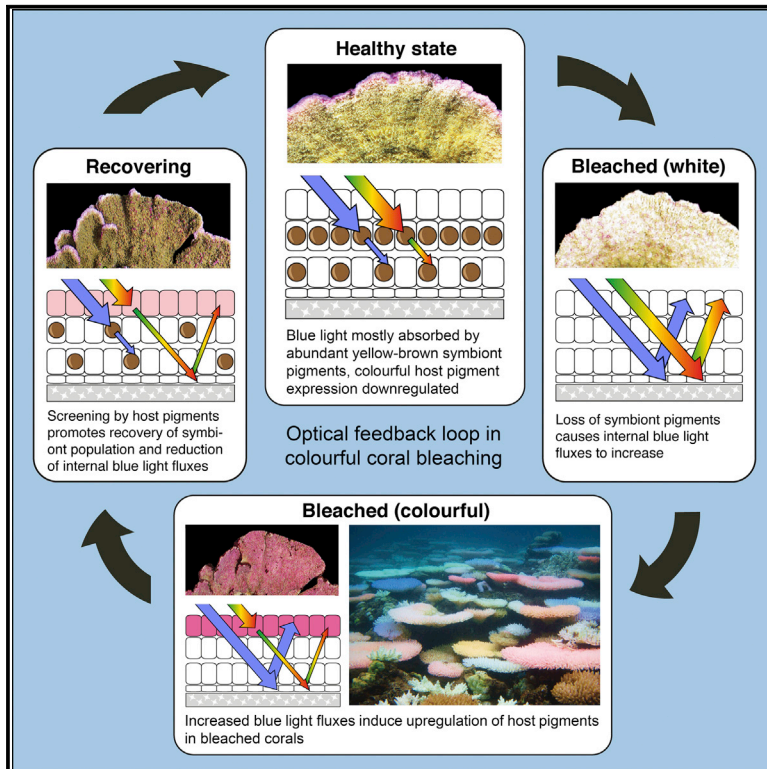


Optical Feedback Loop Involving Dinoflagellate Symbiont and Scleractinian Host Drives Colorful Coral Bleaching

Graphical Abstract



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In Brief

Bollati et al. document the global incidence of colourful bleaching events and describe the physiological mechanism behind the striking phenomenon. Extreme colors result from upregulation of photoprotective host pigments driven by increased internal light fluxes following symbiont loss.

Highlights

- Colorful bleaching is a recurring phenomenon in reef regions around the globe
- Colors result from blue-light-driven host pigment upregulation following bleaching
- Photoprotective host pigments in bleached tissue can aid symbiont recolonization
- Colorful bleaching can be indicative of brief or mild heat and/or nutrient stress



Article

Optical Feedback Loop Involving Dinoflagellate Symbiont and Scleractinian Host Drives Colorful Coral Bleaching

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SUMMARY

Coral bleaching, caused by the loss of brownish-colored dinoflagellate photosymbionts from the host tissue of reef-building corals, is a major threat to reef survival. Occasionally, bleached corals become exceptionally colorful rather than white. These colors derive from photoprotective green fluorescent protein (GFP)-like pigments produced by the coral host. There is currently no consensus regarding what causes colorful bleaching events and what the consequences for the corals are. Here, we document that colorful bleaching events are a recurring phenomenon in reef regions around the globe. Our analysis of temperature conditions associated with colorful bleaching events suggests that corals develop extreme coloration within 2 to 3 weeks after exposure to mild or temporary heat stress. We demonstrate that the increase of light fluxes in symbiont-depleted tissue promoted by reflection of the incident light from the coral skeleton induces strong expression of the photoprotective coral host pigments. We describe an optical feedback loop involving both partners of the association, discussing that the mitigation of light stress offered by host pigments could facilitate recolonization of bleached tissue by symbionts. Our data indicate that colorful bleaching has the potential to identify local environmental factors, such as nutrient stress, that can exacerbate the impact of elevated temperatures on corals, to indicate the severity of heat stress experienced by corals and to gauge their post-stress recovery potential.

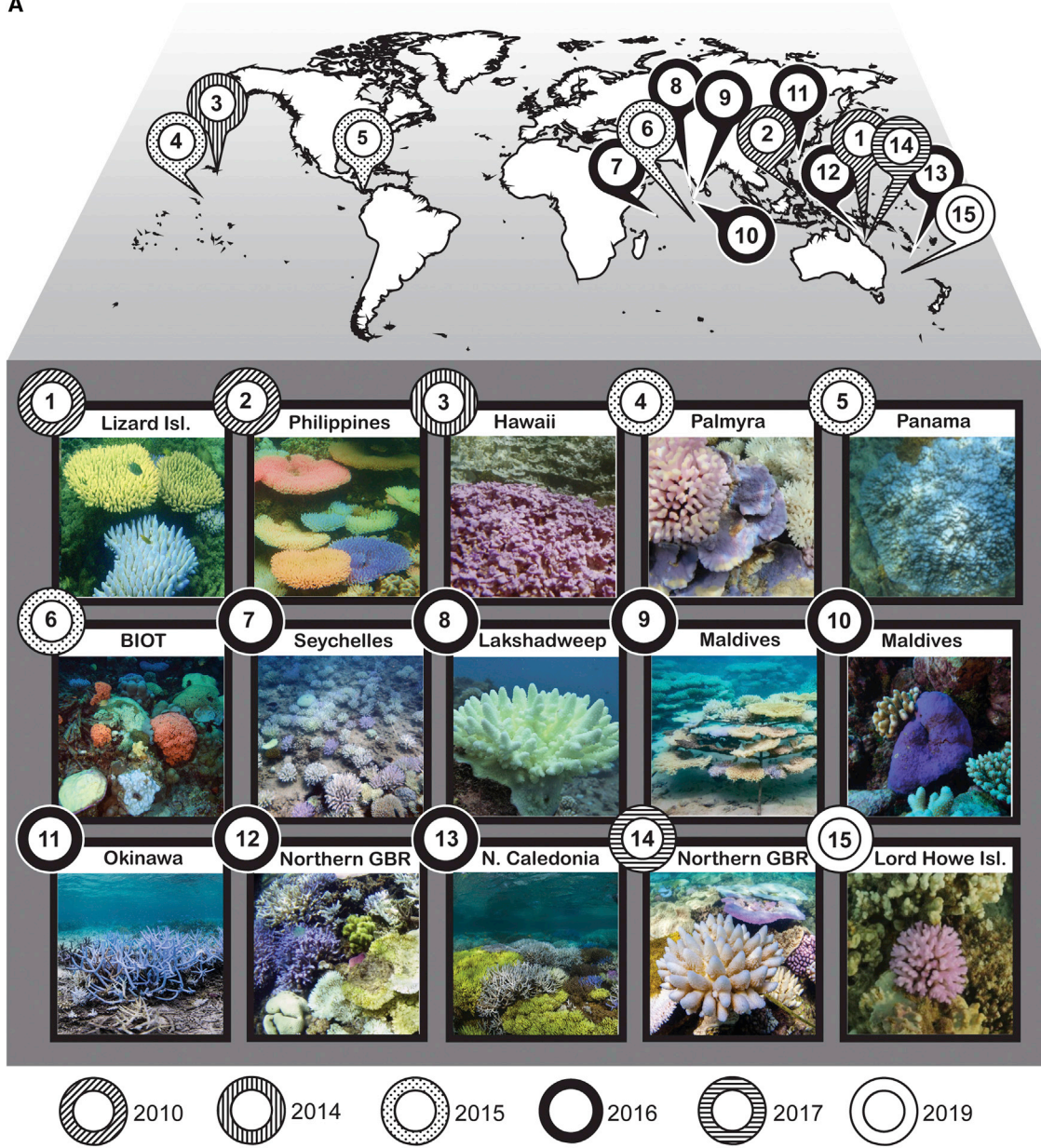
INTRODUCTION

In 2015–2017, the world's coral reefs experienced the most widespread and devastating mass coral bleaching ever recorded [1]. The increasing frequency and extent of these events has been linked to anthropogenic climate change and poses a major threat to coral reef functioning, productivity, and biodiversity [1]. Rising sea water temperatures are the main driver of this phenomenon [2], although a number of environmental factors, including nutrient stress, are known to induce bleaching and/or increase the susceptibility of corals to thermal stress [3–5]. Bleaching is the breakdown of the symbiosis between reef-building corals and unicellular dinoflagellates of the family Symbiodiniaceae [6], which are harbored in the host gastrodermal cells and supply vital parts of the host's metabolic requirement via photosynthate translocation [7]. The malfunctioning of the symbiosis can result in the loss of the dinoflagellate partner and their photosynthetic pigments chlorophyll and peridinin [8],

