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Optical feedback loop involving dinoflagellate symbiont and scleractinian host drives colourful coral bleaching

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Abstract:	Coral bleaching, caused by the loss of brownish-coloured dinoflagellate photosymbionts from the host tissue of reef-building corals, is a major threat to reef survival. Occasionally, bleached corals become exceptionally colourful rather than white. These colours derive from photoprotective green fluorescent protein (GFP)-like pigments produced by the coral host. There is currently no consensus regarding what causes colourful bleaching events and what the consequences for the corals are. Here, we document that colourful bleaching events are a recurring phenomenon in reef regions around the globe. Our analysis of temperature conditions associated with colourful bleaching events suggests that corals develop extreme colouration within 2-3 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 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 colourful 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.

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Dear Editors, dear Reviewers,

We thank you for the constructive comments on our paper titled **"Optical feedback loop involving dinoflagellate symbiont and scleractinian host drives colourful coral bleaching"**, authored by Elena Bollati, Cecilia D'Angelo, Rachel Alderdice, Morgan Pratchett, Maren Ziegler, and Jörg Wiedenmann.

We are pleased that the reviews find that our *"data are solid, and are of interest to the coral reef community"* (Reviewer 1), that we provide a "*compelling and fascinating story*" (Reviewer 2), that "*the evidence presented in the manuscript is fully consistent with the conclusions of the authors"* and that *"considering the importance of the phenomenon described, the results are key to our understanding of coral bleaching and more importantly, to the processes that lead to the recovery of coral populations after experiencing bleaching conditions*" (Reviewer 3).

The reviewers have highlighted areas where our manuscript could be improved and we thank the editor for the opportunity to revise the manuscript accordingly. We include the initial reviews and address them point-by-point, referring also to the new material included in the revised version of the manuscript.

We appreciate your continued support!

Yours sincerely on behalf of the authors,

Prof. J. Wiedenmann

Point-by-point response to reviewer comments

We thank the reviewers for their helpful comments and address them here point-by-point.

Reviewer #1:

Bleaching is an increasingly common and devasting phenomenon on coral reefs worldwide, and there have been numerous papers describing the mechanisms by which corals lose their photosymbiotic dinoflagellates resulting in a loss of pigmentation. However, in a few cases bleaching has been observed to result in coloration from photoprotective GFP-like proteins. To date the mechanisms of colorful bleaching have been overlooked. This study demonstrates that colored bleaching in Porites lichen and Montipora foliosa is due to overexpression of protective fluorescent compounds rather than an optical effect of decreasing symbiont densities in the host tissue. The study further tested this bioindicator relative to nutrient stress to demonstrate that the colored bleaching response is likely a response to sub-lethal stressors rather than acute thermal events. This was an interesting paper, with a reasonably well-designed set of experiments designed to tease apart the aforementioned alternate hypotheses. Given the results I am reasonably convinced that the data are solid, and are of interest to the coral reef community. I do have a couple of problems with the study as described, and wonder if it is of broad enough interest to the readers of Current Biology.

First, while observations (and lovely photos in Fig 1…) indicate that colored bleaching can occur in the field, this is largely a laboratory study. The authors make a point of discussing the biological relevance of their results, but less effort is dedicated to discussing the ecological relevance. Specifically, which species exhibited colored bleaching in the field, what proportion of individuals within populations, do all individuals of a species exhibit the same colored proteins, at what concentrations, etc etc.

We agree that relevance to the natural environment is important and have therefore added new data and evidence to address these comments. Specifically, we have included a new figure 1, supported by a supplementary figure S1 and a supplementary table S1 and demonstrate that colourful bleaching is a recurring phenomenon of global reach that affects key reef building coral genera including *Acropora, Montipora, Porites*, and *Pocillopora*. We have included data that demonstrate that >50% of the individuals within populations have the potential to show enhanced colourations and also show that chromoproteins can make up 3.5% of the total host protein in colourfully bleached corals. These colours vary in a species-dependent manner. Furthermore, we have expanded the discussion to bring in additional references to underpin the ecological relevance of our findings.

Moreover, these data should be discussed relative to the bleaching conditions in the field (the NOAA satellite data are informative, but they aren't really discussed). This information is critical because the laboratory data all seem to be based on a single (cloned) genotype. How do populations vary? Are these observations relevant to the field?!?

We have analysed three additional, well-documented colourful bleaching events (Figure 2). These data are now aligned with the case studies from the initial submission to facilitate comparison, to establish trends in temperature conditions that lead to colourful bleaching and to highlight their similarities to our laboratory experiments. Therefore, our experimental data are ideally suited to provide a mechanistic explanation of the field observations.

I am also concerned that the authors don't "close the loop" with P lichen; they switch to M foliosa for recovery experiments. There appears to be some lip service to the value of this alternate model, but I would ideally like to see this story in a single "lab rat", and then compared to other species for the broader implications.

In the revised version of the manuscript, we show the recovery not only for *Montipora foliosa*, but also for *Pocillopora damicornis* and for *Porites lichen*, the main model coral used in the present study", demonstrating the full cycle for all three coral species (Figure S6).

I would also like to see the experiment conducted on a reef flat, with multiple genotypes, but I gather that isn't possible.

We agree that a field experiment of comparable complexity is not possible because of the difficulties to precisely control environmental conditions and to conduct detailed spectroscopic measurements over the required time period. Therefore, our laboratory study fills in an important knowledge gap and with the added material presented in revised manuscript, we can now also provide evidence for the relevance of the mechanism to the natural reefs.

Finally, this appears to be a specialized case of bleaching (in 1-2 corals). While there is some intriguing mechanistic data, I'm not sure it has broad enough interest (or is contextualized against thermal bleaching well-enough) to inform a broader CB community. I would recommend sending this to Coral Reefs or Limnology & Oceanography.

Our new data highlight that colourful bleaching is a recurring phenomenon of global reach that affects key genera of reef building corals. Furthermore, with the mechanistic understanding generated in our laboratory experiments, we can now explain natural colourful bleaching events as consequence of episodes of brief or mild heat stress exposure and / or the influence of interacting stressors. Therefore, colourful bleaching has the potential to identify mild bleaching conditions with good recovery prospects and to pinpoint events during which the temperature tolerance of corals may be compromised by interacting regional stressors.

Colourful bleaching has been prominently showcased in the Netflix documentary "Chasing Coral" (https://www.chasingcoral.com/2019/07/25/chasing-coral-turns-2/) and has been widely reported in the news. The documentary has been shown in +100 countries and is used in public screenings to raise awareness about coral reef decline. The producers of the documentary, the international nonprofit marine conservation organisation "The Ocean Agency" were appointed to coordinate the "International Year of the Reef 2018" by the International Coral Reef Initiative, working in collaboration with the United Nations Environment Programme. Inspired by the colourful bleaching phenomenon, they launched the "GLOWING GLOWING GONE" campaign, aiming to protect coral reefs through global public engagement and action (https://www.glowing.org/the-warning). International support for the initiative comes from "Adobe" and "Pantone" amongst numerous companies and initiatives (https://www.glowing.org/whos-glowing). As acknowledged in the reviews, our manuscript describes the elusive mechanism underlying colourful bleaching. Therefore, we are convinced that there is a broad general interest in the subject that goes well beyond specialist research communities and that "Current Biology" is the ideal platform to explain the phenomenon.

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Reviewer #2:

In this paper, the authors assessed what putatively causes colorful bleaching events and propose that the increase of light in the absence of symbionts (bleaching) induces expression of coral host protective pigments that cause the colorful bleaching. In itself, it is a compelling and fascinating story, but several of the connections the authors make are not backed up by data:

1. the authors didn't validate their findings in 3 coral species, but rather used a combination of 3 different species to follow distinct responses: photo acclimation/bleaching, photo protective chromoproteins, host symbiont feedback loop; as such, it is uncertain whether a single coral species adheres to all the results shown here; this is further complicated by the fact that the authors used a single biological replicate

We agree. In the revised version of the manuscript, we show the recovery not only for *Montipora foliosa*, but also for *Pocillopora damicornis* and for *Porites lichen* (Figure S6), the main model coral used in the present study, demonstrating the full cycle for all three coral species.

2. further: above is an issue, since the 'biomarkers' consist of low light and high light proteins that are not identified; that is to say, the number of CFPs and GFPs in each coral under investigation may as well be very different; while this doesn't necessarily take away from the overall finding, it needs to be stated explicitly and implications discussed

In the revised manuscript, we clearly state that these proteins have not been classified at their sequence level:

"…*While 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 characterised 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)…"*

Importantly, we establish through our experiments that their tissue concentrations clearly follows the previously established, species independent trends that GFP/CP production in shallow water corals increases along with the light exposure, whereas CFP production levels out or decreases at high light level (References D'Angelo et al 2008, Smith et al 2013, Gittins et al 2015 of the submitted manuscript and Roth et al 2010, JEB 213: 3644-3655).

3. The authors have no 'proof' of an optical feedback loop and a concomitant increase of CFPs sensu stricto; that is to say: the observed data correlates to the light in coral tissue available, but it's not clear whether light availability is 'sensed' or whether any other mechanisms is employed by the coral; this needs to be rephrased or stated

In the revised manuscript, we state that future research should identify the mechanism by which the corals sense the increased light fluxes:

"…Hence, future work should refine the temperature threshold for colourful 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..."

Importantly, in the context of this manuscript, we demonstrate experimentally that the differential regulation of FPs/CPs is a (blue) light driven process in agreement with published literature. Furthermore, the extended discussion of the role of light reflection by the coral skeleton will further clarify the concept of optical feedback in bleached corals (Enriquez et al., 2005).

3. based on the numbers in the figures, the authors have extreme cases of coral bleaching: almost all symbionts are lost from the tissue; commonly, about a 50%-70% loss of symbionts results in visually pale colonies; how are the differences in remaining symbiont density might be related to the phenomenon here?

The inclusion of an extended discussion of the increase of light fluxes in bleaching corals through skeletal backscattering / reflection (Enriquez et al. 2005) clarifies that the high light-driven FP/CP expression will increase along with the internal light fluxes (D'Angelo et al 2008). Furthermore, in the new material included the in the revised manuscript, we demonstrate for *Porites lichen* that a strong colour response is also induced in corals that are less bleached than the examples mentioned by the reviewer.

4. Figure 3: symbiont cell numbers are unclear. is this in million per cm2? This should be amended; symbiont cell numbers for Figure 3b B2 are zero obtained from a single replicate?

We acknowledge that the distribution of scale units on the y-axis made it difficult to appreciate that these numbers had been normalised to the coral surface (cm^2) and then scaled to "1". This has been rectified in the revised manuscript. The scaling facilitates comparison with the dimensionless fluorescence levels that change over the time course of the experiment. Please note that for timepoint B2 (now Fig. 4), three replicates were analysed, all of which returned non-detectable symbiont numbers. Accordingly, there are no visible errors bars from replicate measurements.

Reviewer #3 (Professor Roberto Iglesias-Prieto, The Pennsylvania State University)

In this manuscript Bollati and coworkers present the results of a study aimed to understand the functional significance of the over expression of pigments related to green fluorescent proteins (GFP) of animal origin, in the modulation of the internal light fields of corals during bleaching. The authors distinguished between two different types of animal chromoproteins, the fluorescent ones (CF's) and the non-fluorescent (CP's). Their main finding indicate that the overexpression of these pigmented proteins provides a layer of photoprotection during bleaching events facilitating the recolonization of coral tissues by symbiotic algae. The basic concept is that the reduction in symbiont densities and the concomitant reduction in photosynthetic pigment absorption characteristic of coral bleaching generate an optical feedback loop increasing local irradiance. The authors hypothesize that the colorful bleaching results from the induction of expression and accumulation of FP's and CP's. To test their main hypothesis, they employed a "biomarker" approach to indirectly detect the increase in local irradiance resulting from bleaching.

In the first part of the manuscript, Bollani et al, validated their approach using non-invasive fluorescence spectroscopy. The results presented, clearly indicate that the expression of CFP (Cyan Fluorescent Protein (CFP) relative to GFP is driven solely by light and not by the thermal stress resulting from bleaching. Using this information Bollati and coworkers estimated that the increases in animal pigment contents results from increases of the local light fields and not by the thermal stress, furthermore the authors tested the effects of other stressors on coral pigmentation. In particular they tested if bleaching resulting from phosphate starvation elicit the same phenotype as thermal stress in terms of animal pigmentation. The results presented are consistent with the conclusion regarding the control by light of the expression of the animal chromoproteins. The colorful bleaching phenotype is fully reversible after bleaching recovery. The actual biological relevance of their observation is suggested after the observation that corals expressing the colorful phenotype allow their symbiont population to recover at faster rates than those not presenting high concentrations of animal pigments. Finally, Bollini et al discuss the implications of the heterogeneity in color responses of corals during natural bleaching events. The fact that the genes encoding the chromoproteins are also thermally sensitive, may be a key factor determining the development of either a white or a colorful bleaching. This observation may have very important implications regarding the future of coral reefs under climate change.

