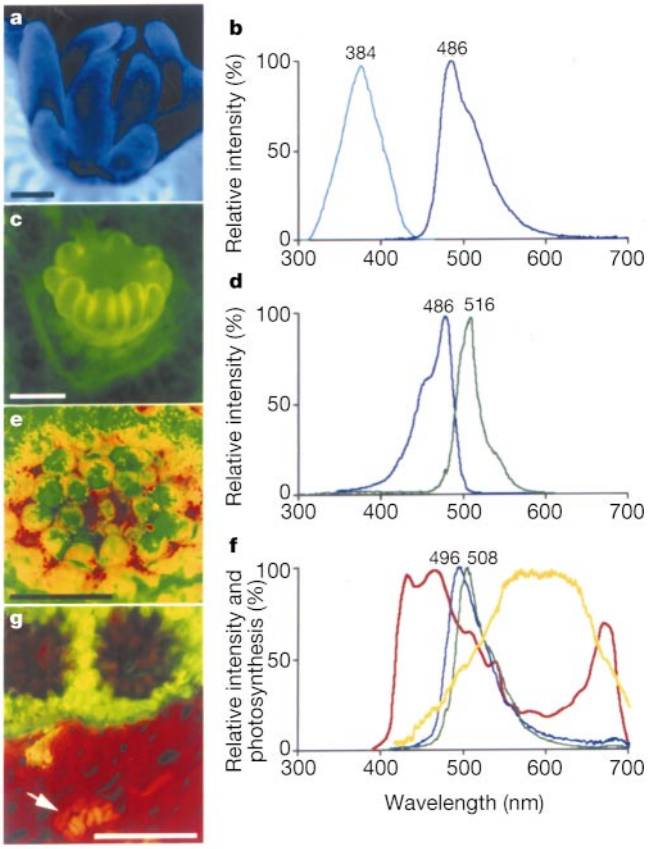

Figure 1 Main types of fluorescent pigments (FPs) in coral polyps. These pigments are found in blue, green, yellow and red combinations (**a**, **c**, **e**, **g**) with overlapping excitation and emission spectra (**b**, **d**, **f**). **a**,**b**, Mainly blue, in *Acropora nobilis*. **c**, **d**, Mainly green, in *Pocillopora damicornis*. **e**,**f**, Emissions of outer blue/green and underlying yellow FPs in 'sun' *Porites cylindrica*. Coral photosynthetic action spectrum[24] (red line) shows that much of the energy is emitted at wavelengths not usable in photosynthesis. **g**, Sub-surface red FPs in green *Montipora digitata*. Arrow, red FPs in mesenterial filaments. Scale bars, 0.5 mm. 'Sun' refers to corals from high-sunlight habitats, 'shade' refers to corals or parts of corals from shaded habitats.

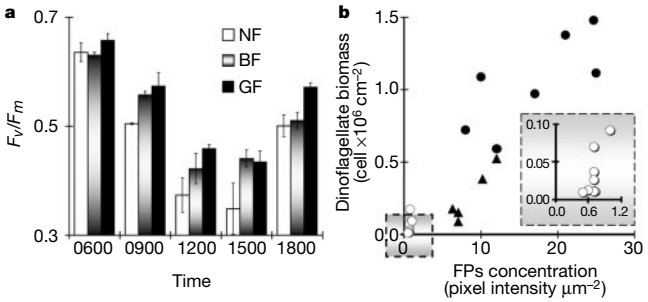

Figure 2 Photoinhibition and bleaching responses of corals. **a**, Maximal potential quantum yield (F_v/F_m) of dinoflagellates in green highly fluorescent (GF), brown medium fluorescent (BF) and non-fluorescent (NF) *Acropora palifera*. Results are means ± s.e. for 3 sub-colonies × morph × 2 tanks × time interval (that is, the samples (270) taken from 3 colonies for each of the 3 morphs, divided randomly between 2 tanks, and killed for PAM fluorescence measurement at 5 given times). **b**, Dinoflagellates per cm^2 and relative concentration of fluorescent pigments per μm^2 of sampled corals: open circles, bleached; triangles, part-bleached; filled circles; unbleached. Inset, enlarged section of graph marked in square.

analysis with a pulse amplitude modulation (PAM) fluorometer[19,20]. As expected[15,16], corals showed pronounced photoinhibition during periods of peak irradiance; non-fluorescent morphs, however, were significantly more photoinhibited and recovered to pre-inhibition rates slower than fluorescent morphs ($P < 0.001$, analysis of variance) (Fig. 2a). Similar measurements with other polymorphic species (*Acropora nobilis*, *Pocillopora damicornis*, *Goniastrea retiformis*) also indicated that FPs are correlated with reduced photoinhibition.