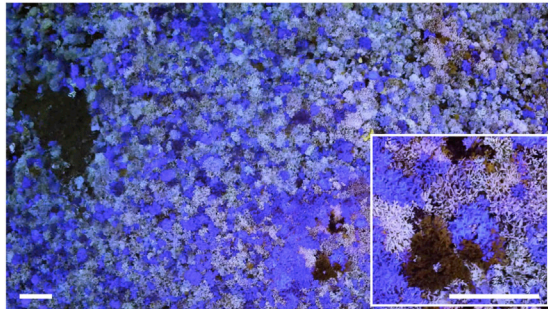
which are mostly responsible for the brownish coloration of unbleached corals [9, 10]. In symbiont-depleted corals, the incident light that would otherwise be absorbed by the photosynthetic symbiont pigments is backscattered very efficiently by the highly reflective, white coral skeleton, resulting in enhanced internal light fluxes in the overlying animal tissue [11] and the bleached appearance of corals. If bleached corals are not able to recover their symbiont populations quickly, they are subject to starvation and diseases. The resulting increased mortality rates are a major contributor to reef decline [1]. In some instances, bleaching renders corals vibrantly green, yellow, or purple-blue rather than white, a phenomenon which reportedly affects key reef building genera, such as *Porites*, *Pocillopora*, *Montipora*, and *Acropora* [12, 13]. The green, red, and pink to purple-blue colors of scleractinian corals involved in these colorful events derive from green fluorescent protein (GFP)-like pigments found in the host tissue of many reef-building corals [10, 14, 15]. This group of homologous pigments includes fluorescent proteins (FPs)



A



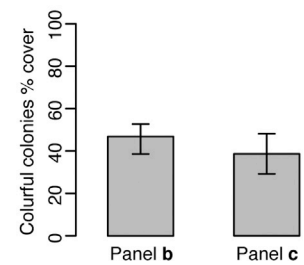
B



C



D



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containing a light-absorbing chromophore, which emits red-shifted wavelengths [16], and chromoproteins (CPs) that strongly absorb light in the visible range but emit few or no photons [17]. Many FPs and CPs found in shallow water corals are localized in the ectoderm of the host coral tissue and are transcriptionally regulated by light intensity, specifically in the blue spectral range [18, 19]. Previous work has established that, in common shallow water corals, host pigments show two major types of light regulation response: low threshold and high threshold. The low-threshold response group is represented mostly by cyan fluorescent proteins (CFPs). The expression of these CFPs is upregulated already at low photon irradiance $<100 \mu\text{mol m}^{-2} \text{s}^{-1}$ but stagnates or decreases at high light intensities [18]. In contrast, members of the high-threshold group are not or only minimally expressed at photon irradiance $<100 \mu\text{mol m}^{-2} \text{s}^{-1}$, but pigment production increases continuously with increasing light exposure of the corals [18]. In shallow-water corals, the high-threshold group consists of green and red FPs (GFPs and RFPs) and pink to purple-blue CPs [18]. Despite their differing optical properties, representatives of all color types of high-threshold pigments have been shown to provide photoprotection for the gastrodermal symbionts via direct or indirect screening of excess sunlight [14, 19–22]. Notably, FPs in coral species commonly found in lower light habitats are biochemically, photophysically, and functionally distinct [14, 17, 22–26]. The visibility and fluorescence of existing host pigments can be enhanced due to the loss of symbionts from the tissue and associated reduced absorption by their photosynthetic pigments. In this case, corals can appear more colorful, even in the absence of upregulation of host pigment expression in response to an environmental trigger [27].

Despite the striking incidence of brightly colored corals during certain mass coral-bleaching events, the environmental conditions that trigger color changes, as well as the involved mechanisms and consequences for the corals, are not understood. Specifically, it is unclear whether the enhanced coloration is due to an active accumulation of pigments, indicative of a functioning cellular machinery, or just a better pigment visibility in the stressed coral tissue that is losing its symbionts. This knowledge is the key prerequisite to interpret the color changes as indicators of specific environmental conditions during those bleaching events or to unravel the biological significance of colorful bleaching for the coral-dinoflagellate symbiosis.

We hypothesized that colorful coral bleaching is caused by the reduction in symbiont pigment absorption in bleached corals and the associated increase in internal light fluxes due to back scattering of incident light by the highly reflective coral skeleton [11], which results in the accumulation of FPs and CPs in the coral tissue driven by high light-induced host pigment gene expression [18, 19].

To test this hypothesis, we established a biomarker system that relies on light-regulated FPs and CPs as indicators for altered photon fluxes in coral tissue [18]. We then applied this biomarker approach to coral colonies undergoing bleaching and recovery during controlled laboratory experiments. The results of the experimental study of three representative model coral species were aligned with field observations to propose a mechanistic model of colorful bleaching that is based on an optical feedback loop involving coral host and dinoflagellate symbiont.

RESULTS

Colorful Bleaching as a Global Phenomenon

We compiled and analyzed photographic evidence and eyewitness statements (Figure 1A; Table S1). The spatial and temporal distribution of these events clearly shows that colorful bleaching is a recurring phenomenon of global reach that affects key reef-building coral genera, including *Acropora*, *Montipora*, *Porites*, and *Pocillopora*. Furthermore, we analyzed aerial images recorded during colorful bleaching events in New Caledonia and the Great Barrier Reef (Figures 1B–1D). In both cases, colorfully bleached corals cover $\sim 40\%$ of the reef surface in shallow water, confirming the significance of colorful bleaching for large parts of coral populations. The high cover of colorfully bleached corals is well aligned with the results of our surveys of shallow water populations of representative reef-building coral species (*Acropora* sp., *Stylophora pistillata*, and *Pocillopora damicornis/verrucosa*), among which $>50\%$ of individuals have the capacity to express high levels of host pigments (Figure S1).

Heat Stress Conditions Associated with Colorful Bleaching Events

We then used satellite data to reconstruct the heat stress levels experienced by corals in the wake of colorful bleaching events. Temperature traces for the different regions were aligned using the local bleaching thresholds (Figures 2A and 2B). In the case of Lizard Island, New Caledonia, and Okinawa, colorful bleaching was reported 17–25 days after temperatures started to return to ambient values after excursions above the bleaching threshold (Figures 2A, 2C–2E, and S2A). During the Palmyra 2015 event, temperatures fluctuated marginally above and below the regional bleaching threshold over ~ 10 weeks (Figures 2B and 2F). Colorful bleaching was documented ~ 12 days after temperatures started falling after their last maximum. During the Philippines 2010 event, colorful bleaching was observed after temperatures marginally exceeded the local bleaching threshold for 3 weeks (Figures 2B and 2G). Later, temperatures in this location rose further, resulting in 100% coral mortality (Figure S2B). In contrast, the other colorful bleaching events resulted in no or low-to-moderate mortality (Figure S2B).

Figure 1. Global Incidence of Colorful Bleaching Events

(A) Colorful bleaching episodes between 2010 and 2017. The pins on the map indicate the spatial distribution of colorful bleaching events with pin head patterns encoding years of occurrence. Details about the events, sources, and image credits are reported in Table S1.

(B–D) Aerial views of reefs covered by colorful colonies during bleaching events in New Caledonia (B; March 2016; image credit: Richard Vevers, Ocean Agency) and the Great Barrier Reef (C; 2017; image credit: Ed Roberts, Tethys Images) and percentage cover by colorful colonies calculated by image analysis (D; mean \pm SD; n = 3 replicate areas per image). Scale bars: ~ 1 m.

See also Figure S1 and Table S1.

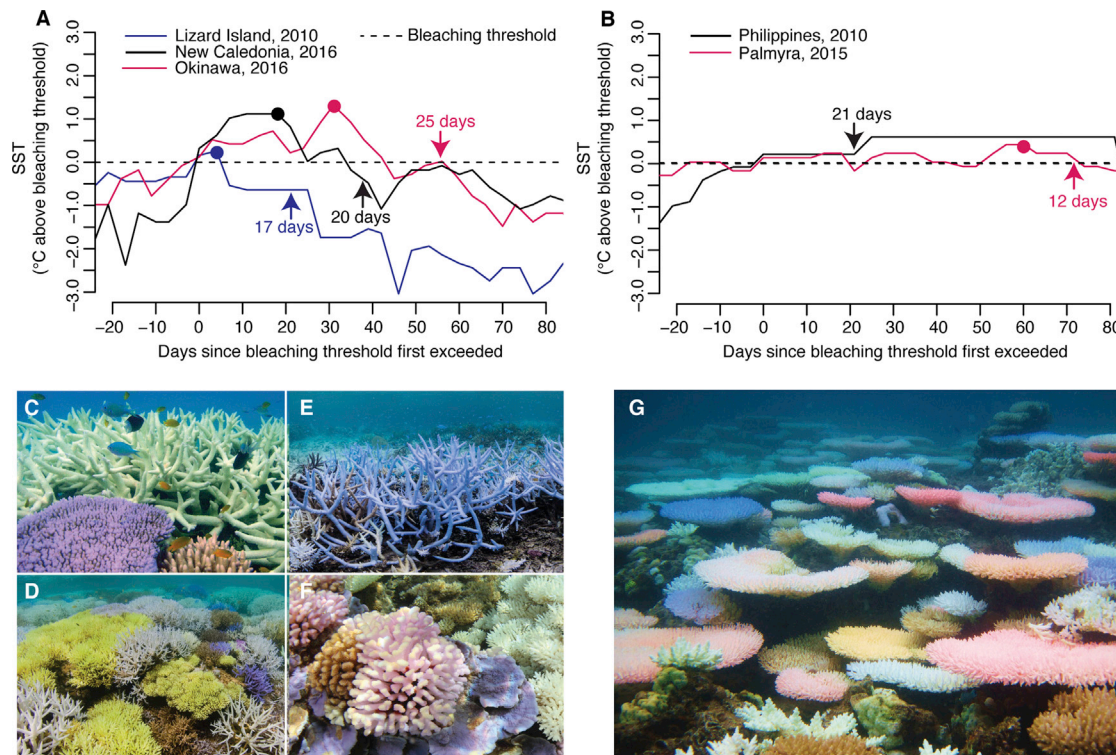


Figure 2. Temperature Conditions during Colorful Bleaching Events

(A–G) Satellite sea surface temperature (A and B) and field images (C–G) for colorful bleaching events on Lizard Island (A and C), New Caledonia (A and D), Okinawa (A and E), Palmyra (B and F), and Palawan, Philippines (B and G). The reefs in (C–F) suffered no or low-to-moderate post-bleaching mortality, although high mortality was recorded for (G). In (A) and (B), arrows show the time the corresponding photographs (C–G) were recorded. The numbers above the arrows indicate days elapsed after temperatures decreased after the heat stress maximum (spheres; A and Palmyra in B) or exceeded the local bleaching threshold (Philippines in B). Image credits: Darren Coker, JCU Townsville (C); Richard Vevers, The Ocean Agency (D and E); Brian Zgliczynski, Scripps Institution of Oceanography (F); Ryan Goehring, University of Washington (G). See also [Figure S2](#) and [Tables S1](#) and [S6](#).