The evidence presented in the manuscript is fully consistent with the conclusions of the authors, considering the importance of the phenomenon described, the results are key to our understanding of coral bleaching and more importantly, to the processes that lead to the recovery of coral populations after experiencing bleaching conditions.

My main criticism is centered on the narrative of the manuscript. The development of the "optical feedback loop" is not a direct result of the removal of algal pigmentation solely, as suggested by the authors. The actual optical loop is the result of back scattering of the highly reflective coral skeleton as described by Enríquez et al (2005). The skeleton acts as a secondary light source increasing the local irradiance and therefore the probability of absorption. Furthermore, as indicated by Enríquez et al (2005), once a coral is bleach, the increase in the local light fields results in a metastable state where recovery without any kind of "optical damper" could be very difficult.

Acknowledging this implication of the role of scattering on the optical loop increases the relevance of the research presented in the manuscript. Although several authors have challenge the role of the skeleton in producing the scattering (ie Wangpraseurt et al, 2012), even in figure 6 the role of the skeleton is highlighted, although the role of the skeleton is ignored in the entire manuscript. I found difficult to understand in the context of Bollani et al manuscript why a critical piece of information is ignored and the only reference to Enríquez et al (2005) is at the end of the manuscript in the context of how to estimate in vivo absorption from reflectance data in corals.
"In habitats with high levels of solar radiation, the presence of pigments such as green fluorescent proteins or pocilloporins (Salih et al. 2000; Dove 2004), functioning as "optical dampers," can modulate the internal light fields, reducing the enhancement effect of the skeleton. Although the photoprotective role of the pocilloporins and related proteins has been recently challenged (Mazel et al. 2003), our results suggest that the specific absorption coefficient of any pigment, including pocilloporins and mycosporine-like amino acids, would be augmented as a result of the interaction between solar radiation and the coral skeleton."

As illustrated by the quote from Enríquez et al (2005), the role of the skeleton in enhancing the absorptivity of the pigments, including the photoprotective animal proteins, should not be ignored in the context of the present manuscript. Putting the manuscript in a larger context will be beneficial to the manuscript and in my opinion will enlarge the scope of the results.

We wholeheartedly agree with what is said in this review and apologise that we did not convey the information clearly that the Enriquez et al 2005 paper provides a key foundation for the proposed mechanism. We demonstrate in the revised manuscript that reflection increases in bleached corals and that CPs can act as efficient optical dampers in bleached corals. We have added and discussed further reflection data and have expanded the introduction and conclusion section as suggested by the reviewer.

Summary: ***"****…We demonstrate that the increase of light fluxes in symbiont-depleted tissue promoted by reflection of the incident from the coral skeleton induces strong expression of the photoprotective coral host pigments..."*

Introduction: "…*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 (Enriquez et al 2005)] and the bleached appearance of corals…"*

"…We hypothesised that colourful 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(Enriquez et al 2005)], which results in the accumulation of FPs and CPs in the coral tissue driven by high light-induced host pigment gene expression [18,19]."

Figure 6: *Reference Enriquez et al 2005 included in scheme and legend.*

Conclusion: *"…This process may represent an adaptive mechanism to minimise high light stress due to increased light fluxes in the bleached host tissue caused by back scattering of the highly reflective coral skeleton [11(Enriquez et al 2005)] and promote recolonization with symbionts after sublethal stress* events…"

I think a quantitative description of the changes in concentration of the chromoproteins is very important, specially as we still do not know the effects of scattering in modulating the specific absorption coefficient in vivo.

We agree. We used the extinction coefficient of the pink CP of *P. damicornis* ("Pocilloporin", Dove et al 1995) to calculate the absolute content of the chromoprotein in tissue extracts and normalised these values to the total protein content for comparison (Supplementary Fig. S5):

"…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 (Figure S5C-D). In contrast, the pink CP made up only ~0.7% of the host protein in unbleached colonies kept in nutrient replete conditions…"

These data confirm that the increase in pink colouration in specimens exposed to nutrient stress-induced bleaching is due to a significantly increased accumulation of chromoproteins in the tissue in comparison to the non-bleached controls and not due to an increased visibility of the host pigment in the bleached coral. These findings are in line with published measurements that found higher CP concentrations in growth regions devoid of symbionts such as axial polyps in *Acropora* spp. (D'Angelo et al 2012, Smith et al 2013). Furthermore, the quantitative spectroscopic analyses of tissue extracts (Fig. 3e, 4e; Fig. S5c) confirm that the colour differences in bleached *P. lichen* and *M. foliosa in vivo* are caused by corresponding difference in pigment content.

Specific comments:

1) In lines 90-92 there is a casual reference about the role of FPs optimizing "the internal light environment for the benefit of the photosynthetic symbionts through scattering or wavelength-transformation" I still do not understand why the symbionts will need light to be transformed from Blue (with high absorption) to orange where dinoflagellates absorption is minimal" This information is not relevant to the manuscript and I have not seen any evidence of the "benefit" to the symbiont, although it should be relatively easy to obtain.

We have shortened the paragraph and only point the reader in direction of the relevant references. We feel that this is important to present a balanced overview of the topic:

"…Notably, FPs in coral species commonly found in lower light habitats are biochemically, photophysically and functionally distinct [14,17,22–26]."

Please refer to our previous letter for further explanations.

2) In the text presented between lines 115-151 there are numerous references to high light intermediate light and low light that require quantitative descriptions

We have included the corresponding values in the revised paragraph:

"…CFPs are usually expressed at low light intensity (~80 µmol photons m^{-2} s^{-1}) and their concentration in coral tissue becomes saturated or reduces at high light intensities (>400 µmol photons m^{-2} s^{-1}) [18]. In contrast, CPs and GFPs in shallow water species are commonly expressed

only under higher photon irradiance (>100 µmol photons m^{-2} s^{-1}) and their tissue concentrations increase with the intensity of incident blue light [18]. At low light levels (~80 µmol photons m^{-2} s^{-1}), our model coral Porites lichen expresses a CFP with a 489 nm emission peak (Figure 3A). In contrast, under high light conditions (~290 µmol photons m^{-2} s^{-1}) the tissue fluorescence is dominated by a 519 nm emitting GFP (Figure 3B). At intermediate light intensity (~150 µmol photons m^{-2} s^{-1}), both FPs are expressed…"

3) Lines 184-187 "Together, these data provide evidence that extreme colouration of bleached corals is mostly due to increased pigment accumulation in the host tissue triggered by increased internal light fluxes caused by the absence of symbionts, and only to a limited extend caused by an optical enhancement of pigment visibility" this is misleading as discussed previously, multiple scattering is responsible for the formation of the optical loop. A reference to the work from M. Kuhl or Marcelino labs is required. Removal of the algal cells in the absence of a source of scattering will increase 100% the light field, a flat coral surface will result in 300% increase and this percentage can be has high as 800% in realistic corals.

We acknowledge that this sentence can be easily misunderstood. When discussing "optical enhancement", we were referring to the better visibility of the coloured pigments against a white, highly reflective background (bleached coral) as opposed to a brown, highly absorbing background (unbleached coral). We have rephrased this paragraph to read:

" …Together, these data provide evidence that extreme colouration of bleached corals is mostly due to increased pigment accumulation in the host tissue triggered by increased internal light fluxes caused by the light reflection and scattering by the coral skeleton and tissue in the absence of symbionts [11,22,33] (Enriquez et al 2005, Marcelino et al 2013, Lyndby et al 2016)…"

In the blue spectral range (450nm) responsible for the regulation of FP/CP expression (D'Angelo et al 2008), reflectivity increases about 10-fold in bleached *Porites lichen* and *Pocillopora damicornis* (Supplementary Figs S4, S5 & S6), indicating a significant enhancement of internal light fluxes. This increase is in good agreement with the numbers provided by the reviewer.

Lines 245 -247 "…the efficiency of symbiont photosynthesis indicated by photosystem II maximum quantum efficiency (Fv/Fm) was significantly higher in areas that had a higher CP content" Fv/Fm is not a descriptor of the efficiency of photosynthesis, only of the probability of charge separation at Photosystem II under dark adaptation, although it indicates a recovery of the algal population.

We have rephrased this statement in the revised manuscript to read:

"…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 (

TITLE

Optical feedback loop involving dinoflagellate symbiont and scleractinian host drives colourful coral bleaching

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SUMMARY

Coral bleaching, caused by the loss of brownish-coloured dinoflagellate photosymbionts from the host tissue of reef-building corals, is a major threat to reef survival. Occasionally, bleached corals become exceptionally colourful rather than white. These colours derive from photoprotective green fluorescent protein (GFP)-like pigments produced by the coral host. There is currently no consensus regarding what causes colourful bleaching events and what the consequences for the corals are. Here, we document that colourful bleaching events are a recurring phenomenon in reef regions around the globe. Our analysis of temperature conditions associated with colourful bleaching events suggests that corals develop extreme colouration within 2-3 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 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 colourful 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 harboured 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 colouration 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 colours of scleractinian corals involved in these colourful 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) 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 localised 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 µmol m^{-2} 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 µmol m^{-2} 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 colour 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 colourful even in the absence of upregulation of host pigment expression in response to an environmental trigger [27].

Despite the striking incidence of brightly coloured corals during certain mass coral bleaching events, the environmental conditions that trigger colour changes, as well as the involved mechanisms and consequences for the corals, are not understood. Specifically, it is unclear whether the enhanced colouration 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 colour changes as indicators of specific environmental conditions during those bleaching events or to unravel the biological significance of colourful bleaching for the coral-dinoflagellate symbiosis.

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

RESULTS AND DISCUSSION

Colourful bleaching as a global phenomenon.

We compiled and analysed photographic evidence and eyewitness statements (Figure 1A, Table S1). The spatial and temporal distribution of these events clearly shows that colourful bleaching is a recurring phenomenon of global reach that affects key reef building coral genera including *Acropora, Montipora, Porites, and Pocillopora*. Furthermore, we analysed aerial images recorded during colourful bleaching events in New Caledonia and the Great

Barrier Reef (Figure 1B-D). In both cases, colourfully bleached corals cover ~40% of the reef surface in shallow water, confirming the significance of colourful bleaching for large parts of coral populations. The high cover of colourfully 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*, *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 colourful bleaching events

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

A biomarker approach to report changes in the internal light field in symbiotic corals.

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

While 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 characterised 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 colour in response to irradiation with strong near-UV (~410 nm) or with blue (~465 nm) light (Figure S3B,C). 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 CPF 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 (Figure 3C,D). To demonstrate unambiguously that the differential changes in host pigment expression are not an intrinsic response 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 colours. First, we bleached designated regions of interest by exposure to focussed red light. While 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 (Figure 3E,F). In contrast, no increase in tissue fluorescence was detected in the corals exposed to green light (Figure 3E,F), 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 25-26°C to gradually increasing temperatures up to 31-

32°C (Δ~0.5°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 ≥31°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 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 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 timepoint 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 (Figure 4A-C). 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 normalised to areal total protein content or the

corresponding coral surface area) showed a significantly increased value, indicating the enhanced colouration was due to host pigment accumulation in the tissue (Figure 4E). Importantly, tissue fluorescence exceeded 100x pre-treatment values ~3 weeks only after the acute heat stress treatment was terminated (Figure 4A-C). The timescale of this response is consistent with the interval between the timepoint when temperatures started to return to ambient levels and observations of colourful 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 colouration of bleached corals is mostly due to increased pigment accumulation in the host tissue triggered by increased internal light fluxes caused by the light reflection and scattering 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]. Since colourful bleaching relies on this response, the enhanced coral colour indicates that while the corals are stressed, the expression machinery for at least some proteins is functional and responsive.

Colourful bleaching in the absence of thermal stress

To test whether colourful 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 (Figure 5A,B). GFP

emission *in vivo* and tissue concentration quantified in extracts, increased during bleaching (Figure 5A,C,E). Accordingly, 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 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 (Figure S5A,B). 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 (Figure S5C-D). In contrast, the pink CP made up only ~0.7% of the host protein in unbleached colonies kept in nutrient replete conditions. Therefore, the colourful 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 ~4x higher blue light fluxes detected at the surface of bleached colonies (Figure S5E). As for the GFP in *P. lichen* (Figure S4B), the optical dampening 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 (Figure S5F,G), tips or areas of wound regeneration [36]. In these areas, the CPs are thought to reduce the internal light fluxes to facilitate the colonisation of newly formed tissue with symbionts [20,36]. After the corals were bleached by phosphate starvation, the colour 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 colourful bleaching

Next, we tested whether the recolonization of bleached corals by symbionts can reverse the colouration response for our three model species. First, we documented the recovery of *P. lichen* and *P. damicornis* that were bleached by red light exposure as introduced in Figure 3E,F, and Figure S5A,B. *In vivo* spectroscopic measurements clearly show that the host pigments content in colourfully bleached corals reduces as they recover their symbiont population (Figure S6B,E). 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 colourfully bleached state (Figure S5C,F). 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 colourfully bleached *Montipora foliosa*, the corals

recovered and their colour 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 colour 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).