As high solar radiation is a factor in the widely observed mass bleaching of corals[17,21,22], FPs might affect susceptibility to bleaching. Bleaching occurs as a consequence of damage to dinoflagellate photosynthesis caused by the combined effects of thermal stress and sunlight[18,19,22]; the dinoflagellates either degrade or are expelled from the host. During the severe 1998 mass bleaching event on the GBR, we sampled 21 common coral species affected by bleaching to varying degrees. We found a significant correlation ($r^2 = 0.9471$; $P < 0.0001$) between bleaching resistance (that is, high tissue dinoflagellate biomass) and the concentration of FPs within the tissue (Fig. 2b).

Light scattering is an important factor linked to the sun-screening function of FPs. We measured spectral reflective properties of coral tissues with fibre-optic microprobes[23,24] positioned over specific parts of single coral polyps. The highly reflective bare coral skeleton was used as a reflection standard (100%). FPs greatly modified the surface light environment, not only by their emissions but also by light scattering and reflectance, which was higher in areas with high FPG concentrations (Fig. 3a). White-pigmented regions of tissues, formed by dense layers of FP chromatophores, had 60–100% reflectivity (Fig. 3a, b). The most pigmented, and most reflective, parts of colonies—first, branch tips and colony edges, and second, the oral disk/cone and tentacle tips, which on polyp retraction form a sun-screening polyp 'plug'[7] (Fig. 4c)—correspond respectively to known areas of highest cell division and areas immediately above reproductive organs. This distribution points to a photoprotective role of FPs in screening sensitive coral tissues as well as symbionts.

Our observations also indicate that corals actively vary the areal density of pigment chromatophores by polyp expansion and contraction. During expansion, more light penetrates into the tissues through the gaps between FPGs. Under high light, polyp contraction leads to denser concentration of tissue FPGs and cytoplasmic FPs, forming a thicker and a quasi-continuous FP layer; this layer acts as an effective sunscreen (Fig. 3b, c) by light scattering and by radiant fluorescence energy transfer from shorter to longer wavelengths. We also found that FP-containing polyps of shade-adapted and high-light-adapted corals exhibited differences in spectral reflectivity. Shade-adapted polyps absorbed most of the incident light, in line with previous observations[25], whereas polyps that had adapted to high light levels were generally 20–100% more reflective (Fig. 3b).

What are the causes of such different optical properties of tissue from shade- and high-light-adapted corals? The three-dimensional (3D) cellular localization of FPs in corals showed a clear difference in the distribution of FPs relative to the layers of endosymbionts[7]. In high-light-acclimated corals, FPs are localized above the endosymbionts (Fig. 4a–c), and are in a position to screen them from excess sunlight. In shade-adapted corals from light-limited habitats, FPs are localized endodermally, among or below the layers of endosymbionts (Fig. 4d), consistent with their proposed function of light enhancement for photosynthesis by way of wavelength-transformation and back-scattering[4,5].