A Biomarker Approach to Report Changes in the Internal Light Field in Symbiotic Corals

To test our hypothesis that extreme coral coloration during bleaching results from a light-driven upregulation of host pigments, we established a novel bioindicator test relying on the coral's own CFPs and GFPs as intrinsic, intracellular markers to visualize changes in the internal light fluxes during bleaching.

CFPs are usually expressed at low light intensity ($\sim 80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), and their concentration in coral tissue becomes saturated or reduces at high light intensities ($>400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) [18]. In contrast, CPs and GFPs in shallow-water species are commonly expressed only under higher photon irradiance ($>100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and their tissue concentrations increase with the intensity of incident blue light [18]. At low light levels ($\sim 80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), our model coral *Porites lichen* expresses a CFP with a 489-nm emission peak (Figure 3A). In contrast, under high light conditions ($\sim 290 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), the tissue fluorescence is dominated by a 519-nm-emitting GFP (Figure 3B). At intermediate light intensity ($\sim 150 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), both FPs are expressed.

Although these GFP-like proteins show the same light regulation patterns as representatives of these pigment groups from other species, their primary structure has not been characterized yet. Therefore, we conducted a differential precipitation assay

using organic solvents, an established approach to separate GFP-like proteins [28], and confirmed that the CFP and the GFP contained in raw tissue extracts of *P. lichen* are indeed biochemically distinct proteins (Figure S3A). In contrast to a bioengineered cyan fluorescent variant of a GFP-like protein from a jellyfish [29], the CFP of *P. lichen* does not show changes in emission color in response to irradiation with strong near-UV ($\sim 410 \text{ nm}$) or with blue ($\sim 465 \text{ nm}$) light (Figures S3B and S3C). We therefore ruled out the possibility that the switch of cyan to green tissue fluorescence observed in the high light acclimation response of *P. lichen* is the result of a photoswitching process involving a single pigment. Our biochemical analyses confirmed that the CFP and GFP in this species belong to the groups with a low light (CFP) or high light (GFP) expression threshold, common in many species from shallow reefs [17, 18], making them promising biomarkers for light fluxes in coral tissue.

As predicted, during high light acclimation of unbleached *P. lichen*, the GFP content strongly increases, whereas the CFP content decreases (Figure 3C). Accordingly, the 519 nm to 489 nm ratio of fluorescence emission increases over time, following a saturating exponential function, reflecting the changes in the tissue concentration of both proteins (Figures 3C and 3D). To demonstrate unambiguously that the differential changes in host pigment expression are not an intrinsic response

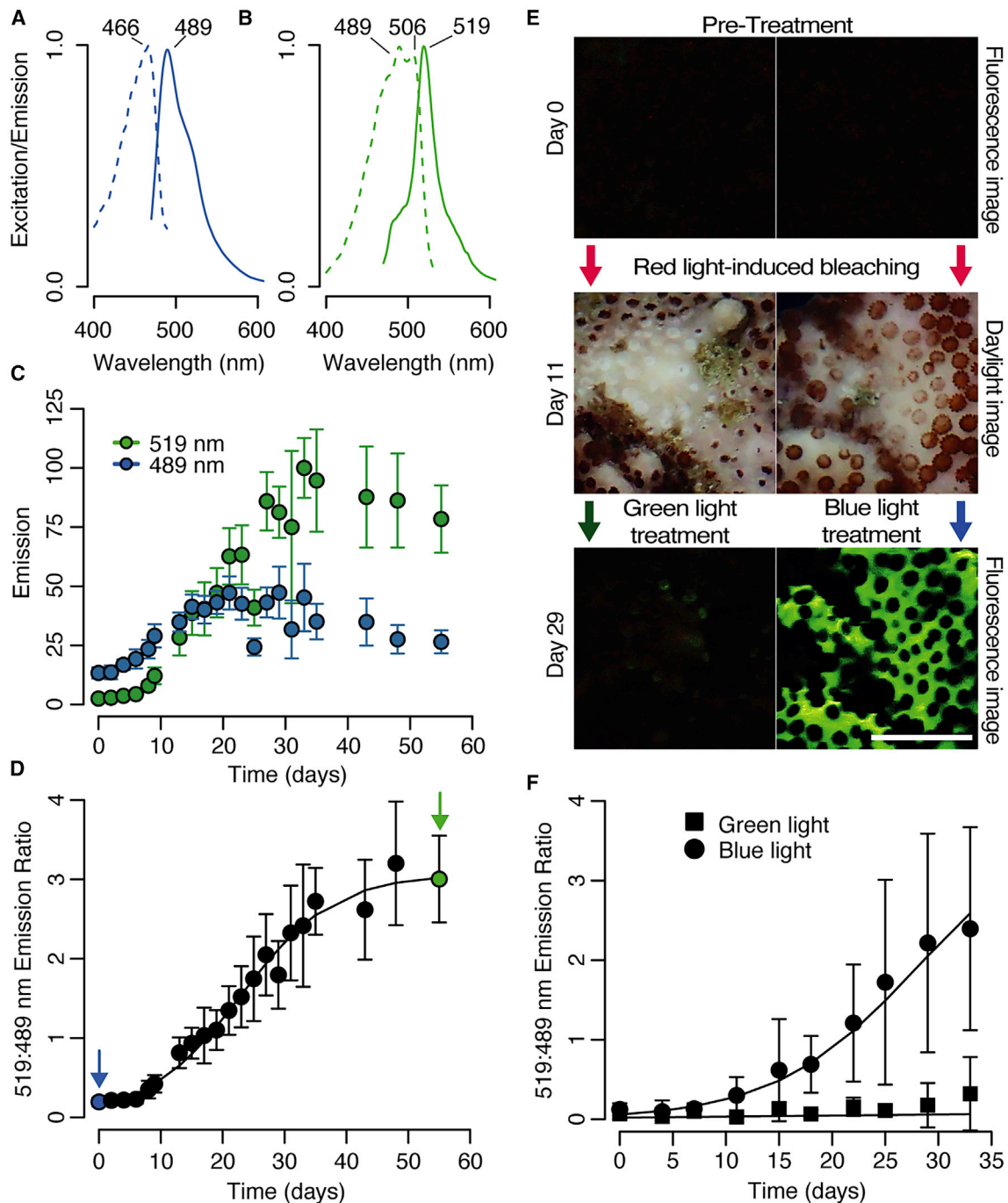


Figure 3. Photoacclimation of *Porites lichen* Indicated by Changes in Host Pigment Levels

(A and B) *In vivo* spectral characterization of *P. lichen* under low (A) and high (B) light. Dashed lines, excitation (emission = 550 nm); solid lines, emission (excitation = 450 nm).

(C) Time course of green (519 nm) and cyan (489 nm) emission during acclimation of unbleached *P. lichen* to $\sim 290 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

(D) Time course of green:cyan emission ratio during high light acclimation of unbleached *P. lichen*. Arrows indicate time points for collection of spectra in (A) (cyan) and (B) (green). In (C) and (D), spheres show mean \pm SD; $n = 12$ areas (2 replicate colonies).

(E) Photographs of *P. lichen* before bleaching (day 0, fluorescence image), after bleaching with focused red light (day 11, white light image), and after exposure to green or blue light (day 29, fluorescence image). Scale bar: 10 mm.

(F) Time course of green:cyan emission ratio for bleached *P. lichen* exposed to green or blue light. Mean \pm SD; $n = 3$ replicate colonies.

See also Figures S3 and S6 and Table S4.