Is colourful bleaching biologically relevant?

Our observation of colourful 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 (Figure 6, Figure S5F-G) where this pigment naturally facilitates the colonization with symbionts under ambient conditions [20,36]. Hence, the increased host pigment levels in colourfully bleached corals have clear potential to aid recovery of bleached corals by damping light fluxes in the symbiont depleted tissue (Figure S4B, S5E, S6C,F). To provide experimental support for such a protective function, we locally bleached *P. damicornis* by focussed red light stress, then promoted or prevented the production of the photoprotective CP by exposure to blue or green light, respectively (Figure 7A). Afterwards, 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 timepoint 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]. Furthermore, *Porites* colonies that had developed brilliant blue and green colours during a 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 colour polymorphisms in coral species [10,19,39,40]. Depending on the genetic background of the colour 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 optimise 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 colouration response under relevant stress conditions, we determined the proportion of colour 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 (<5m) of all examined species (*Acropora* sp., *Stylophora pistillata, Pocillopora damicornis*) consisted of >50% coloured individuals (Figure S1). In line with published literature [19], expression levels among coloured 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 <5m accumulate visible amounts of pink CP, while ~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 colourful 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 colourful bleaching events ~40% of the reef surface in shallow water can be covered by brightly coloured coral colonies (Figure 1B-D).

Why do not all bleaching events provoke extreme coral colouration?

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 coloured during mass bleaching episodes. This colouration response relies on an upregulation of pigment production and, hence, a functional expression machinery. Since several experimental studies have reported that the expression and accumulation of coral host pigments is inhibited by temperatures ≥31.5˚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, while those that were bleached by nutrient stress at ambient temperatures showed enhanced pigmentation and recovered when returned to ambient conditions (Figure S7).

Consequently, colourful 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 colourful bleaching events, characterized and classified as DHW (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 colourful bleaching) over high (90%, New Caledonia, Palmyra [49]) to moderate-to-high (location dependent, 65-98%, Okinawa [50]). During earlier colourful bleaching events on Tahiti, survival rates rates ranged from 80-85% (1991) to >>85% (1994) [13,48]. These observations suggest that colourful bleaching can be predictive of sublethal stress conditions and mortality rates of <20%. Together with the potential protective function of the enhanced pigmentation, colourful bleaching could therefore signal enhanced recovery prospects of the affected reefs. Critically, after documentation of the colourful 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 colourful bleaching event needs to be considered when evaluating post-colourful 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 GBR [51]. According to the DHW stress classification, this episode ranks just above the threshold for “bleaching possible” (Figure S2B), still, colourful bleaching was documented (Figure 1, 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

386 in 2010 (Figure S2A). However, no bleaching was reported [51]. Either these bleaching
387 episodes went unnoticed or other environmental parameters may have increased the
388 bleaching susceptibility and resulted in extreme colouration of corals in 2010. In this
389 scenario, colourful bleaching events could provide a useful visual warning that reefs are
390 impacted by local stressors in combination with heat stress.
391
392 **CONCLUSION**
393 We conclude that colourful bleaching is an emergency response of symbiotic corals driven by
394 an optical feedback loop involving host and symbionts. This process may represent an
395 adaptive mechanism to minimise high light stress due to increased light fluxes in the
396 bleached host tissue caused by back scattering of the highly reflective coral skeleton [11] and
397 promote recolonization with symbionts after sublethal stress events. We have also shown the
398 potential of colourful bleaching to identify mild bleaching conditions with good recovery
399 prospects and events during which the temperature tolerance of corals may be compromised
400 by interacting stressors such as nutrient stress. In contrast to climate change related warming
401 of seawater that needs to be addressed at a global level, the latter stressor can be managed at
402 the regional scale, supporting efforts to mitigate the coral reef crisis in a two-pronged
403 approach [52]. Hence, future work should refine the temperature threshold for colourful
404 bleaching, investigate how this is affected by underlying nutrient stress and detail how the
405 changes in internal light fluxes are translated in altered pigment expression of the coral host.

AUTHOR CONTRIBUTIONS

E.B., C.D.A. and J.W. designed the study, performed the analysis and wrote the manuscript. C.D.A. performed the *M. foliosa* bleaching experiment; J.W. contributed the biochemical and photophysical characterisation of the CFP and GFP from *P. lichen*. R.A. performed the red light bleaching and recovery experiment; C.D.A and J.W. quantified the proportion of colour morphs for *Acropora* sp., *S. pistillata* and *P. damicornis* in the Northern Red Sea whereas M.Z. classified the *P. damicornis* colour 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.

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DATA AVAILABILITY

The satellite heat stress data is available from NOAA Coral Reef Watch, http://coralreefwatch.noaa.gov/satellite/hdf/index.php. All other data sets generated and/or analysed during the study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare no competing interests.

FIGURE LEGENDS

Figure 1. Global incidence of colourful bleaching events.

(**A**) Colourful bleaching episodes between 2010 and 2017. The pins on the map indicate the spatial distribution of colourful 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 colourful 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 colourful colonies calculated by image analysis (**D**, mean ± s.d. n=3 replicate areas per image). Scale bars: ~1 m.

Figure 2. Temperature conditions during colourful bleaching events.

Satellite sea surface temperature (**A**, **B**) and field images (**C-G**) for colourful bleaching events on Lizard Island (**A**, **C**), New Caledonia (**A**, **D**), Okinawa (**A**, **E**), Palmyra (**B**, **F**), and Palawan, Philippines (**B**, **G**). The reefs in **C**-**F** suffered no or low to moderate post-bleaching mortality, while 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 indicates days elapsed after temperatures decreased after the heat stress maximum (spheres) (**A**, Palmyra in **B**) or exceeded the local bleaching threshold (Philippines in **B**). Image credits: Morgan Pratchett, JCU Townsville (**C**), Richard Vevers, The Ocean Agency (**D**, **E**), Brian Zgliczynski, Scripps Institution of Oceanography (**F**), Ryan Goehrung, University of Washington (**F**).

Figure 3. Photoacclimation of *Porites lichen* indicated by changes in host pigment levels. (**A**, **B**) *In vivo* spectral characterisation 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 ~290 μmol photons m^{-2} 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 ± s.d., n = 12 areas (2 replicate colonies). (**E**) Photographs of *P. lichen* before bleaching (Day 0, fluorescence image), after bleaching with focussed red light (Day 11, white light image), and after exposure to green or blue light (Day 29, fluorescence image). Scale 10 mm. (**F**) Time course of green:cyan emission ratio for bleached *P. lichen* exposed to green or blue light. Mean ± s.d., n = 3 replicate colonies.

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. H: healthy; B1: partially bleached; B2: bleached. (**B**) Symbiont cell density normalised to coral surface area (arbitrary unis, a.u.). Timepoint H was set to 1 and the other timepoints 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), GFP concentration in host tissue homogenate measured by emission and normalised to coral surface area (middle) and to total

host protein (bottom). In (**B**) and (**E**), error bars show mean ± s.d., n = 3 replicate colonies; spheres indicate individual data points; asterisks signify $p<0.05$.

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) ± s.d. (dashed lines), n = 3 replicate colonies. (**B**) Post-treatment symbiont cell counts normalised to coral surface area. (**C**) Post-treatment photographs (white light or 450 nm excitation). Scale 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), GFP concentration measured as emission of host tissue homogenate normalised to coral surface area (middle) and to total host protein (bottom). In (**B**) and (**E**), bars show mean ± s.d., n = 3 replicate colonies; spheres indicate individual data points; asterisks signify $p<0.05$.

Figure 6. The host-symbiont feedback loop of light-mediated pigment expression in reef corals.

Photographs show changes in colouration of *Montipora foliosa* during bleaching and recovery. Schematic drawings conceptualise 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.

[1] References [11,32]

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 ± s.d., n = 3 replicate colonies; spheres indicate individual data points; asterisks signify $p<0.05$; letters show $p<0.05$ in post-hoc pairwise comparison.

METHODS

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 colourful bleaching across the globe and resulting survival rates were obtained courtesy of the authors detailed in Table S1.

Abundance of colour morphs

The abundance of colour 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 <5m to analyse 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 colour. The percentage of colourful 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 <5m. The intensity of pink pigmentation was scored directly against reference colour scales under artificial light provided by a while light diving torch to avoid the underwater light conditions to influence colour perception.

551 **Coral propagation and aquarium set up**

552 Colonies of *Porites lichen* (Dana 1846), *Pocillopora damicornis* (Linnaeus 1758), and
553 *Montipora foliosa* (Pallas 1766), were initially obtained from the UK ornamental trade and
554 propagated in the Coral Reef Laboratory mesocosm facility for >10 years [53]. Replicate
555 colonies for experimental treatment were mounted flat on ceramic tiles to ensure an even
556 distribution of incident light. The corals were left to recover and grow for at least a month in
557 this position before the start of experimental treatments. The acclimation phase was
558 completed under replete nutrient conditions for all corals [4], 24-26°C water temperature, and
559 12h:12h light:dark cycle under white (10,000 Kelvin) metal halide lamps (Aquamedic,
560 Coalville, UK).

561

562 **Replication**

563 For each species, all samples were derived by asexual propagation from the same mother
564 colony. Therefore, replicate colonies, as defined here, are small colonies with the full
565 capacity for autonomous growth. While being genetically identical, they have experienced
566 independent life histories. This approach was necessary in order to exclude the confounding
567 effects by intraspecific colour polymorphisms due to variations in the number of active gene
568 copies that can result in highly different levels of individual GFP-like protein expression
569 [19,41]. While the dynamic response to environmental light stimuli such as upregulation of
570 the pigment expression under high light conditions are the same for different colour morphs,
571 the absolute values can differ substantially [19]. For the optical time series measurements
572 presented in Figs. 2 and 3, corals were kept intact and measurements were performed on
573 replicate areas; this was preferable to an identical protocol performed after sub-
574 fragmentation, because i) it ensured minimum variability in the incident light field, critical
575 for GFP-like protein regulation [18], and ii) it minimised 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 μmol photons $m^{-2}s^{-1}$ from a metal halide lamp for 35 days, then exposed to 290 μmol photons $m^{-2}s^{-1}$ for 55 days. The experiment was performed at 25±1°C. Per colony, fluorescence spectra were recorded for three replicate areas.

Partially purified tissue extracts of *P. lichen* from low light treatments that contained predominantely CFP were exposed to high intensity light (~1000 μmol photons $m^{-2}s^{-1}$) produced by near UV (peak wavelength λ=410nm, FWHM~40) or blue (peak wavelength λ=465 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). Timecourses 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 μmol photons $m^{-2}s^{-1}$ of red light [30] (Lumileds, San Jose, CA, USA; peak wavelength λ=660 nm, full width at half maximum FWHM=40 nm) over 7 days. Replicate colonies were then either placed under 100 μmol photons $m^{-2}s^{-1}$ of blue light (Lumileds, λ=450 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, λ=530 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 μmol photons $m^{-2}s^{-1}$ of white light from a metal halide lamp (Aquamedic) for 26 days.

Heat-induced bleaching

To monitor changes in coral colour 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 μmol photons $m^{-2}s^{-1}$) for at least 20 days. Afterwards, temperatures were ramped up to 31-32°C over ~2 weeks (Δ~0.5°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 μmol photons $m^{-2}s^{-1}$).

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. Recovery of was photographically document 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 μmol photons $m^{-2}s^{-1}$ in either replete (high nitrate / high phosphate; HN/HP) or imbalanced (high nitrate / low phosphate; 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 μmol photons $m^{-2}s^{-1}$ 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 μmol photons $m^{-2}s^{-1}$ for 56 days, then left to recover in HN/HP conditions for a further 56 days.

626 Margins (HM) and inner regions (HI) of three unbleached replicate colonies were sampled
627 before HN/LP treatment, while recovering inner regions (RI) were sampled after 28 days
628 HN/HP recovery.**Quantification of *in vivo* fluorescence and reflectance**
629 All i*n vivo* fluorescence emission measurements were performed with a fluorescence
630 spectrophotometer (Varian, Palo Alto, CA, USA) equipped with a fibre optic probe [18],
631 using 450 nm excitation light. For *P. lichen*, spectra were unmixed using the least-squares
632 method [54] into 3 endmembers: CFP (489 nm), GFP (519 nm) and symbiont pigments (680
633 nm). Endmember contributions to measured spectra were used to plot time series.
634 Fluorescence images were taken with a 450 nm LED (Aquaray Fiji Blue, Tropical Marine
635 Centre, London, UK) for excitation through a 500 nm long-pass emission filter (Nightsea,
636 Lexington, MA, USA). Photographs were obtained by imaging corals side by side with a
637 digital compact camera (Olympus, Shinjuku, Japan).
638 Reflectance spectra were collected with a USB4000 modular spectrometer and a tungsten
639 halogen light source connected to a dip probe (Ocean Optics, Largo, FL, USA), using a
640 Spectralon 99% reflectance standard (Labsphere, North Sutton, NH, USA) as reference.
641 Absorbance of *P. damicornis* was calculated from reflectance spectra as log(1/R) [11];
642 spectra were unmixed into two endmembers, pink CP (565 nm) and intact symbionts (665
643 nm). The Photosystem II maximum quantum efficiency (Fv/Fm) of *P. damicornis* was
644 recorded after 12h dark acclimation with a DIVING-PAM (Walz, Effeltrich, Germany) under
645 exposure to dim light (<5 μmol photons $m^{-2}s^{-1}$) prior to and during measurements [38].
646
647 **Quantification of symbiont cell numbers and host pigment content in tissue extracts**
648 Tissue was harvested by airbrushing corals with artificial seawater. Surface area sampled was
649 measured from skeleton photographs using image analysis software Fiji [55]. 50 μL of each
650 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 BCA colorimetric assay (Pierce, Waltham, MA, USA) against BSA standard. For *P. lichen*, fluorescence was measured by loading 0.25 $\mu g \mu L^{-1}$ protein diluted in artificial seawater (total volume 200 µL) in a fluorescence spectrophotometer (Varian) equipped with 96-well plate reader; emission spectra (ex = 450 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 ~150 µmol photons $m^{-2}s^{-1}$ 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 $\mu g \mu L^{-1}$, 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 normalised to the total protein content of the sample.