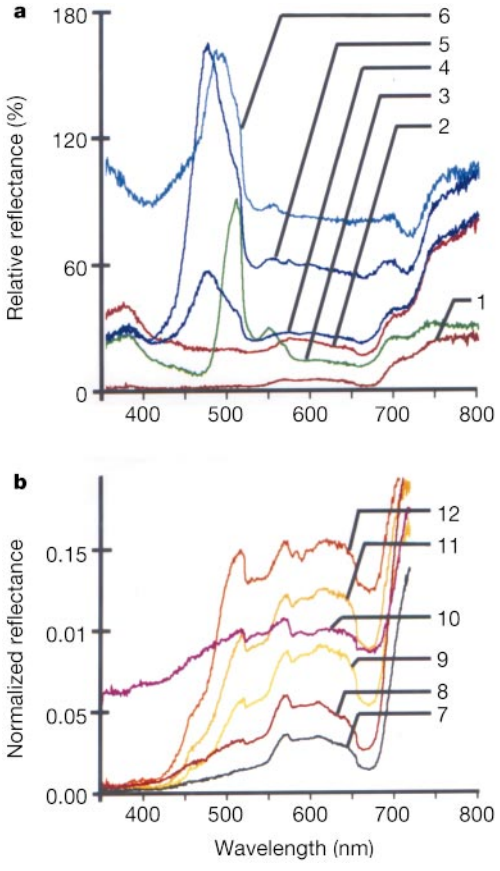

Figure 3 Apparent reflectance. **a**, *Plesiastrea versipora*: tissues lacking FPs (1); tissue with blue FPs overlying skeletal ridge (2); contracted tentacle with green FPGs (3); blue FPGs in expanded (4) and contracted (5) oral disk; dense blue FPGs in white oral disk of shallow-water *Platygyra daedalea* (6). **b**, Intertidal yellow *Porites cylindrica*: 'shade' expanded (7); contracted (8); and 'sun' expanded (11), contracted (12) tentacles; edge of polyp calyx (9); septal skeleton with thin tissue (10). Dips in spectra are due to absorption by photosynthetic pigments. Spectral peaks are due to FPs emission and reflection.

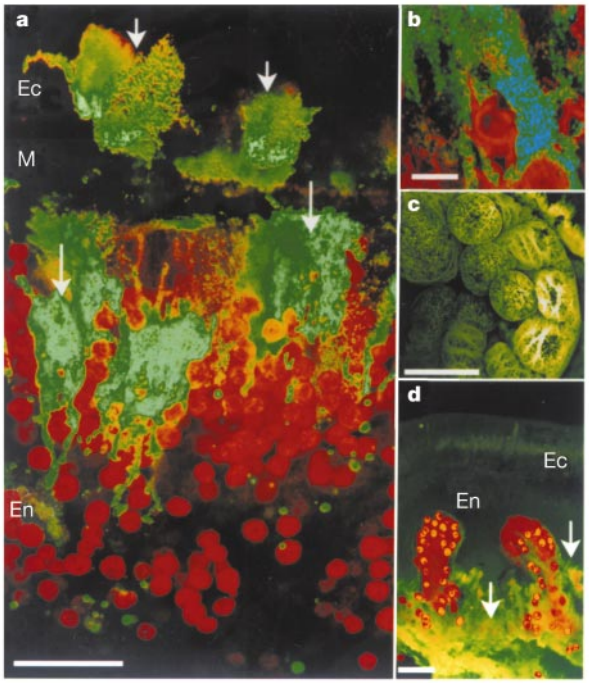

Figure 4 Reconstruction of serial confocal sections through tissues of 'sun' and 'shade' corals. **a**, FPs in chromatophores above endosymbionts in polyp tentacle of 'sun' intertidal *Goniopora tenuidens*. Scale bar, 50 μm. **b**, FPG-filled chromatophores with long extensions enveloping endosymbionts. Scale bar, 10 μm. **c**, Retracted tentacles with dense FPGs form a 'plug over polyp. Scale bar, 1 mm. **d**, Endodermal FPs below dinoflagellates in 'shade' *Lobophyllia corymbosa*. Scale bar, 50 μm. FPs shown in green/yellow, symbiotic dinoflagellates in red. Arrows, FP chromatophores. Ec, ectoderm (epidermis); M, mesogloea; En, endoderm (gastrodermis).

The present results suggest that FPs reduce the photoinhibitory effect of high levels of solar radiation, which in conjunction with thermal stress leads to bleaching. These findings improve our understanding of the causes of observed inter- and intraspecific variability in bleaching[17,26], and may provide an insight into how changing global climate conditions would influence the species diversity and rate of change of coral reef communities[22].

The evidence presented here indicates that the role of FPs in photoprotection in shallow water, hitherto neglected, is at least as significant as the function of light capture in deep water previously assigned to them. Dinoflagellate photosynthesis is vulnerable to both UV[27,28] and high levels of PAR[16–19]. While accessory pigments in dinoflagellates can dissipate excess PAR as heat[29], FPs can dissipate excess light energy through fluorescence and light scattering. FPs may also supplement screening of UV radiation by mycosporin-like amino acids[30], as some FPs can transform absorbed UVA radiation to longer non-actinic wavelengths through fluorescence. By screening chlorophylls and peridinin from high levels of solar radiation and by absorbing UVA, FPs thereby decrease the likelihood of irreversible photoinhibition, photooxidation and subsequent coral bleaching. By changing their optical properties with the help of these GFP-like pigments, coral polyps are able to optimize the photosynthetic activity of their tissues for the better survival of the organism. □