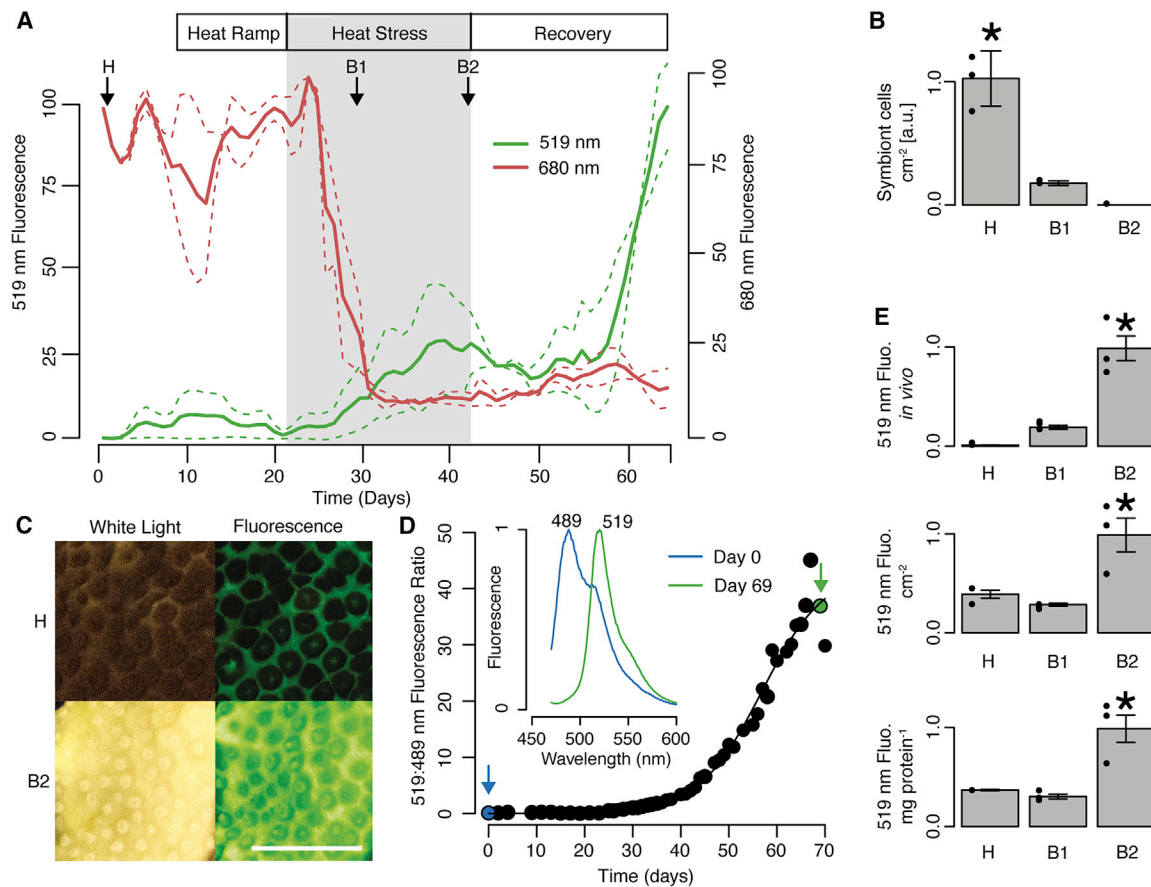


Figure 4. Fluorescence of *Porites lichen* Undergoing Thermal Bleaching

(A) Time series of *in vivo* host GFP (519 nm) and chlorophyll (680 nm) fluorescence emission during treatment. Dashed lines show means of two fully independent experiments (each $n = 6$ areas); solid lines show midpoint. B1, partially bleached; B2, bleached; H, healthy.

(B) Symbiont cell density normalized to coral surface area (arbitrary units [a.u.]). Time point H was set to 1, and the other time points were scaled accordingly.

(C) Representative photographs of coral during stages H and B2 (white light or 450-nm excitation); scale bar: 10 mm.

(D) Ratio of mean GFP (519 nm) to mean CFP (489 nm) fluorescence emission during treatment, with *in vivo* spectra taken at time points indicated.

(E) Host GFP (519 nm) fluorescence measured *in vivo* (top) and GFP concentration in host tissue homogenate measured by emission and normalized to coral surface area (middle) and to total host protein (bottom).

In (B) and (E), error bars show mean \pm SD; $n = 3$ replicate colonies; spheres indicate individual data points; asterisks signify $p < 0.05$. See also Figure S4 and Tables S2 and S4.

to bleaching stress but an independent bioindicator for changes in the quantity and spectral quality of the light experienced by corals, we monitored changes in fluorescence in bleached tissue exposed to different light colors. First, we bleached designated regions of interest by exposure to focused red light. Although these long wavelengths efficiently induce light stress in symbionts [30], they do not induce an upregulation of high-threshold GFPs, which is reliant on blue light exposure [18]. Subsequently, the bleached areas were exposed to either blue or green light. Only the blue-light-exposed *P. lichen* replicates showed an increase in green tissue fluorescence and the characteristic changes in 519 nm to 489 nm fluorescence ratio indicative of a high light acclimation response (Figures 3E and 3F). In contrast, no increase in tissue fluorescence was detected in the corals exposed to green light (Figures 3E and 3F), confirming that the CFP/GFP expression is driven solely by light and not by the bleaching process per se. In the present study, we used this

novel reporter system for non-invasive monitoring of changes in the internal light climate in bleaching corals.

Light-Mediated Upregulation of Pigment Content in Bleached Corals

To assess the changes in host and symbiont pigments during thermal bleaching and recovery, we exposed *P. lichen* acclimated to 25°C to 26°C to gradually increasing temperatures up to 31°C to 32°C ($\Delta \sim 0.5^\circ\text{C d}^{-1}$). We used 680-nm fluorescence as an *in vivo* indicator of changes in the amount of symbiont chlorophyll in the coral tissue [31]. Fluorescence spectra collected from heat-stressed corals showed a rapid drop in 680-nm emission (ex = 450 nm) at temperatures $\geq 31^\circ\text{C}$ (Figure 4A), indicating a loss of symbiont pigment from the host tissue. Symbiont cell counts confirmed that this drop in chlorophyll fluorescence was due to a loss of symbionts and corresponded to the onset of bleaching (Figure 4B). The absence of

photosynthetic pigments of the symbionts resulted in strongly increased light fluxes at the colony surface measured as spectral reflectivity [32] (Figure S4A). In particular, blue light fluxes in the spectral range around 450 nm, responsible for the upregulation of FPs and CPs, were ~20-fold increased, an order of magnitude that has been shown to promote strong pigment expression in shallow-water corals [18].

In vivo fluorescence emission of the high-threshold GFP in the intact coral tissue showed a small initial increase after the drop in symbiont chlorophyll (680 nm) fluorescence (Figure 4A). In contrast, when the areal GFP content was determined at this time point using symbiont-free tissue extracts, the average GFP concentration did not change compared to the pre-bleaching values (Figure 4E). These findings suggest that, during the early stages of bleaching, an optical enhancement of tissue fluorescence can result from the reduction in competing absorption of incident light by photosynthetic symbiont pigments, as reported by an earlier study [27].

Notably, a major increase in GFP fluorescence started only ~3 weeks after the symbionts were lost (Figures 4A–4C). The fluorescence intensity ratio of GFP (high expression threshold) and CFP (low expression threshold) increased following a saturating exponential function (Figure 4D), as recorded for unbleached corals during high light acclimation (Figure 3D) and for bleached corals exposed to blue light (Figure 3F). Furthermore, areal GFP content measured in tissue extracts (when normalized to areal total protein content or the corresponding coral surface area) showed a significantly increased value, indicating the enhanced coloration was due to host pigment accumulation in the tissue (Figure 4E). Importantly, tissue fluorescence exceeded 100× pre-treatment values only ~3 weeks after the acute heat stress treatment was terminated (Figures 4A–4C). The timescale of this response is consistent with the interval between the time point when temperatures started to return to ambient levels and observations of colorful bleaching under natural conditions on Lizard Island in 2010 and in New Caledonia and Okinawa in 2016 (~2.5–3.5 weeks; Figure 2A). Together, these data provide evidence that extreme coloration of bleached corals is mostly due to increased pigment accumulation in the host tissue triggered by increased internal light fluxes caused by the reflection and scattering of the incident light by the coral skeleton and tissue in the absence of symbionts [11, 22, 33].

Importantly, a light-driven upregulation of host-pigment expression is the natural response of healthy corals to adjust to changes in their light environment [18–20]. Because colorful bleaching relies on this response, the enhanced coral color indicates that, while the corals are stressed, the expression machinery for at least some proteins is functional and responsive.

Colorful Bleaching in the Absence of Thermal Stress

To test whether colorful bleaching may indicate also other forms of environmental stress, we applied nutrient stress as a heat-independent stressor to induce bleaching. Already at ambient temperature, phosphate starvation caused by elevated levels of dissolved inorganic nitrogen has been shown to cause chemically imbalanced growth of the photosymbionts, resulting in alteration of the membrane lipid complement, impairment of photochemistry, and eventually bleaching [4, 34]. Accordingly,

P. lichen kept under high-nitrate/low-phosphate (HN/LP) conditions [4], bleached gradually as signified by the loss of symbionts from the tissue and the corresponding decrease of chlorophyll fluorescence (Figures 5A and 5B). GFP emission *in vivo* and tissue concentration quantified in extracts increased during bleaching (Figures 5A, 5C, and 5E). At the same time, fluorescence intensity ratio of high (GFP) to low (CFP) induction threshold pigments increased exponentially (Figure 5D). As expected, the overall light fluxes in the bleached tissue increased as indicated by the higher surface reflectivity (Figure S4B). However, in contrast to *P. lichen* bleached by acute heat stress (Figure S4A), the concentration of host pigments in the coral tissue increased over the same time period during which the symbiont pigmentation was lost (Figure 5A). Furthermore, in *P. lichen* bleached by nutrient stress, the light fluxes in the blue spectral range up to ~500 nm are strongly reduced due to the high GFP content of the host tissue (Figure S4B), indicating the mitigating effect of the host pigments on internal light stress.