676 **Statistical analysis**

677 The 519:489 nm ratio time series data were fitted with logistic functions parameterised using
678 non-linear least squares, with the exception of the HN/LP *P. lichen* dataset which was
679 cropped at 80 days and fitted with an exponential function; this was due to the 489 nm
680 contribution reaching zero at this point, and driving the ratio to infinity. Endpoint
681 measurements were tested for significant difference in means using ANOVA, with α=0.05;
682 where more than two samples were compared, Tukey's HSD was used for post-hoc testing
683 (α=0.05) upon detection of a significant difference. All analysis was performed using R.
684 Results of statistical analysis and model equations are presented in Tables S2-4.
685

686 **Satellite heat stress data**

687 Sea surface temperature and DHW data for Lizard Island (GBR), Amedee (New Caledonia),
688 Okinawa (Japan), Palmyra and El Nido (Philippines) virtual stations and local bleaching
689 thresholds were obtained from NOAA Coral Reef Watch (2000, updated twice-weekly.
690 *NOAA Coral Reef Watch 50-km Satellite Virtual Station Time Series Data for Lizard Island*
691 *and El Nido*, Jan. 1, 2002-Dec. 31, 2016. Silver Spring, Maryland, USA: NOAA Coral Reef
692 Watch. Data set accessed 2019-11-15 at
693 http://coralreefwatch.noaa.gov/satelline/vs/index.php). The bleaching threshold was
694 calculated as maximum monthly mean temperature +1°C (NOAA Coral Reef Watch) and set
695 to "0" to facilitate comparison between regions. Timelines were aligned by setting the day
696 when the local bleaching threshold was clearly exceeded to "0". Bleaching years for Lizard
697 Island were identified based on data for "Australia, GBR Northern" in reference [51].
698 Mortality rates were expressed as a function of DHW using an exponential function (Table
699 S5).

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Figure 1

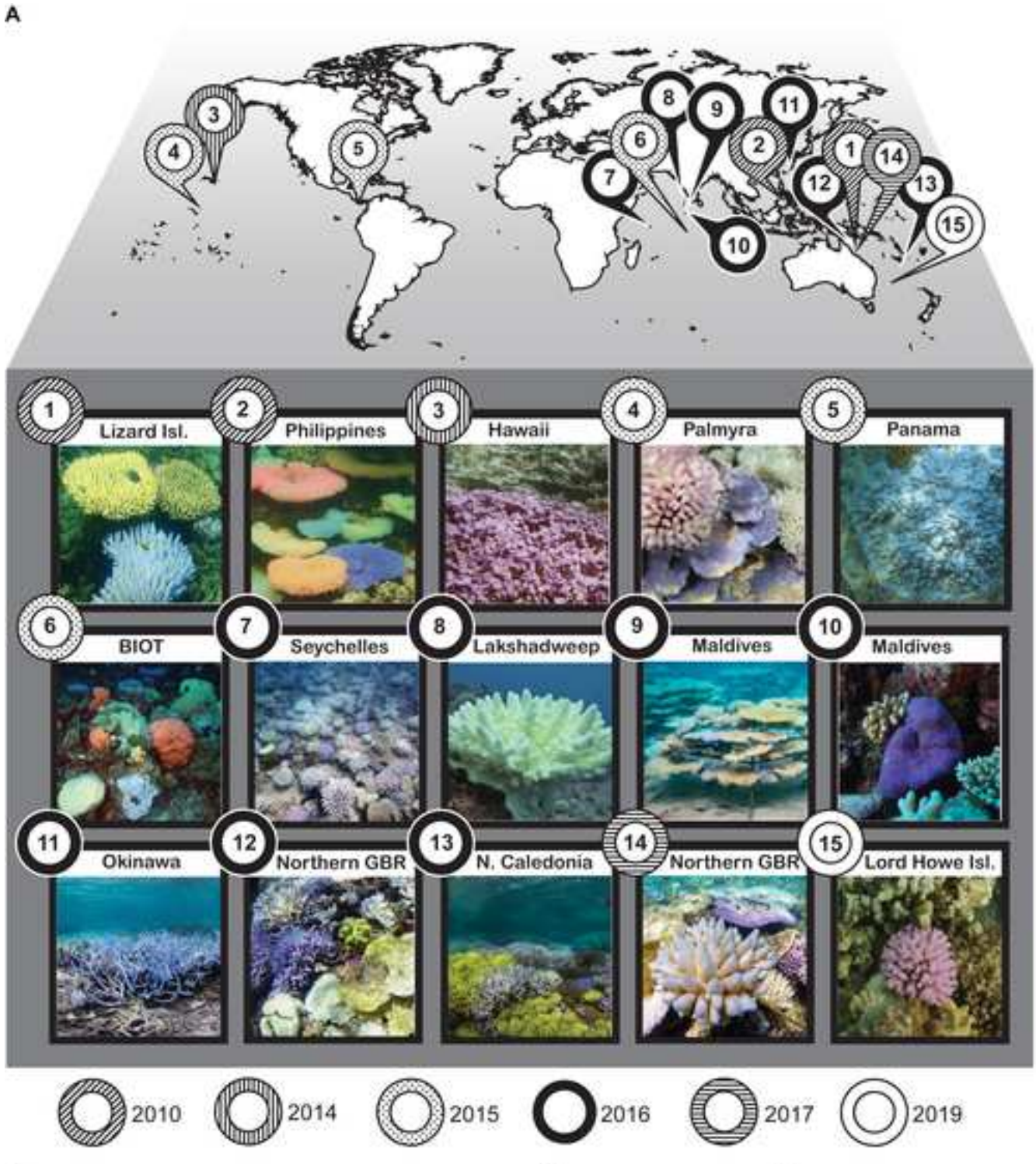

Figure 2

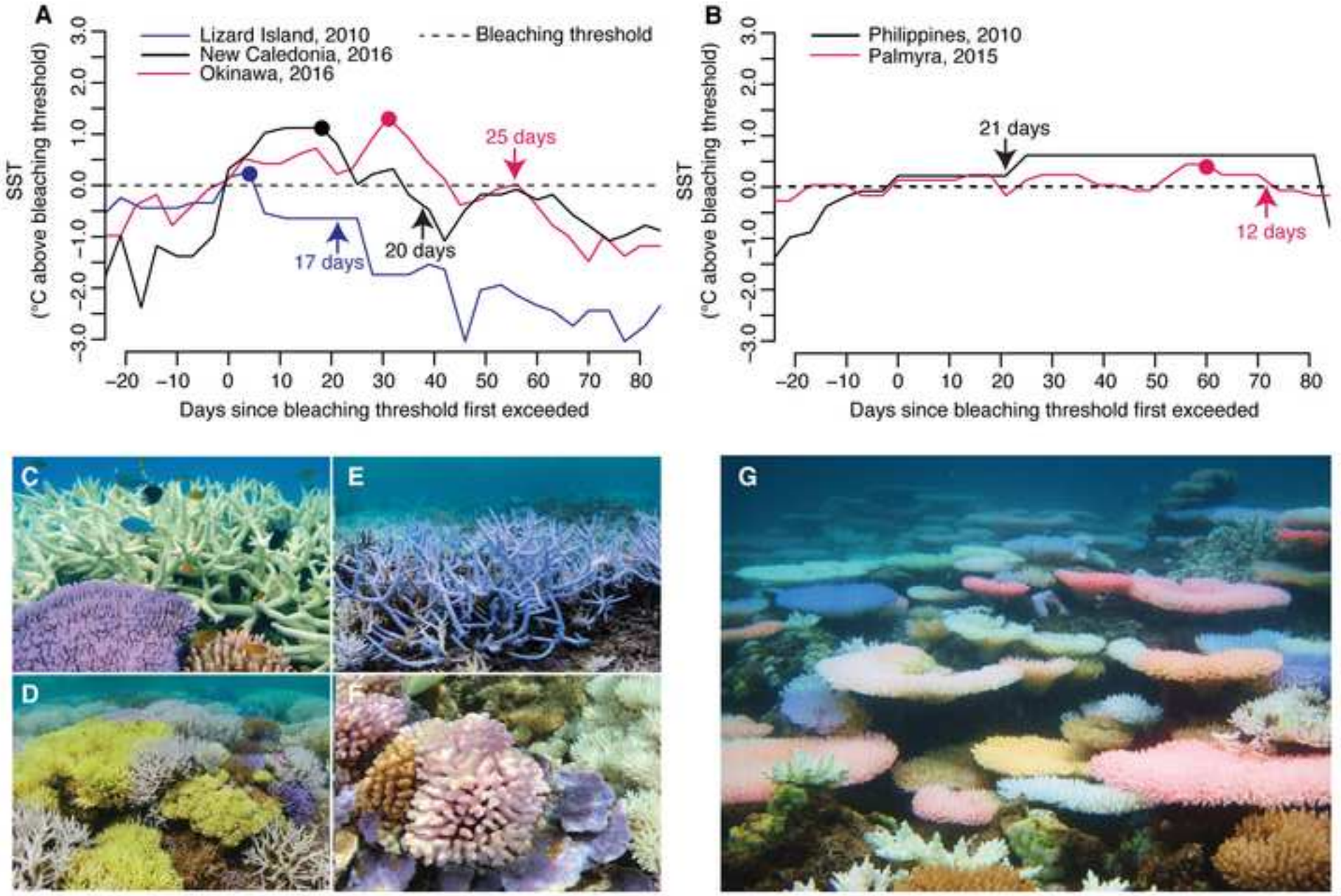

Figure 3

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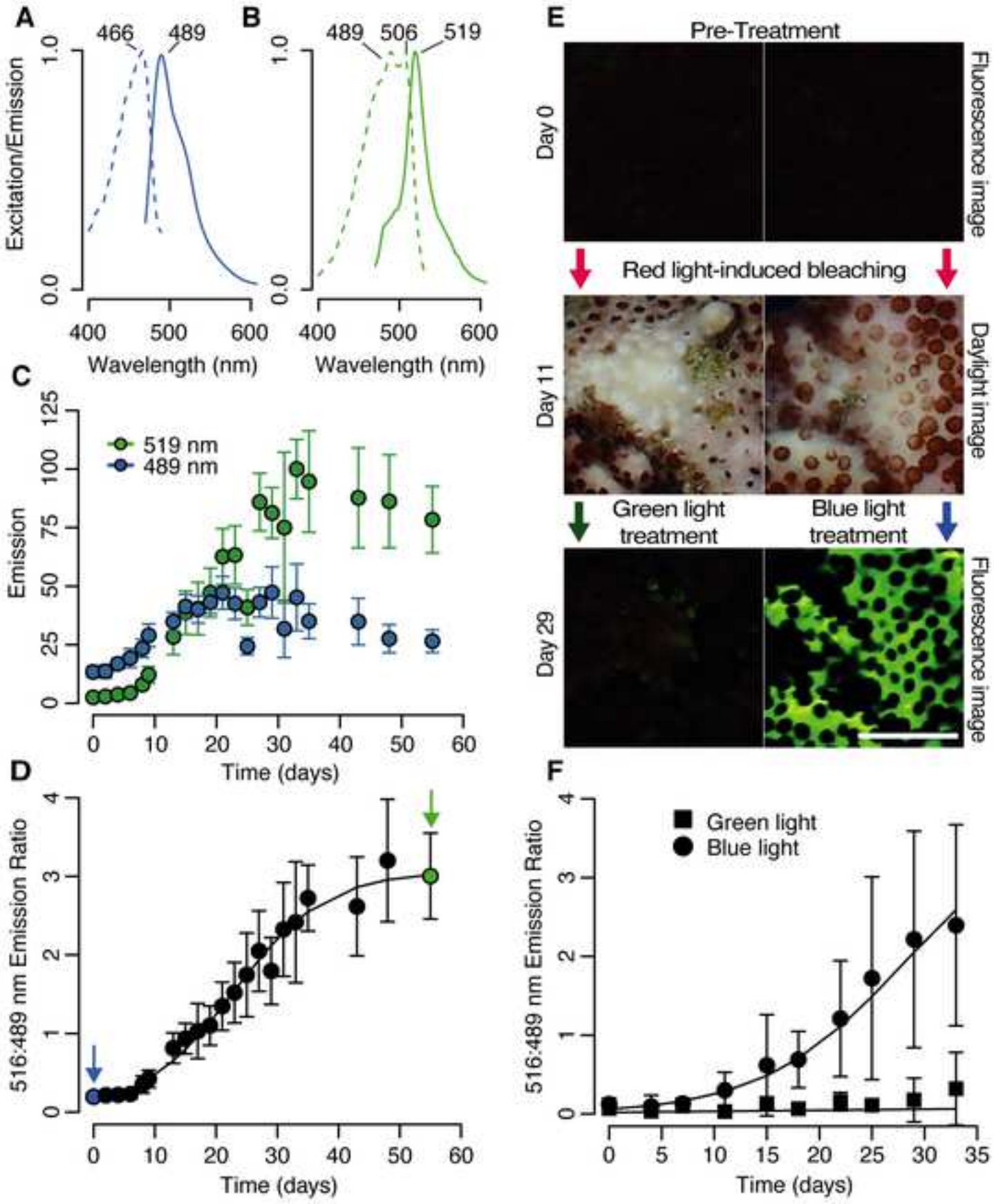