Methods

Survey sites, sampling and manipulations

Surveys of fluorescent corals were made at the inter-tidal lagoon, reef flat and inner and outer slope of Heron Island (23° 26′ S, 151° 55′ E) and One Tree Island (OTI) (23° 30′ S, 152° 06′ E), and at several GBR mid-shelf-reefs (1–20 m depth). Corals were sampled by chiselling pieces from replicate colonies (n = 3–6). Each sample was broken in two, and, subsequently, one subsample was frozen and the other was chemically fixed as described previously[7] for microscopy. Polymorphic *A. palifera* colonies used in photoinhibition experiments were collected from the OTI lagoon from ~1 m depth. Three colonies of each colour morph were broken into replicate sub-colonies, and fixed in horizontal position in flowing sea water (27–28 °C) with one side exposed to sunlight and the other shaded. Controls were kept at 50–80 μmol photons m^{-2} s^{-1}. During the March 1998 bleaching event, samples were taken at Coats (17° 28′ S, 146° 30′ E) and Cayley (18° 30′ S, 147° E) mid-shelf reefs from depths of 1–6 m. Replicate samples (n = 3–6) were taken from species selected by susceptibility to bleaching: 9 bleached (including non-fluorescent *A. nobilis* morph); 5 partially bleached (including fluorescent *A. nobilis*); 7 unbleached.

Microscopy

Frozen, glutaradehyde-fixed and live coral samples were analysed by fluorescence wide-field and confocal (CLSM) microscopy as described previously[7]. Fluorescence characteristics of FPs were not substantially affected by glutaraldehyde fixation. Confocal imaging used 488-nm excitation, with detection at 520–550 nm (FPs) and >585 nm (chlorophyll). 3D reconstruction from optical sections was done with VoxelView Ultra 2.1.2 (Vital Images). A fluorescence microscope fitted with a cooled CCD camera (PCO Sensicam) was used to test energy transfer from blue to green FPGs, both extracted from *Plesiastrea versipora*. Intensity (in the green) was measured as the ratio of emission excited at 330–380 nm to the emission excited at 450–490 nm, thereby compensating for differences between granules. Relative FP concentrations in light- and shade-adapted samples—as well as in post-bleaching samples—were measured semi-quantitatively by CLSM as the fluorescence intensity per μm^2 of imaged coral surface (replicate 3–6 colonies per specimen). Zooxanthellae were extracted from samples, and their biomass per cm^2 of coral surface was determined microscopically and correlated to the relative concentration of FPs in surface tissue as determined by CLSM. Coral surface areas were measured as described previously[18].

Spectroscopy

Fluorescence excitation and emission spectra of FPGs isolated by homogenization and repeated centrifugation of coral tissues in phosphate buffer (0.1 M, pH 7.2) were determined by a Perkin Elmer Luminescence Spectrometer S50B. All spectra were normalized to their peaks. Reflectance spectra were measured on live corals in sea water by a tapered (40-μm tip) fibre-optic field radiance microprobe[23] positioned ~100 μm above the coral surface. The microprobe was connected to a fibre-optic diode-array spectrometer (Hamamatsu PMA-11) with a 300–800 nm spectral range. A micromanipulator was used to position the microprobe tip above specific single polyp regions, as viewed under a dissection microscope. Samples were illuminated by a UV–visible metal halide light source through a 1-mm quartz fibre equipped with a collimator at the output end. Spectra of reflected light from the corals were normalized to the spectrum of reflected light from a reflectance standard in order to obtain reflectance spectra corrected for the spectral composition of incident light (normalized reflectance). Data were subsequently expressed as percentages of surface downwelling radiance reflected from a cleaned coral skeleton (relative reflectance).

Active fluorescence measurements

Throughout the day (06:00, 09:00, 12:00, 14:00, 18:00), photoinhibition of light-exposed and shaded portions of sub-colonies (n = 3 per morph), and shaded controls, was measured as decrease in the maximal potential quantum yield (F_v/F_m) of PSII by a pulse amplitude modulation fluorometer (DIVING-PAM)[19,20] after 30 min dark-adaption (where F_v is the variable fluorescence yield and F_m is the maximum dark-adapted fluorescence yield). Photosynthetically active radiation (400–700 nm) during the experiment was measured by an LI-190SA quantum sensor (Li-Cor-Inc.).

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