We also assessed the host pigment response to bleaching in *Pocillopora damicornis*, a species capable of expressing high levels of a pink CP [35] that belongs to the group of high-light-induced, photoprotective pigments of shallow water corals [18, 20]. As observed for green FPs in *P. lichen*, the pink CP content of *P. damicornis* increases strongly after bleaching only in the presence of blue light (Figures S5A and S5B). When *P. damicornis* was exposed to nutrient starvation stress, the CP concentration in the coral tissue increased to 3.5% of the total host protein content (Figures S5C and S5D). In contrast, the pink CP made up only ~0.7% of the host protein in unbleached colonies kept in nutrient replete conditions. Therefore, the colorful bleaching response is indeed the result of significantly higher host pigment concentrations in the tissue that can be attributed to an upregulation of pigment expression in response to the ~4× higher internal blue light fluxes detected at the surface of bleached colonies (Figure S5E). As for the GFP in *P. lichen* (Figure S4B), the optical damping effect of the CP on internal light fluxes in the bleached coral is clearly visible in the reflection spectrum (Figure S5E).

Finally, we used a third species, *Montipora foliosa*, to confirm that our findings are representative for a range of key reef-building corals. This species can serve as a model for corals that show a strong expression of photoprotective CPs in symbiont-free growth zones, such as colony margins (Figures S5F and S5G), tips, or areas of wound regeneration [36]. In these areas, the CPs are thought to reduce the internal light fluxes to facilitate the colonization of newly formed tissue with symbionts [20, 36]. After the corals were bleached by phosphate starvation, the color of the whole colonies changed to purple (Figure S5F). The CP concentrations in the inner, previously non-pigmented areas of the corals matched those in the healthy growth margins of unbleached individuals (Figure S5H). These findings further confirm that the enhanced coloration of bleached corals is caused by increased light fluxes in symbiont-free tissue, regardless of the stress that caused loss of symbionts.

Reversibility of Colorful Bleaching

Next, we tested whether the recolonization of bleached corals by symbionts can reverse the coloration response for our three model species. First, we documented the recovery of *P. lichen*

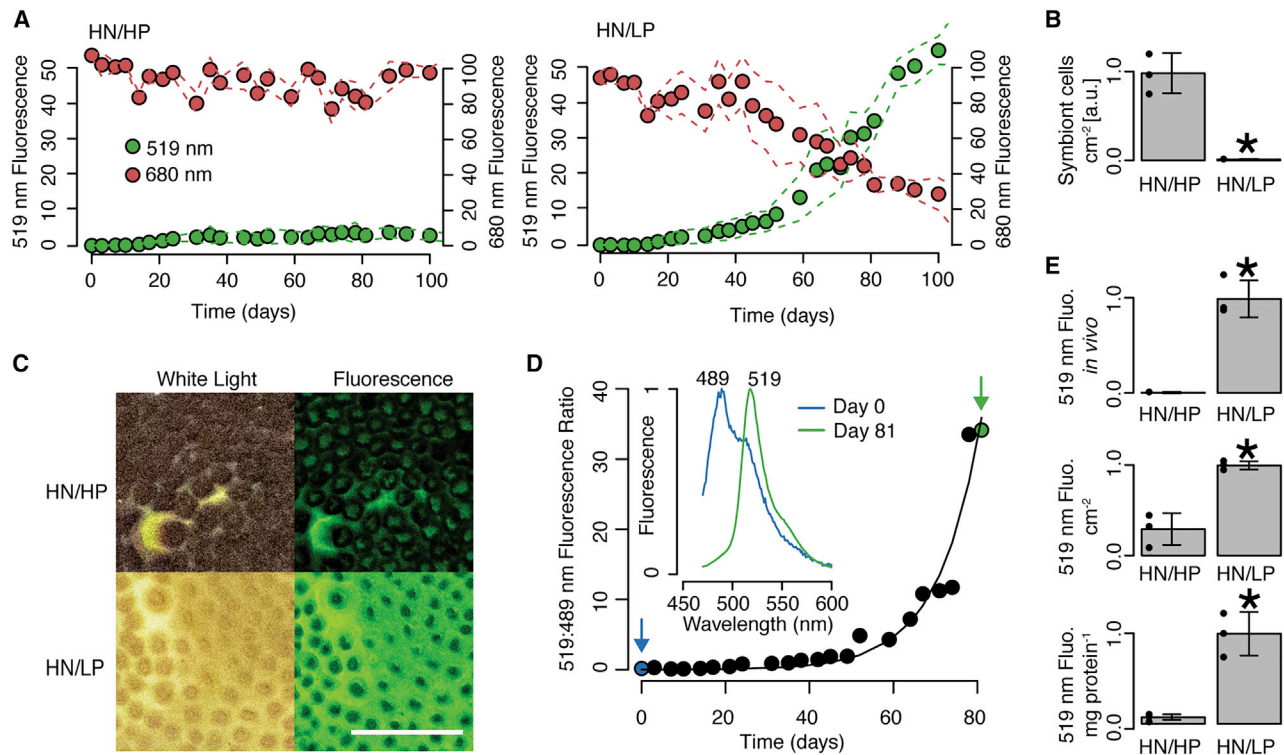


Figure 5. Fluorescence of *P. lichen* during Nutrient-Stress-Induced Bleaching

(A) Time series of *in vivo* host GFP (519 nm) and chlorophyll (680 nm) fluorescence emission for *P. lichen* kept in replete nutrient conditions (high nitrate/high phosphate [HN/HP]) or transferred to imbalanced nutrient conditions (high nitrate/low phosphate [HN/LP]). Means (spheres) \pm SD (dashed lines); $n = 3$ replicate colonies.

(B) Post-treatment symbiont cell counts normalized to coral surface area.

(C) Post-treatment photographs (white light or 450-nm excitation). Scale bar: 10 mm.

(D) Ratio of mean GFP (519 nm) to mean CFP (489 nm) fluorescence emission for HN/LP coral, with *in vivo* spectra taken at time points indicated.

(E) Host GFP (519 nm) fluorescence measured *in vivo* (top) and GFP concentration measured as emission of host tissue homogenate normalized to coral surface area (middle) and to total host protein (bottom).

In (B) and (E), bars show mean \pm SD; $n = 3$ replicate colonies; spheres indicate individual data points; asterisks signify $p < 0.05$. See also Figures S4 and S5 and Tables S3 and S4.

and *P. damicornis* that were bleached by red light exposure as introduced in Figures 3E, 3F, S5A, and S5B. *In vivo* spectroscopic measurements clearly show that the host pigment content in colorfully bleached corals reduces as they recover their symbiont population (Figures S6B and S6E). As expected, the reduction of internal light fluxes in *P. lichen* through the competing absorption by the symbiont pigments results in a reverse trend in the accumulation of our high- and low-threshold-induced biomarkers, manifesting as decrease in the GFP:CFP emission ratio. The reflectance spectra recorded over the complete bleaching and recovery cycle of *P. lichen* and *P. damicornis* further emphasize the key role of reflection of incident light by the skeleton in enhancing internal light fluxes in the bleached corals and the role of the host pigments as optical dampers in the colorfully bleached state (Figures S5C and S5F). The reflection spectra provide also further evidence of the return of the host pigment levels to the pre-bleaching values in the recovered specimen. When nutrient-replete conditions were restored in the case of colorfully bleached *Montipora foliosa*, the corals recovered and their color reverted to show the initial state where the CP expression is

limited to the symbiont-free growth margins (Figure S5F). Taken together, our observations illustrate that the color changes observed during bleaching are part of a light-mediated feedback loop in which the expression of host pigments is influenced by the presence of the symbiont and vice versa (Figure 6). We note that, depending on the nature of stress, the colorful bleached state may develop after (Figure 4A) or along with (Figure 5A) the loss of symbionts.

Is Colorful Bleaching Biologically Relevant?