Figure 4

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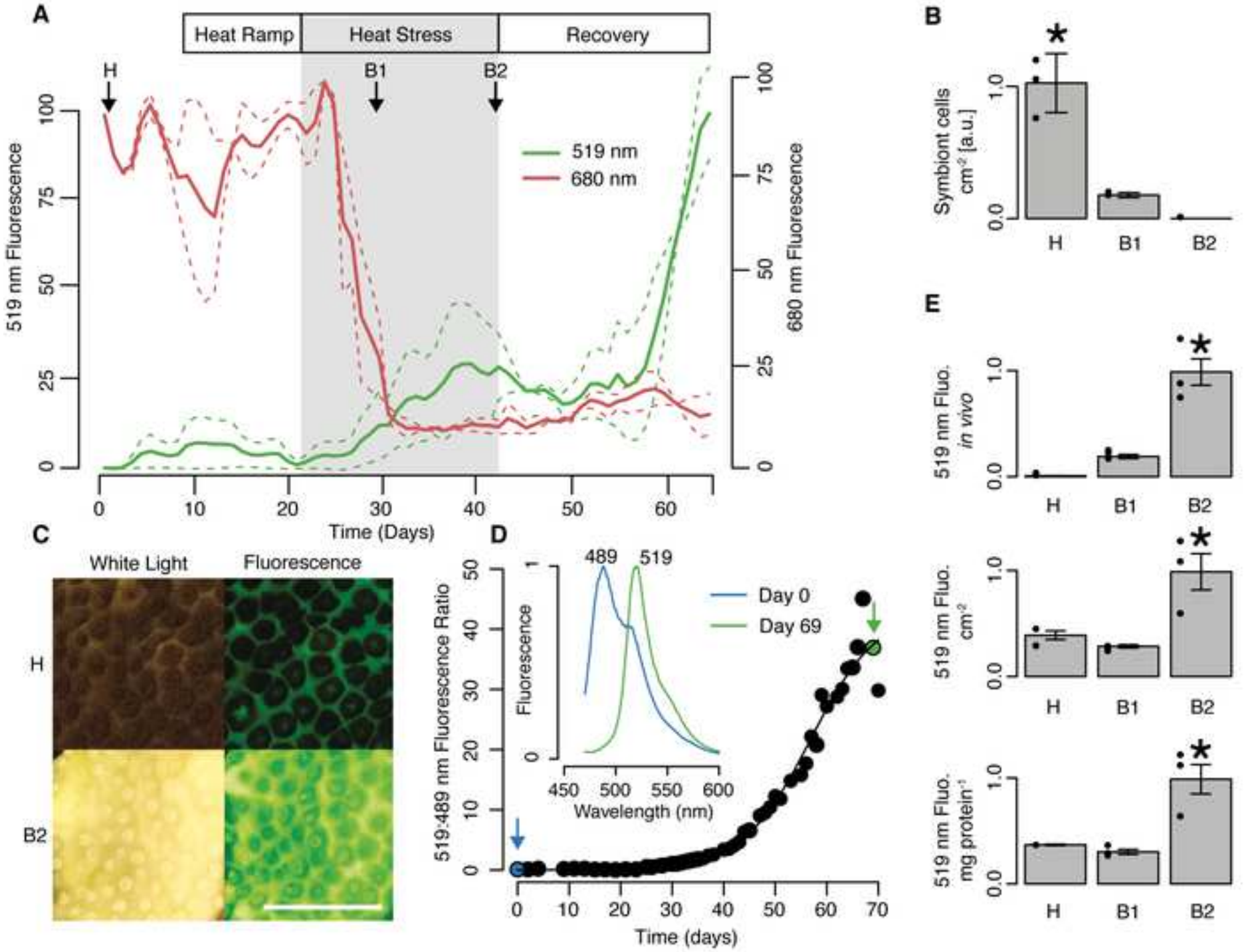


Figure 5

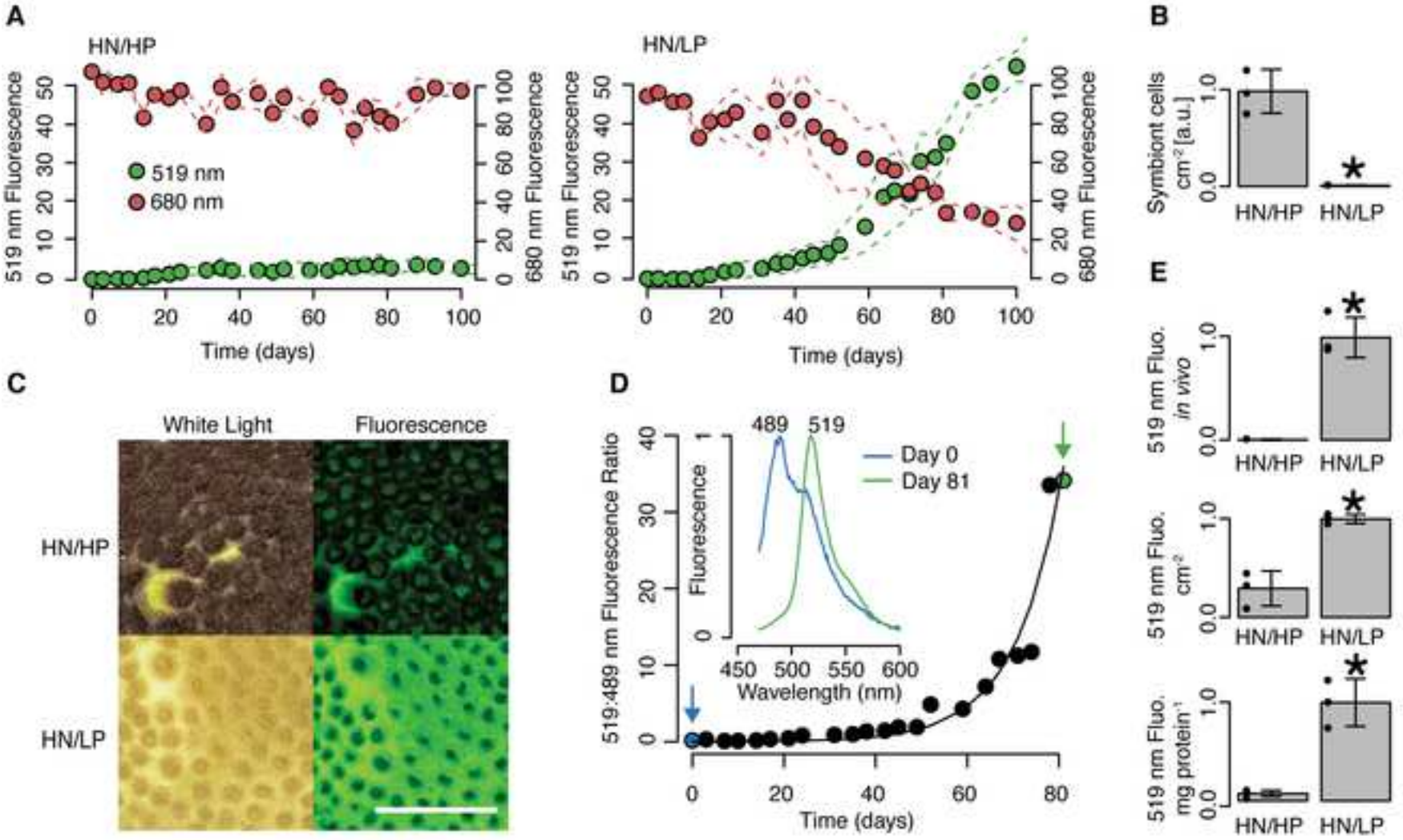
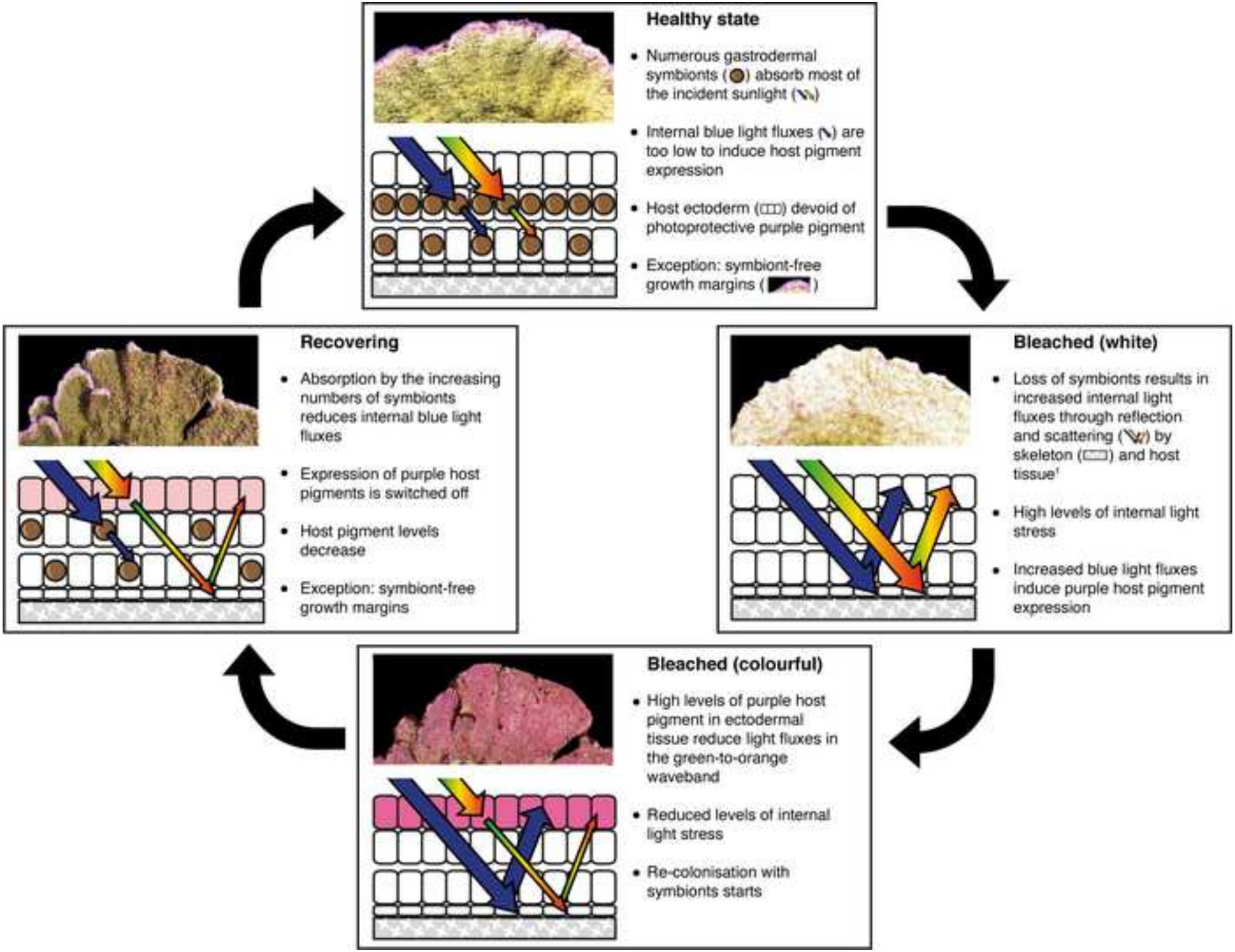
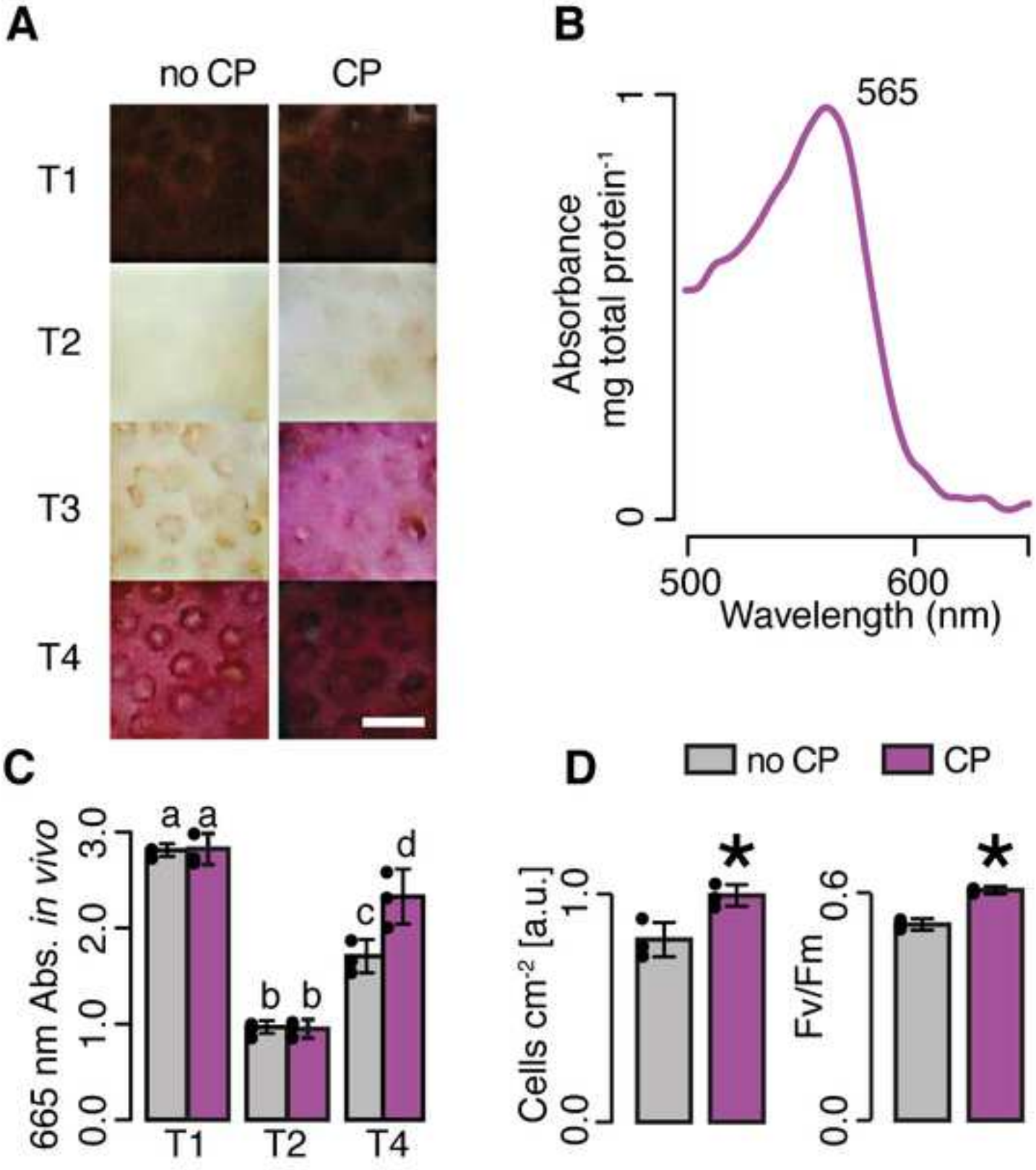


Figure 6





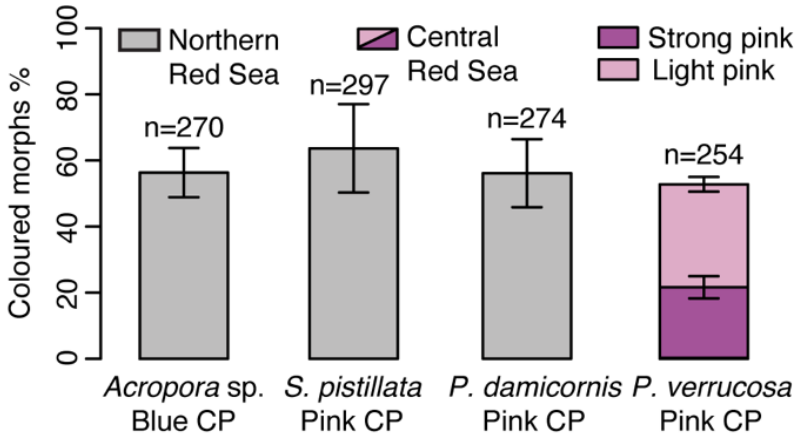


Figure S1. Related to Figure 1. Prevalence of colourful corals under non-bleaching conditions. Occurrence of coral colour morphs expressing blue or pink CPs for common reef-building coral species in reefs of the Northern Red Sea (Eilat, Israel) and Central Red Sea (Saudi Arabia). Mean ± s.d., n=3 (Central Red Sea) to n>6 (Northern Red Sea) belt transects per site and species.

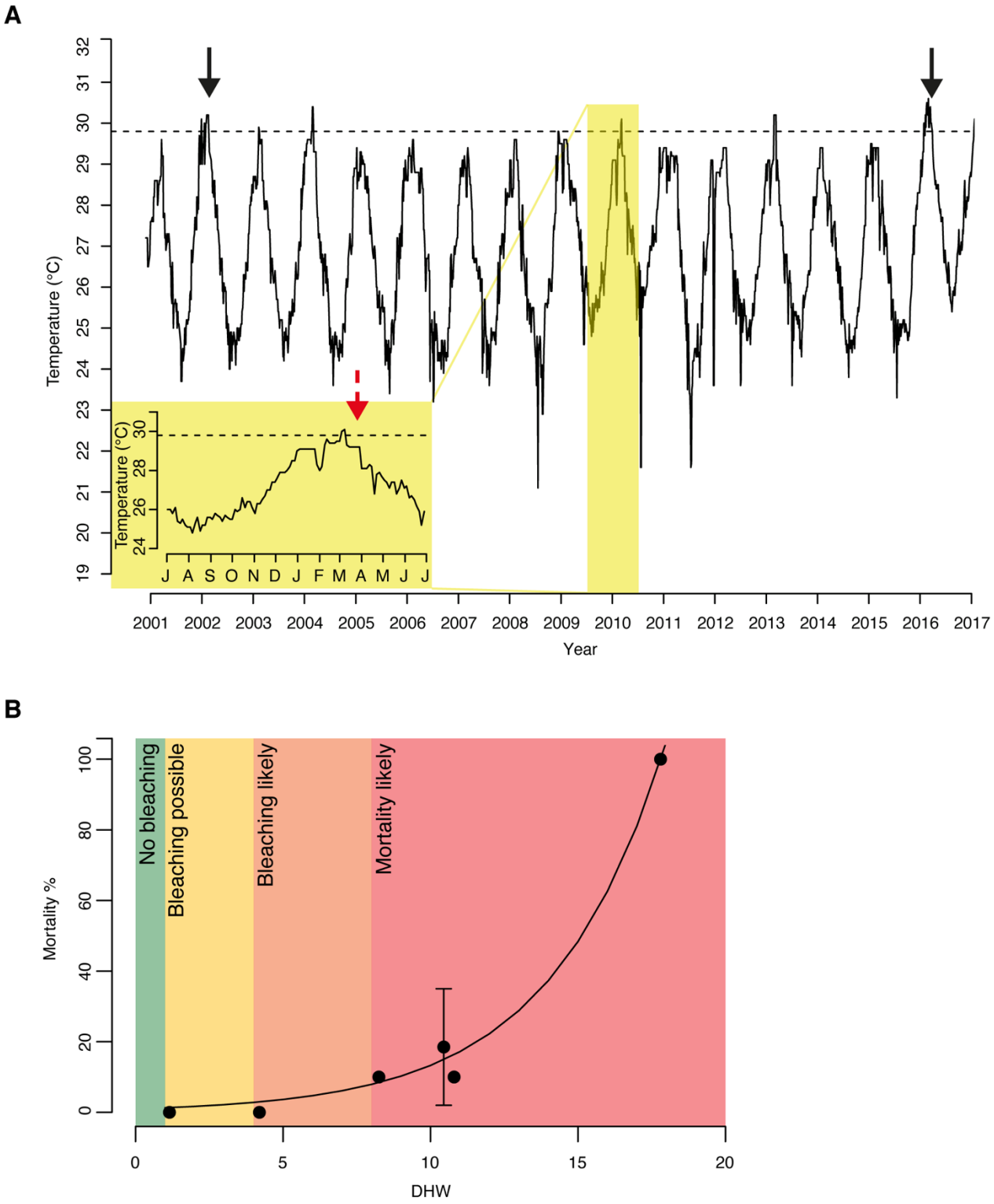


Figure S2. Related to Figure 2. Heat stress and colourful bleaching events. (**A**) Time series of satellite 50 km sea surface temperature for Lizard Island, Great Barrier Reef, Australia. Black arrows show years of severe bleaching reported in reference [51]; dashed red arrow indicates time of colourful bleaching shown in Figure 1A, 2C. Dashed line shows bleaching threshold temperature (maximum monthly mean temperature + 1°C). Data: NOAA Coral Reef Watch. (**B**) Heat stress as Degree Heating Weeks (DHW) and reported mortality rates (Table S1) in the aftermath of colourful bleaching events. Error bar represents range.

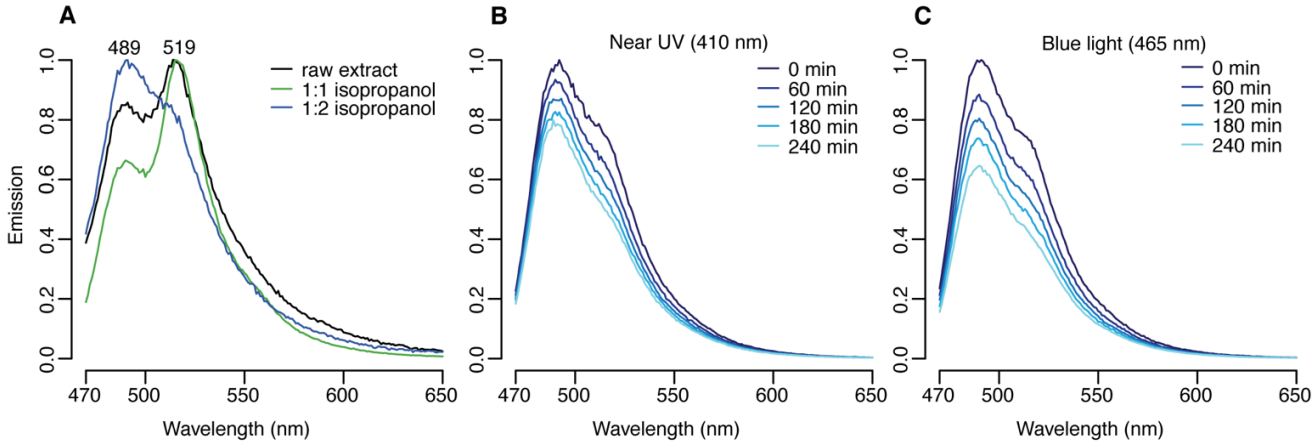


Figure S3. Related to Figure 3. Biochemical and photophysical characterisation of *P. lichen* host pigments. (**A**) Emission spectra of fluorescent proteins in clarified tissue extract of *P. lichen* after 8 weeks acclimation to medium light conditions (170 µmol m^{-2} s^{-1}). Raw extract and following partial separation of cyan (~489 nm) and green (~519 nm) emitting proteins by differential precipitation with 1:1 and 1:2 v/v isopropanol, and subsequent dissolution in phosphate buffer (pH 6.8). (**B**,**C**) Time course of photobleaching of the purified ~489 nm emitting CFP under exposure to strong near UV (410 nm, **B**) and blue (465 nm, **C**) light.