Our observation of colorful bleaching of *M. foliosa* demonstrates that the host pigment concentrations in the tissue of bleached corals can reach the same levels as in the healthy yet symbiont-free growth margins of this species (Figures 6, S5F, and S5G), where this pigment naturally facilitates the colonization with symbionts under ambient conditions [20, 36]. Hence, the increased host pigment levels in colorfully bleached corals have clear potential to aid recovery of bleached corals by damping light fluxes in the symbiont-depleted tissue (Figures S4B, S5E, S6C, and S6F). To provide experimental support for such a protective function, we locally

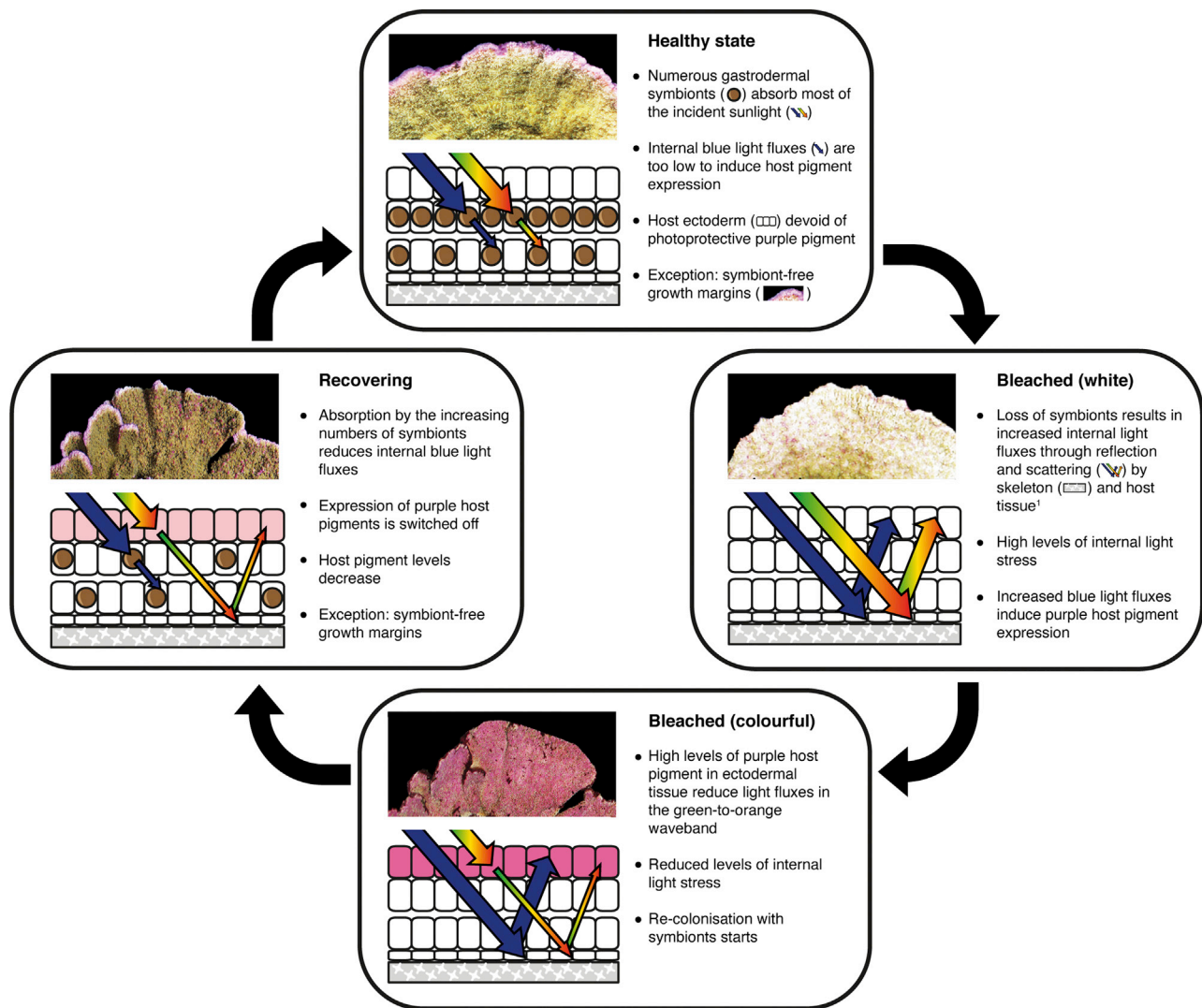


Figure 6. The Host-Symbiont Feedback Loop of Light-Mediated Pigment Expression in Reef Corals

Photographs show changes in coloration of *Montipora foliosa* during bleaching and recovery. Schematic drawings conceptualize the changes in light fluxes and host pigment production in dependence of the concentration of symbiont cells and the photoprotective purple host pigment in the coral tissue.

¹See [11, 32].

bleached *P. damicornis* by focused red light stress and then promoted or prevented the production of the photoprotective CP by exposure to blue or green light, respectively (Figure 7A). Afterward, the corals were exposed to white light illumination to monitor the recovery of the symbiont population in the presence or absence of enhanced levels of light-screening host pigments. The absorption properties of the corals indicated that tissue areas expressing high initial levels of pink CP (Figure 7B) show a significant increase in amount of symbiont pigments after 25 days (Figure 7C). Measurements conducted at this time point revealed that the changes in tissue absorption properties in the areas of increased levels of CP-mediated photoprotection held higher symbiont cell densities, indicative of a faster recovery of the symbiont population (Figure 7D). In line with previous light-stress experiments [20], the photosystem II maximum quantum efficiency (Fv/Fm) of the

symbionts was significantly higher in areas that had a higher CP content (Figure 7D), indicating a recovery of the algal population [37, 38]. We also consistently observed recovery of pink colonies of *P. damicornis* that had been experimentally bleached by nutrient stress (Figure S7). These findings are further underpinned by observations during natural bleaching events that report enhanced survival of coral colonies containing high levels of FPs and CPs [12, 14]. Specifically, *Porites* colonies that had developed brilliant blue and green colors during the bleaching event in Panama were reported to be spared from mortality [12].

Why Do Not All Corals Bleach Colourful?

The question why only some corals produce high levels of photoprotective pigments when being bleached can be answered by considering the frequent occurrence of color polymorphisms in

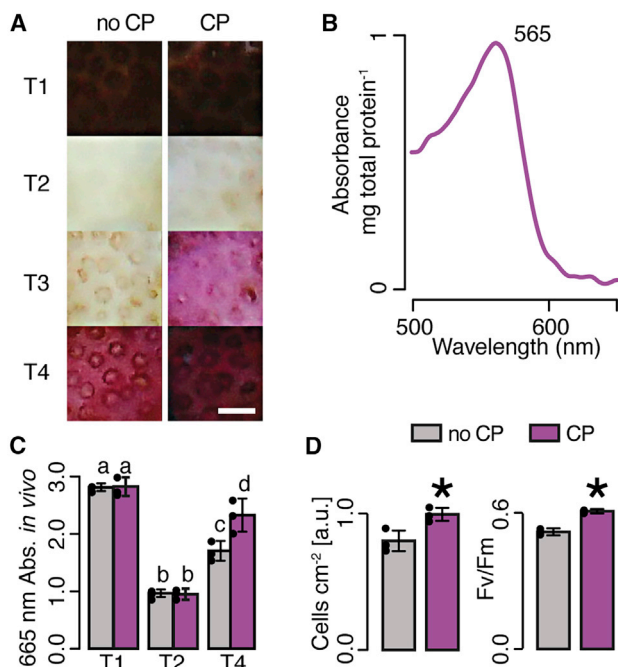


Figure 7. Influence of Coral Chromoprotein Content on Recovery from Bleaching

(A) Representative photographs of *P. damicornis* with and without CP expression during the time course of bleaching and recovery. T1, pre-treatment; T2, bleached; T3, after CP induction; T4, after 26 days recovery. Scale bar: 3 mm.

(B) Absorbance spectrum of *P. damicornis* CP.

(C) Contribution of symbiont pigments to *in vivo* absorbance.

(D) Symbiont cell counts and their *in hospite* photosystem II maximum quantum efficiency (Fv/Fm) for corals sampled at T4.

In (C) and (D), bars show mean \pm SD; $n = 3$ replicate colonies; spheres indicate individual data points; asterisks signify $p < 0.05$; letters show $p < 0.05$ in post hoc pairwise comparison. See also Figure S7 and Table S3.

coral species [10, 19, 39, 40]. Depending on the genetic background of the color morphs, their maximal capacity to produce pigments in response to light stress can differ significantly between individuals [19, 41]. Accordingly, symbiotic corals are able to pursue different strategies to deal with environmental stress and optimize the exploitation of different niches along the steep light gradients characteristic for coral reefs [41]. To quantify the potential of natural populations of key reef-building coral species to exhibit a coloration response under relevant stress conditions, we determined the proportion of color morphs of natural populations that express visible amounts of blue and pink CPs [17, 35]. In a reef system in the Northern Red Sea, shallow-water populations (<5 m) of all examined species (*Acropora* sp., *Stylophora pistillata*, and *Pocillopora damicornis*) consisted of >50% colored individuals (Figure S1). In line with published literature [19], expression levels among colored morphs can vary. In three reefs spread over 26 km in the central Red Sea, we found that ~50% of *P. verrucosa* populations in depths <5 m accumulate visible amounts of pink CP, although ~20% contain high pigment amounts (Figure S1). We conclude that more than 50% of the surveyed corals have the genetic background that can render them more colorful during relevant

bleaching conditions. These data are in excellent agreement with earlier observations of 45% of *Pocillopora verrucosa* and 40%–85% of the *Acropora* sp. colonies on a reef in Tahiti developing enhanced coloration and fluorescence during a bleaching event in 1994 [13]. The high proportion of morph with the capacity to express GFP-like pigments can also explain our finding that, during colorful bleaching events, ~40% of the reef surface in shallow water can be covered by brightly colored coral colonies (Figures 1B–1D).

Why Do Not All Bleaching Events Provoke Extreme Coral Coloration?

Our study shows that an optical feedback loop involving the scleractinian host and the dinoflagellate symbiont drives increased expression of photoprotective pigments, rendering some corals brightly colored during mass bleaching episodes. This coloration response relies on an upregulation of pigment production and, hence, a functional expression machinery. Because several experimental studies have reported that the expression and accumulation of coral host pigments is inhibited by temperatures $\geq 31.5^\circ\text{C}$ [27, 42–44], we suspected that the intensity of the stress may play a critical role. The lack of light-mediated upregulation at higher temperatures may indicate that the host pigment production is failing or that energy reserves are allocated to other stress responses, such as the expression of heat shock proteins [45]. As a result, the corals will bleach mostly white under the latter conditions. In support of this hypothesis, our heat stress experiments with *P. lichen* revealed that corals accumulated large amounts of host pigments only after the experimental specimen were relieved from heat stress (Figure 4A). Furthermore, we consistently observed that replicate colonies of our *P. damicornis* model exposed to acute heat stress bleached white and died, although those that were bleached by nutrient stress at ambient temperatures showed enhanced pigmentation and recovered when returned to ambient conditions (Figure S7). Consequently, colorful bleaching indicates that corals were exposed only to mild or short episodes of heat stress or that interacting stressors, such as nutrient stress, have caused a breakdown of the symbiosis at temperatures around or below the thermal bleaching threshold.

Indeed, with exception of the Okinawa 2016 event, heat stress conditions during the colorful bleaching events, characterized and classified as DHWs (degree heating weeks), can be considered mild (Figure S1B) [46, 47]. This is confirmed by high-level coral survival reported for these events (Table S1; Figure S1B) [12, 48–50]. Survival rates ranged from 100% (Lizard Island long-term observation; Philippines at the time of colorful bleaching) through high (90%, New Caledonia, Palmyra) [49] to moderate-to-high (location dependent; 65%–98%; Okinawa) [50]. During earlier colorful bleaching events on Tahiti, survival rates ranged from 80%–85% (1991) to >>85% (1994) [13, 48]. These observations suggest that colorful bleaching can be predictive of sublethal stress conditions and mortality rates of <20%. Together with the potential protective function of the enhanced pigmentation, colorful bleaching could therefore signal enhanced recovery prospects of the affected reefs.

Critically, after documentation of the colorful bleaching event in the Philippines, temperatures increased further and remained elevated for >7 weeks. As predicted by the DHW stress

conditions, corals experienced ultimately complete mortality (Figure S2B; Table S1). Hence, the full temperature history of a colorful bleaching event needs to be considered when evaluating post-colorful bleaching mortality and recovery prospects.

In 2010, Lizard Island experienced a minor heat stress episode that was short and mild compared to other years (Figure S2A), during which widespread coral bleaching and mortality was reported from the Great Barrier Reef (GBR) [51]. According to the DHW stress classification, this episode ranks just above the threshold for “bleaching possible” (Figure S2B); still, colorful bleaching was documented (Figures 1 and 2). Notably, in two other years, 2004 and 2013, heat stress exposure of corals on Lizard Island, also deduced from satellite data, was higher than in 2010 (Figure S2A). However, no bleaching was reported [51]. Either these bleaching episodes went unnoticed or other environmental parameters may have increased the bleaching susceptibility and resulted in extreme coloration of corals in 2010. In this scenario, colorful bleaching events could provide a useful visual warning that reefs are impacted by local stressors in combination with heat stress.

DISCUSSION

We conclude that colorful bleaching is an emergency response of symbiotic corals driven by an optical feedback loop involving host and symbionts. This process may represent an adaptive mechanism to minimize high light stress due to increased light fluxes in the bleached host tissue caused by back scattering of the highly reflective coral skeleton [11] and promote recolonization with symbionts after sublethal stress events. We have also shown the potential of colorful bleaching to identify mild bleaching conditions with good recovery prospects and events during which the temperature tolerance of corals may be compromised by interacting stressors, such as nutrient stress. In contrast to climate-change-related warming of seawater that needs to be addressed at a global level, the latter stressor can be managed at the regional scale, supporting efforts to mitigate the coral reef crisis in a two-pronged approach [52]. Hence, future work should refine the temperature threshold for colorful bleaching, investigate how this is affected by underlying nutrient stress, and detail how the changes in internal light fluxes are translated in altered pigment expression of the coral host.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.cub.2020.04.055>.

A video abstract is available at <https://doi.org/10.1016/j.cub.2020.04.055#mmc4>.

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AUTHOR CONTRIBUTIONS

E.B., C.D., and J.W. designed the study, performed the analysis, and wrote the manuscript. C.D. performed the *M. foliosa* bleaching experiment. J.W. contributed the biochemical and photophysical characterization of the CFP and GFP from *P. lichen*. R.A. performed the red light bleaching and recovery experiment. C.D. and J.W. quantified the proportion of color morphs for *Acropora* sp., *S. pistillata*, and *P. damicornis* in the Northern Red Sea, whereas M.Z. classified the *P. damicornis* color morphs in the Central Red Sea. E.B. performed all other experiments. M.P. provided documentation of the Lizard Island bleaching event and information on wider GBR bleaching.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
Pierce BCA Protein Assay Kit	Thermo Fisher Scientific	Catalog number 23227
Deposited Data		
NOAA Coral Reef Watch 50-km Satellite Virtual Station Time Series	http://coralreefwatch.noaa.gov/satellite/vs/index.php	N/A
Software and Algorithms		
R version 3.5-3.6	https://www.r-project.org/	N/A
ImageJ version 2	https://imagej.net/	N/A

RESOURCE AVAILABILITY

Lead Contact

Requests for further information and data should be directed to and will be fulfilled by the lead contact, Jörg Wiedenmann (joerg.wiedenmann@noc.soton.ac.uk).

Materials Availability

This study did not generate new unique materials.

Data and Code Availability

The satellite heat stress data is available from NOAA Coral Reef Watch, <http://coralreefwatch.noaa.gov/satellite/hdf/index.php>. All other datasets generated and/or analyzed during the study are available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Coral propagation and rearing conditions

Colonies of *Porites lichen* (Dana 1846), *Pocillopora damicornis* (Linnaeus 1758), and *Montipora foliosa* (Pallas 1766), were initially obtained from the UK ornamental trade and propagated in the Coral Reef Laboratory mesocosm facility for > 10 years [53]. Replicate colonies for experimental treatment were mounted flat on ceramic tiles to ensure an even distribution of incident light. The corals were left to recover and grow for at least a month in this position before the start of experimental treatments. Ambient conditions for all experimental models consisted of replete nutrients (high nitrate: ~6.5 μM / high phosphate: ~0.3 μM) [4], 24–26°C water temperature, and 12h:12h light:dark cycle under white (10,000 Kelvin) metal halide lamps (Aquamedic, Coalville, UK).

METHOD DETAILS

Field data collection

Image acquisition

Coral colonies displaying enhanced host pigmentation were photographed on reefs on Lizard Island, Northern Great Barrier Reef, Australia (14°40'S, 145°07'E) on 25/03/2010 at a depth of ~4 m. Images and eyewitness descriptions of colorful bleaching across the globe and resulting survival rates were obtained courtesy of the authors detailed in Table S1. Aerial footage of the New Caledonia bleaching event was acquired through Adobe Stock (Adobe Stock License ADB093421050UK).

Abundance of color morphs

The abundance of color morphs of *S. pistillata*, *P. damicornis* and *Acropora* sp. was quantified in fringing reefs in the Northern Red Sea close to Eilat, Israel (29°29'50"N - 34°54'44E to 29°29'44"N - 34°54'32"E). For each species, six to seven coast-parallel > 25 m belt transects were surveyed in a depth < 5 m to analyze at least 250 individuals per species. The number of individuals with clearly detectable pink (*S. pistillata*, *P. damicornis*) or blue (*Acropora* sp.) CP content were recorded and compared to the number of individuals with beige color. The percentage of colorful individuals per total number of representatives of each species per belt transect were determined and used to calculate mean and standard deviation.

Visual surveys of the pink color morphs of *Pocillopora verrucosa* were conducted on the ocean facing side of three mid-shelf reefs off the coast of Saudi Arabia (Qita Al Kirsh: 22°25'48"N, 38°59'29"E; Al Fahal: 22°16'01"N, 38°57'28"E; Um Albalam: 22°12'04"N,

38°57'04''E) in depths < 5 m. The intensity of pink pigmentation was scored directly against reference color scales under artificial light provided by a white light diving torch to avoid the underwater light conditions to influence color perception.

Replication

For each species, all samples were derived by asexual propagation from the same mother colony. Therefore, replicate colonies, as defined here, are small colonies with the full capacity for autonomous growth. While being genetically identical, they have experienced independent life histories. This approach was necessary in order to exclude the confounding effects by intraspecific color polymorphisms due to variations in the number of active gene copies that can result in highly different levels of individual GFP-like protein expression [19, 41]. While the dynamic response to environmental light stimuli such as upregulation of the pigment expression under high light conditions are the same for different color morphs, the absolute values can differ substantially [19]. For the optical time series measurements presented in Figures 3 and 4, corals were kept intact and measurements were performed on replicate areas; this was preferable to an identical protocol performed after sub-fragmentation, because i) it ensured minimum variability in the incident light field, critical for GFP-like protein regulation [18], and ii) it minimized bias from enhanced expression associated with wounding and growth margins [36]. Where end-point destructive sampling was required, this was performed on replicate fragments.

Experimental treatments

High light treatment

Two replicate colonies of *P. lichen* were acclimated to 80 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ from a metal halide lamp for 35 days, then exposed to 290 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for 55 days. The experiment was performed at $25 \pm 1^\circ\text{C}$. Per colony, fluorescence spectra were recorded for three replicate areas.