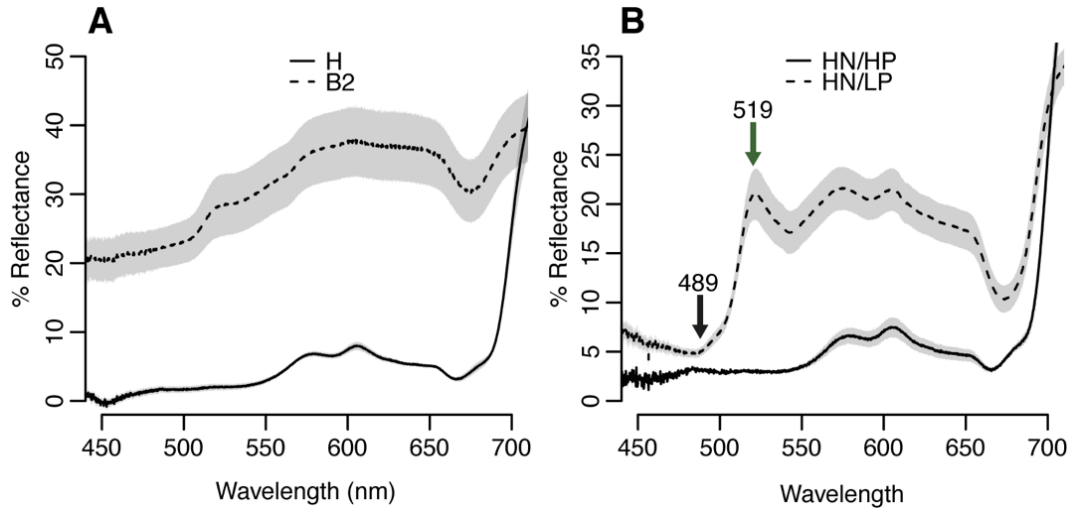


Figure S4. Related to Figure 4 and Figure 5. Spectral reflectance of healthy and bleached *P. lichen*. (**A**) Before (H) and after (B2) 17 days heat stress treatment. (**B**) After 98 days in replete (HN/HP) or imbalanced (HN/LP) nutrient conditions. Mean ± s.d., n = 3 replicate colonies. Number above arrows indicated the position of the absorption (489) and emission (519) maximum of the GFP expressed in imbalanced nutrient conditions, given in nanometres.

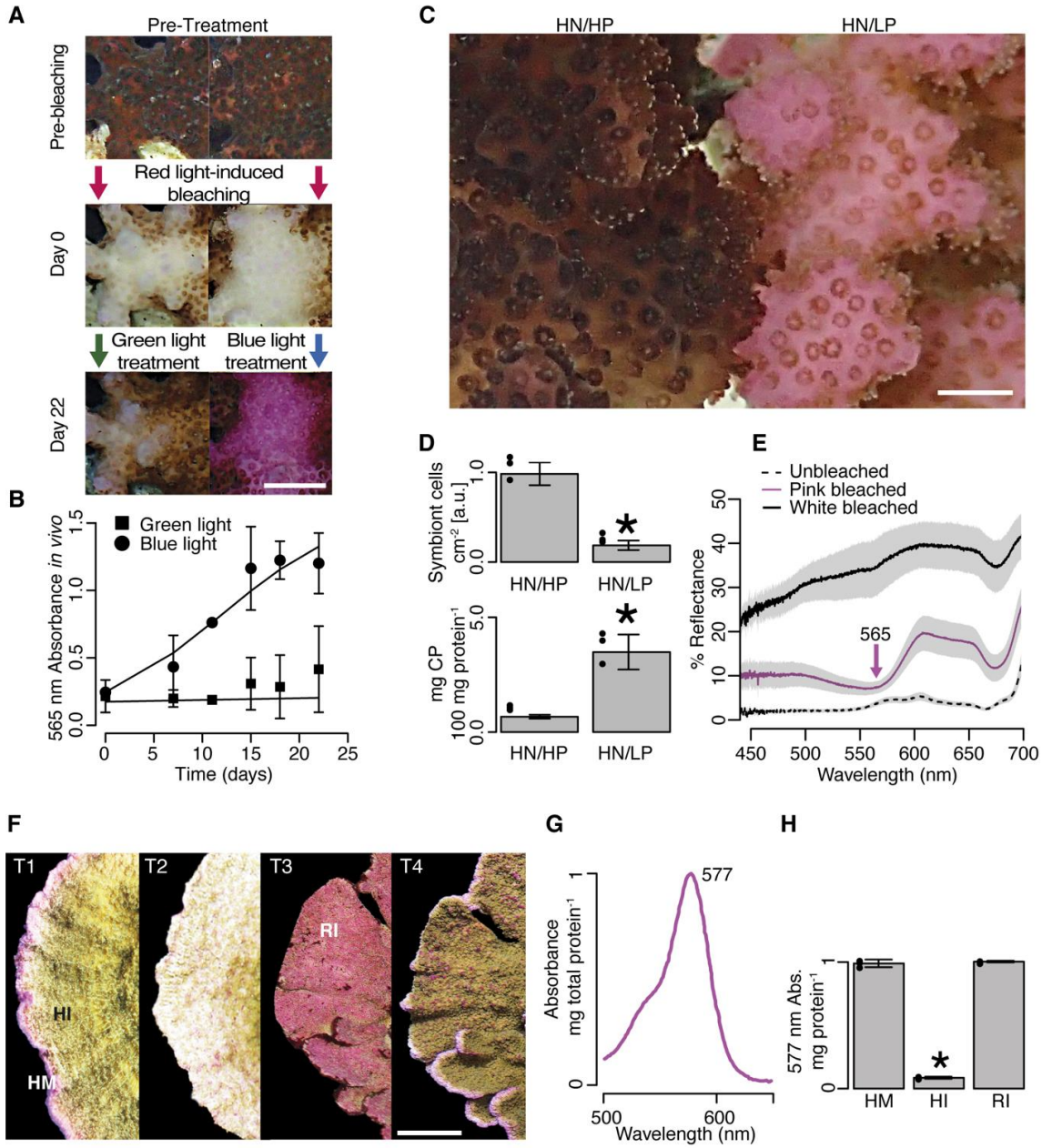


Figure S5. Related to Figure 5. Enhanced accumulation of photoprotective chromoproteins in *P. damicornis* and *M. foliosa* under nutrient stress. (**A**) Photographs of *P. damicornis* before treatment (Pre-bleaching), after bleaching with focused red light (Day 0), and after exposure to green or blue light for 22 days. Scale bar: 10 mm. (**B**) Contribution of pink CP to absorbance spectrum, calculated from *in vivo* reflectance, for bleached *P. damicornis* exposed to green or blue light. Mean ± s.d., n = 3 replicate colonies. (**C**) Photographs of *P. damicornis* kept under replete (HN/HP) and imbalanced (HN/LP) nutrient conditions for ~3.5 months. Scale bar: 5 mm. (**D**) Symbiont cell density determined for tissue extracts normalised to coral surface area (upper graph) and the tissue concentration of the pink CP normalised to total host protein content (mg/100mg) for HN/HP and HN/LP colonies. Mean ± s.d., n = 3 replicate colonies; spheres show individual data points; asterisks show $p<0.05$. (**E**) Reflectance spectra of HN/HP and HN/LP *P. damicornis*. Mean ± s.d., n = 3 replicate colonies. Arrow indicates the position of the CP absorbance maximum. (**F**) Representative images of *M. foliosa* during nutrient-induced bleaching and recovery. T1: Pre-treatment (HM = healthy margin; HI = healthy inner region). T2: Bleached. T3: After 28 days recovery (RI = recovering inner region); T4: Fully recovered coral, after 56 days. Scale bar: 10 mm. (**G**) Absorbance spectrum of *M. foliosa* CP. (**H**) CP concentration in host tissue extracts measured as specific (577 nm) absorbance and normalised to total protein content; mean ± s.d., asterisk shows $p<0.05$.

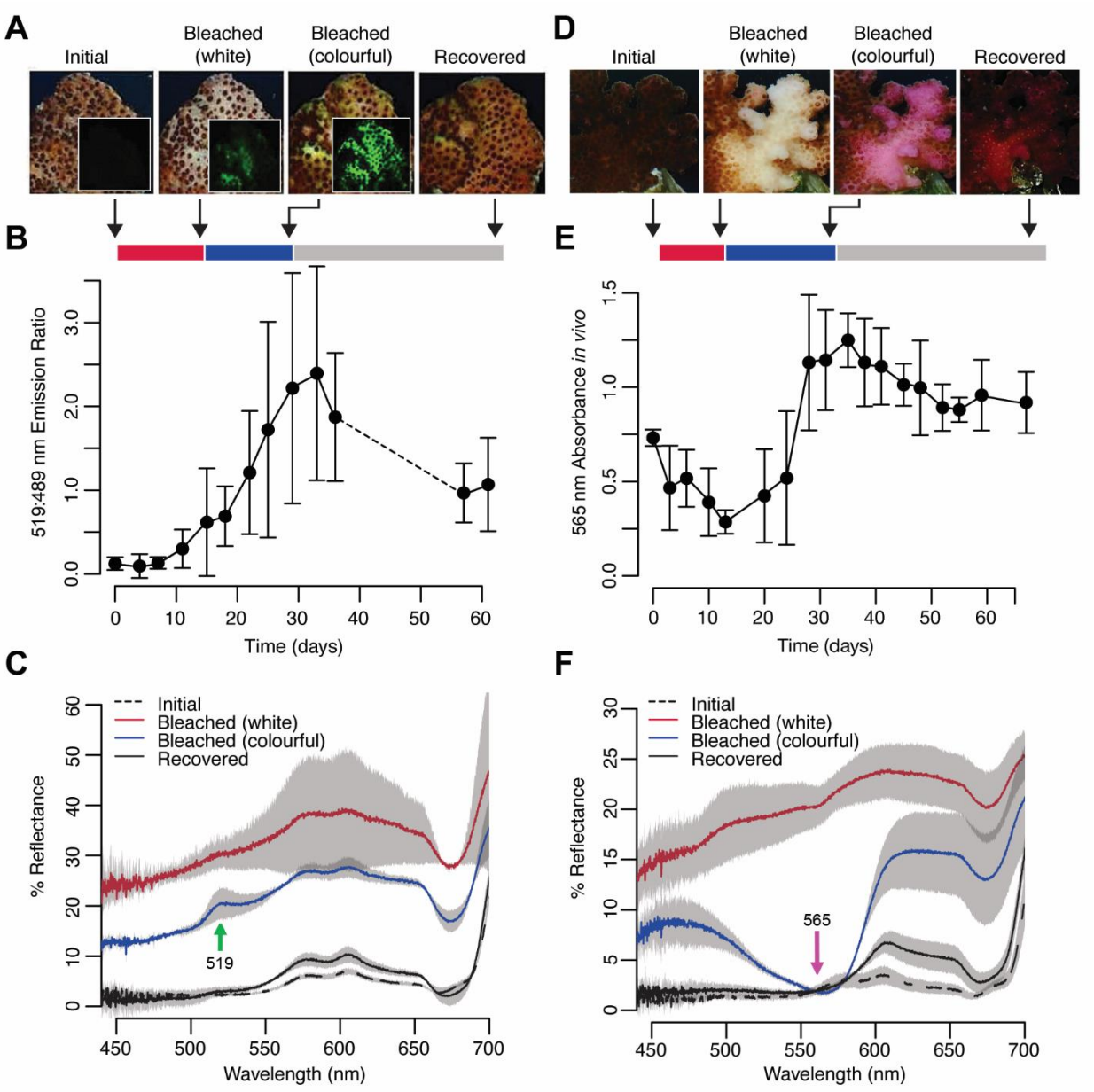


Figure S6. Related to Figure 3, S5. Bleaching and recovery of *P. lichen* and *P. damicornis.* Daylight and fluorescence images (**A, D**), timecourse of *in vivo* changes in GFP:CFP (519:489 nm) emission ratio (**B**) and CP (560nm) absorbance (**E**) and reflectance spectra (**C, F**) of *P. lichen* (**A-C**) and *P. damicornis* (**D-F**) before ("Initial"), after bleaching with focussed red light ("Bleached white"), after induction of FP/CP expression with blue light ("Bleached colourful"), and after recovery under full spectrum light ("Recovered"). In **B** and **E**, spheres show mean ± s.d., n = 3 replicate colonies. In **C** and **F**, lines show mean ± s.d, n = 2 (**C**) or 3 (**F**) replicate colonies. Arrows and numbers indicate the fluorescence emission maximum (**C**) and absorption maximum (**F**) of the dominant host pigments expressed in the colourful bleached state.

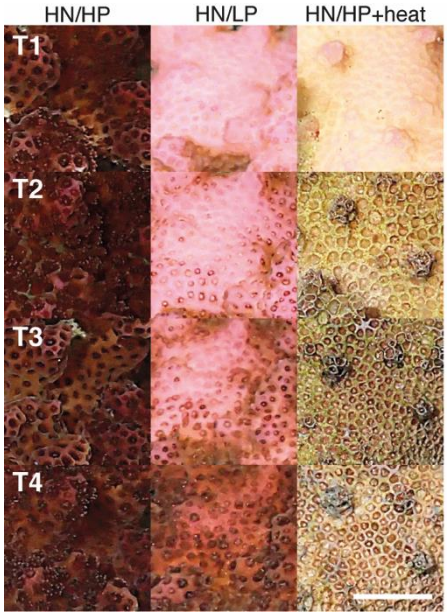


Figure S7. Related to Figure 7. Recovery of *P. damicornis* following heat or nutrient stress-induced bleaching. Representative photographs of *P. damicornis* kept under control (HN/HP, 26°C) conditions, imbalanced nutrients (HN/LP, 26°C) conditions, or heat stress (HN/HP, 31°C for 9 days) conditions. Photographed during recovery in HN/HP conditions after 0 (T1), 34 (T2), 53 (T3) and 77 (T4) days. Scale bar: 10 mm.