Partially purified tissue extracts of *P. lichen* from low light treatments that contained predominantly CFP were exposed to high intensity light ($\sim 1000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) produced by near UV (peak wavelength $\lambda = 410 \text{ nm}$, full width at half maximum [FWHM] ~ 40) or blue (peak wavelength $\lambda = 465 \text{ nm}$, FWHM ~ 40) Aquaray LEDs (Tropical Marine Centre, London, UK) in a quartz cuvette in a Cary Eclipse fluorescence spectrometer (Varian, Palo Alto, CA, USA). Time courses of changes in the fluorescence spectra were recorded as described below.

Light-induced bleaching

Six replicate colonies of *P. lichen* and six of *P. damicornis* were bleached by exposure to 400 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ of red light [30] (Lumileds, San Jose, CA, USA; peak wavelength $\lambda = 660 \text{ nm}$, FWHM = 40 nm) over 7 days. Replicate colonies were then either placed under 100 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ of blue light (Lumileds, $\lambda = 450 \text{ nm}$, FWHM = 40 nm) for 18 (*P. lichen*) or 22 days (*P. damicornis*) to stimulate FP/CP upregulation [18], or placed under an equal photon flux of green light (Lumileds, $\lambda = 530 \text{ nm}$, FWHM = 60 nm) to prevent FP/CP expression [18]. To monitor recovery of *P. damicornis*, replicate colonies previously treated with blue or green light were placed to recover under 200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ of white light from a metal halide lamp (Aquamedic) for 26 days.

Heat-induced bleaching

To monitor changes in coral color during bleaching over time, two separate experiments were performed using intact *P. lichen*. Per experiment, six replicate areas were measured. At the beginning of the experiments, corals were acclimated to the light fluxes in the treatment tanks (200–240 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$) for at least 20 days. Afterward, temperatures were ramped up to 31–32°C over ~ 2 weeks ($\Delta \sim 0.5^\circ\text{C/d}$) and kept stable at the maximal temperature for ~ 3 weeks. Finally, temperatures were ramped down to 25–28°C and kept stable for the remainder of the experiment. Average values were calculated from the two experiments.

Bleached corals were sampled in a third repeat of the experiment after 10 (B1) or 18 (B2) days of heat stress; control corals (H) were sampled from another, identical compartment of the experimental system where the corals were kept in parallel at 26°C under the same light intensity (120 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$).

To monitor the recovery of *P. damicornis* from acute heat stress, temperature was increased from 26°C to 31°C over 8 days, kept stable for 9 days, and ramped down to 26°C over 6 days. The recovery was photographically documented over 77 days.

Nutrient stress-induced bleaching

For the time series data, six replicate colonies of *P. lichen* were kept at 26°C under 180 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ in either replete (high nitrate: $\sim 6.5 \mu\text{M}$ / high phosphate: $\sim 0.3 \mu\text{M}$; HN/HP, or imbalanced (high nitrate: $\sim 6.5 \mu\text{M}$ / low phosphate: $\sim 0.006 \mu\text{M}$; HN/LP) nutrient conditions [4, 53] for 100 days. For endpoint sampling, six replicate colonies of *P. lichen* and *P. damicornis* were kept in HN/HP or HN/LP conditions at 26°C under 190 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for 107 days. Recovery of *P. damicornis* after return in HN/HP conditions was photographically documented over 77 days. For *M. foliosa*, three replicate colonies were kept in HN/LP conditions under 200 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for 56 days, then left to recover in HN/HP conditions for a further 56 days. Margins (HM) and inner regions (HI) of three unbleached replicate colonies were sampled before HN/LP treatment, while recovering inner regions (RI) were sampled after 28 days HN/HP recovery.

In vivo fluorescence and reflectance

All *in vivo* fluorescence emission measurements were performed with a fluorescence spectrophotometer (Varian, Palo Alto, CA, USA) equipped with a fiber optic probe [18], using 450 nm excitation light. For *P. lichen*, spectra were unmixed using the least-squares method [54] into 3 endmembers: CFP (489 nm), GFP (519 nm) and symbiont pigments (680 nm). Endmember contributions to measured spectra were used to plot time series.

Fluorescence images were taken with a 450 nm LED (Aquaray Fiji Blue, Tropical Marine Centre, London, UK) for excitation through a 500 nm long-pass emission filter (Nightsea, Lexington, MA, USA). Photographs were obtained by imaging corals side by side with a digital compact camera (Olympus, Shinjuku, Japan).

Reflectance spectra were collected with a USB4000 modular spectrometer and a tungsten halogen light source connected to a dip probe (Ocean Optics, Largo, FL, USA), using a Spectralon 99% reflectance standard (Labsphere, North Sutton, NH, USA) as reference. Absorbance of *P. damicornis* was calculated from reflectance spectra as $\log(1/R)$ [11]; spectra were unmixed into two end-members, pink CP (565 nm) and intact symbionts (665 nm). The Photosystem II maximum quantum efficiency (F_v/F_m) of *P. damicornis* was recorded after 12h dark acclimation with a DIVING-PAM (Walz, Effeltrich, Germany) under exposure to dim light ($< 5 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) prior to and during measurements [38].

Symbiont cell numbers and host pigment content

Tissue was harvested by airbrushing corals with artificial seawater. Surface area sampled was measured from skeleton photographs using image analysis software Fiji [55]. 50 μL of each sample were separated for symbiont cell counts, performed in 10 μL aliquots with a haemocytometer (Marienfeld, Lauda-Königshofen, Germany) under a fluorescence microscope (Leica, Wetzlar, Germany). The total tissue extract was separated into host and symbiont fraction by slow centrifugation (4°C, 2000 *rcf.*, 2 min followed by 3000 *rcf.*, 5 min), and subsequently remaining cellular debris was removed from the host fraction (4°C, 20,000 *rcf.*, 45 min). Total host protein concentration was measured via Pierce BCA colorimetric assay (Thermo Fisher, Waltham, MA, USA) against BSA standard. For *P. lichen*, fluorescence was measured by loading 0.25 $\mu\text{g}\mu\text{L}^{-1}$ protein diluted in artificial seawater (total volume 200 μL) in a fluorescence spectrophotometer (Varian) equipped with 96-well plate reader; emission spectra ($\text{ex} = 450 \text{ nm}$) were unmixed as described for *in vivo* data. Clarified tissue extracts from *P. lichen* expressing both CFP and GFP after acclimation to light of $\sim 150 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ were subjected to differential precipitation with isopropanol as described [28]. Briefly, the tissue extract was stepwise supplemented with isopropanol to reach concentrations of 50%, 100% and 200%. After each step, the samples were centrifuged (20,000 *rcf.*, 10 min). The supernatant was transferred to a new tube and more isopropanol was added. The protein precipitates resulting from each step were dissolved in phosphate buffer (150 mM, pH 6.8) and subjected to fluorescence spectrometric analysis.

For *P. damicornis*, absorbance of clarified tissue lysate diluted in artificial seawater (1.5 $\mu\text{g}\mu\text{L}^{-1}$, total volume 100 μL) was measured in a 10 mm quartz cuvette using a UV-Vis spectrophotometer (Varian). Spectra were background corrected and unmixed as described for *in vivo* data, to remove contribution of symbiont protein to measured absorbance. Pink CP concentration in tissue extracts was calculated from the specific absorbance using the published molar extinction coefficient and molar mass [35]. For *M. foliosa*, the purple CP was further purified from the cleared extracts by size-exclusion chromatography [36] and normalized to the total protein content of the sample.

Satellite heat stress data

Sea surface temperature and DHW data for Lizard Island (GBR), Amedee (New Caledonia), Okinawa (Japan), Palmyra and El Nido (Philippines) virtual stations and local bleaching thresholds were obtained from NOAA Coral Reef Watch (2000, updated twice-weekly. NOAA Coral Reef Watch 50-km Satellite Virtual Station Time Series Data for Lizard Island and El Nido, Jan. 1, 2002-Dec. 31, 2016. Silver Spring, Maryland, USA: NOAA Coral Reef Watch. Dataset accessed 2019-11-15 at <http://coralreefwatch.noaa.gov/satellite/vs/index.php>). The bleaching threshold was calculated as maximum monthly mean temperature +1°C (NOAA Coral Reef Watch) and set to “0” to facilitate comparison between regions. Timelines were aligned by setting the day when the local bleaching threshold was clearly exceeded to “0.” Bleaching years for Lizard Island were identified based on data for “Australia, GBR Northern” in reference [51].

QUANTIFICATION AND STATISTICAL ANALYSIS

The 519:489 nm ratio time series data were fitted with logistic functions parameterised using non-linear least-squares, with the exception of the HN/LP *P. lichen* dataset which was cropped at 80 days and fitted with an exponential function; this was due to the 489 nm contribution reaching zero at this point, and driving the ratio to infinity. Endpoint measurements were tested for significant difference in means using ANOVA; where more than two samples were compared, Tukey’s HSD was used for post hoc testing upon detection of a significant difference. Results of statistical analysis and model equations are presented in Tables S2–S5. For the satellite heat stress data, mortality rates were expressed as a function of DHW using an exponential function (Table S6). All analysis was performed using R v3.5-3.6. Throughout the analysis, mean and standard deviation were used to describe central tendency and dispersion; sample size and definition of replicates are provided in each figure legend, and significance was defined as $\alpha = 0.05$ for all statistical tests.