Table S1. Reports of colourful bleaching events

	Location	Date	Description	Sources	Image credits	Evidence of colourful bleaching
1	Lizard Island, GBR	Mar 2010	Colourful bleaching, no mortality observed	Morgan Pratchett, this paper.	Morgan Pratchett, James Cook University	Photo evidence
2	Taytay Bay, Philippines	Jun 2010	Colourful bleaching followed by mortality	Ryan Goehrung, Marine Photobank via https://oceanportal.tumblr.com/post/34639028532	Ryan Goehrung, University of Washington	Photo evidence and quote: "The last stunning cry of the living corals and their symbiotic algae before full on bleaching and subsequent coral death during a global bleaching event in the summer of 2010. Upon returning to the site after only a matter of months, all coral pictured here were dead, completely bleached." "At this stage the zooxanthellae algae is experiencing acute heat stress, causing the algae to turn these vibrant shades otherwise not seen in corals. Upon returning to the site after only a matter of months, all coral pictured here were dead, completely bleached."
3	Lisianski Atoll, Hawaii	2014	Moderate event with bleaching at 35% of sites. Incidence up to 90% at one shallow site.	https://phys.org/news/2014-10-scientists-coral-northwest-hawaii.html https://www.theguardian.com/environment/2015/apr/01/south-pacific-coral-die-off-el-nino	AP Photo/ NOAA and Courtney Couch, the Hawaii Institute of Marine Biology	Photo evidence
4	Palmyra	Oct 2015	Bleaching affecting >90% of corals but <10% mortality	Fox, M. D., *et al.* Limited coral mortality following acute thermal stress and widespread bleaching on Palmyra Atoll, central Pacific. *Coral Reefs* (2019), 38(4):701-712 https://scripps.ucsd.edu/news/advanced-virtual-technology-captures-how-coral-reefs-recover-after-bleaching	Brian Zgliczynski, Scripps Institution of Oceanography	Photo evidence
5	Secas Islands, Panama	16/07/2015 onwards	Severe bleaching affecting massive and branching corals. Differential susceptibility and mortality between colour morphs of massive *Porites* sp.	McGraw, B. A coral bleaching event at Secas Islands, Chiriqui Bay, Pacific Coast, Panama. *Reef Encounter* (2016), 31(2):45-48	Bill McGraw, www.newaquatechpanama.com	"Some *Porites* appeared to change color from brilliant blue to tan to green without showing mortality (...)" "Post bleaching dives showed a degree of survival or recovery of the coral community, and notably several new color morphs such as the massive *Porites*, are now conspicuous (…)"

6	Salomon Islands, BIOT	15/04/2015 onwards	Severe bleaching affecting most species above 10 m and >50% below 10 m. Bleaching reported down to 35 m.	http://www.livingoceansfoundation.org/coral-bleaching-colors-biot/	Andy Bruckner, Coral Reef CPR	"I felt like I was at a carnival swimming through a pool of rainbow glazed popcorn. Fluorescent lime green, pink, purple, blue and snow-white corals carpeted the seafloor, each one more colorful than the last. The colorful corals were in stark contrast to the rest of the seafloor" "By April 25, most of the table acroporids were white, light yellow or light blue, and numerous other corals, especially lobe coral (*Lobophyllia*), all of the mushroom corals, short, stout-branched corals (acroporids, *Pocillopora*, and *Stylophora*), and massive species such as *Symphyllia* and *Goniopora* were various fluorescent shades of blue, yellow or green"
7	Seychelles	Feb-Aug 2016	Widespread event with 80-90% mortality	http://blueeconomyseychelles.org/item/79-2016-coral-bleaching-event-one-year-later	Louise Malaisé, Nature Seychelles	Photo evidence
8	Kalpeni, Lakshadweep	Jun 2016	Bleaching and some mortality of pocilloporids and acroporids	http://blog.ncf-india.org/2016/06/08/colours-of-a-catastrophe/	Shreya Yadav, Nature Conservation Foundation	"I am swimming in the unusually warm waters of Kalpeni in the Lakshadweep, overwhelmed by the bizarre colours of this catastrophe. Muted pastel shades have turned violently psychedelic. A branching colony of brown Pocillopora is now lavender, sky-blue fingers of Acropora a radiant white, grey sheets of Montipora fluoresce green and pink." "It is like swimming over the canvas of some hallucinating artist. From the surface, the reef is pockmarked with colour, like a tree trunk covered in spots of glowing lichen."
9	North Malé atoll and Baa atoll, Maldives	17/04/2016 onwards	Bleaching affecting the majority of colonies on transplantation frames and on the reef down to 25 m. >90% mortality.	http://marinesavers.com/2016/07/coral-bleaching-updates/	Reefscapers	Photo evidence
10	South Malé atoll and Baa atoll, Maldives	2016	Widespread bleaching with mortality of branching corals but high overall recovery overall	http://www.coralreefcpr.org/bleaching-blues.html https://carpediemmaldives.com/coral-bleaching-maldives-2016/	Andy Bruckner, Coral Reef CPR	"A few had already turned white and many had become vividly colored. Often the branch tips of cauliflower coral (*Pocillopora*) were blue, pink or purple, the sides and tops of boulder corals (*Porites*) had become a light blue and some staghorn type corals were a vivid fluorescent blue."

11	Okinawa	12/09/2016	Moderate bleaching event affecting acroporids. High site variability in bleaching incidence. Up to 35% mortality at certain sites.	Singh, T. *et al.* Effects of moderate thermal anomalies on Acropora corals around Sesoko Island, Okinawa. *PLoS ONE* (2019), e0210795. https://www.coralreefimagebank.org/fluorescing	The Ocean Agency / XL Catlin Seaview Survey	Photo evidence
12	Northern GBR	2016	Widespread bleaching event. 95% of reefs between Cairns and PNG showed significant signs of bleaching.	https://theconversation.com/coral-bleaching-taskforce-more-than-1-000-km-of-the-great-barrier-reef-has-bleached-57282 https://www.theguardian.com/environment/2017/mar/15/stopping-global-warming-is-only-way-to-save-great-barrier-reef-scientists-warn https://www.flickr.com/photos/great-barrier-reef-marine-park/25755302282/in/photostream/	Chris Jones / Great Barrier Reef Marine Park Authority	Photo evidence
13	New Caledonia	06/03/2016	Moderate bleaching event followed by partial recovery (10% mortality).	Fanny Houlbreque, pers. comm. https://time.com/coral/ https://phys.org/news/2016-10-scientists-caledonia-coral.html	The Ocean Agency / XL Catlin Seaview Survey	"The corals he captures lit up fluorescently as their color left them slowly but surely"
14	Northern GBR	Apr 2017	To be confirmed.	https://www.theverge.com/2018/1/4/16849336/global-warming-coral-reefs-bleaching-rate-climate-change	Ed Roberts, Lancaster University / Tethys Images	Photo evidence and pers. comm.: "The fluorescing on the GBR and in the Coral Sea during the back to back bleachings was quite startling, especially in the top 10 metres. It's been well documented for quite a while, but the extent and severity of it during the 2016/17 global event was something to behold. The fluoro stage seems to be reasonably short, and I've seen a wide array of species from almost all genus doing it. The shallow water acros definitely get most of the attention, but it's not a phenomenon restricted by species identity."

15	Lord Howe Island	Mar 2019	Localised bleaching event with up to 92% incidence at one site, <5% or no incidence at other sites	https://www.abc.net.au/triplej/programs/hack/coral-bleaching-in-lord-howe-island/10960428	Tess Moriarty, University of Newcastle	Photo evidence

Table S2. Related to Figure 4, 5, 7, S5. Analysis of variance (ANOVA) results.

(a) *P. lichen*, heat-induced bleaching (Figure 4B,E)

Variable	F	p	Tukey's HSD	
Symbiont cells cm^{-2}	$F_{2,6}$=53.84	<0.001	H, B1	p<0.001
			H, B2	p<0.001
			B1, B2	p=0.287
519 nm fluorescence *in vivo*	$F_{2,6}$=26.18	0.001	H, B1	p=0.403
			H, B2	p=0.001
			B1, B2	p=0.004
519 nm fluorescence cm^{-2}	$F_{2,6}$=10.08	0.012	H, B1	p=0.890
			H, B2	p=0.025
			B1, B2	p=0.015
519 nm fluorescence mg^{-1}	$F_{2,6}$=14.26	0.005	H, B1	p=0.952
			H, B2	p=0.010
			B1, B2	p=0.007

(b) *M. foliosa*, nutrient-induced bleaching and recovery (Figure S5H)

Variable	F	p	Tukey's HSD	
577 nm absorbance mg^{-1}	$F_{2,6}$=3153	<0.001	HM, RI	p=0.988
			HM, HI	p<0.001
			HI, RI	p<0.001

(c) *P. damicornis*, 665 nm absorbance *in vivo* during light-induced bleaching and recovery (Figure 7C)

	F	p
Treatment	$F_{1,4}$=7.3021	0.054
Time	$F_{2,8}$=200.2141	<0.001
Treatment*Time	$F_{2,8}$=7.4626	0.0148

Tukey's HSD

T1 no CP, T2 no CP	p<0.001
T1 no CP, T3 no CP	p<0.001
T1 no CP, T1 CP	p=1.000
T1 no CP, T2 CP	p<0.001
T1 no CP, T3 CP	p=0.003
T2 no CP, T3 no CP	p<0.001
T2 no CP, T1 CP	p<0.001
T2 no CP, T2 CP	p=1.000
T2 no CP, T3 CP	p<0.001
T3 no CP, T1 CP	p<0.001
T3 no CP, T2 CP	p<0.001
T3 no CP, T3 CP	p<0.001
T1 CP, T2 CP	p<0.001
T1 CP, T3 CP	p=0.002
T2 CP, T3 CP	p<0.001

(d) *P. damicornis*, recovery under white light after light-induced bleaching (Figure 7D)

Variable	F	p
Zooxanthellae cm^{-2}	$F_{1,4}$=14.28	0.019
Fv/Fm	$F_{1,4}$=73.25	0.001

(e) *P. lichen*, nutrient-induced bleaching (Figure 5B,E)

Variable	F	p
Symbiont cells cm^{-2}	$F_{1,4}$=55.42	0.002
519 nm fluorescence *in vivo*	$F_{1,4}$=168.6	<0.001
519 nm fluorescence cm^{-2}	$F_{1,4}$=44.78	0.003
519 nm fluorescence mg^{-1}	$F_{1,4}$=43.34	0.003

(f) *P. damicornis*, nutrient-induced bleaching (Figure S5D)

Variable	F	p
Symbiont cells cm^{-2}	$F_{1,4}$=100.3	<0.001
565 nm absorbance mg^{-1}	$F_{1,4}$=40.35	0.003

Table S3. Related to Figure 3, 4, 5. Model parameterisation and statistical analysis for 519:489 nm fluorescence emission time series data.

Treatment	Model	Parameters	SE	t-value	p
High light acclimation (Figure 3D)	$y = \frac{a}{1 + e^{-\frac{x-b}{c}}}$	a=3.064	0.201	15.24	<0.001
		b=22.889	1.341	17.07	<0.001
		c=7.569	0.441	17.16	<0.001
Green light exposure following bleaching (Figure 3F)	$y = a + bx$	a=0.020	0.025	0.790	0.453
		b=0.001	0.002	0.573	0.582
Blue light exposure following bleaching (Figure 3F)	$y = \frac{a}{1 + e^{-\frac{x-b}{c}}}$	a=3.842	1.703	2.257	0.059
		b=28.082	5.810	4.833	0.002
		c=6.759	1.037	6.515	<0.001
Heat Stress (Figure 4D)	$y = \frac{a}{1 + e^{-\frac{x-b}{c}}}$	a=43.321	2.862	15.14	<0.001
		b=56.828	1.166	48.75	<0.001
		c=6.558	0.600	10.93	<0.001
Nutrient Stress (Figure 5D)	$y = e^{a+bx}$	a=-4.362	0.747	-5.843	<0.001
		b=0.098	0.010	10.221	<0.001

Table S4. Related to Figure S5. Model parameterisation and statistical analysis for *P. damicornis* pink CP absorbance time series data.

Treatment	Model	Parameters	SE	t-value	p
Green light exposure following bleaching (Figure S5B)	$y = a + bx$	a=0.175	0.047	3.691	0.021
		b=0.001	0.004	0.305	0.776
Blue light exposure following bleaching (Figure S5B)	$y = \frac{a}{1 + e^{-\frac{x-b}{c}}}$	a=1.629	0.235	6.924	0.006
		b=11.880	1.935	6.138	0.009
		c=6.865	0.455	15.069	0.001

Table S5. Related to Figure S2. Model parameterisation and statistical analysis for coral mortality and heat stress data.

Model	Parameters	SE	t-value	p
$y = e^{ax}$	a=0.259	0.002	127.6	<0.001