J. Phycol. **47,** 846–860 (2011)

DOI: 10.1111/j.1529-8817.2011.01021.x

IMPORTANCE OF MACRO- VERSUS MICROSTRUCTURE IN MODULATING LIGHT LEVELS INSIDE CORAL COLONIES[1]

Paulina Kaniewska[2]

ARC Centre of Excellence, Global Change Institute, The University of Queensland, St. Lucia, Queensland 4072, Australia

Sveinn H. Magnusson

Marine Biological Laboratory, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark

Kenneth R. N. Anthony

ARC Centre of Excellence, Global Change Institute, The University of Queensland, St. Lucia, Queensland 4072, Australia

Ruth Reef

School of Biological Sciences, The University of Queensland, St. Lucia, Queensland 4072, Australia

Michael Kühl

Marine Biological Laboratory, Department of Biology, University of Copenhagen, Strandpromenaden 5, 3000 Helsingør, Denmark
Plant Functional Biology and Climate Change Cluster, University of Technology Sydney, PO Box 123, Ultimo Sydney, New South Wales 2007, Australia

and Ove Hoegh-Guldberg

ARC Centre of Excellence, Global Change Institute, The University of Queensland, St. Lucia, Queensland 4072, Australia

Adjusting the light exposure and capture of their symbiotic photosynthetic dinoflagellates (genus *Symbiodinium* Freud.) is central to the success of reef-building corals (order Scleractinia) across high spatio-temporal variation in the light environment of coral reefs. We tested the hypothesis that optical properties of tissues in some coral species can provide light management at the tissue scale comparable to light modulation by colony architecture in other species. We compared within-tissue scalar irradiance in two coral species from the same light habitat but with contrasting colony growth forms: branching *Stylophora pistillata* and massive *Lobophyllia corymbosa*. Scalar irradiance at the level of the symbionts (2 mm into the coral tissues) were <10% of ambient irradiance and nearly identical for the two species, despite substantially different light environments at the tissue surface. In *S. pistillata*, light attenuation (90% relative to ambient) was observed predominantly at the colony level as a result of branch-to-branch self-shading, while in *L. corymbosa*, near-complete light attenuation (97% relative to ambient) was occurring due to tissue optical properties. The latter could be explained partly by differences in photosynthetic pigment content in the symbiont cells and pigmentation in the coral host tissue. Our results demonstrate that different strategies of light modulation at colony, polyp, and cellular levels by contrasting morphologies are equally effective in achieving favorable irradiances at the level of coral photosymbionts.

Key index words: **irradiance; morphology; photoacclimation; scale; scleractinian coral; *Symbiodinium***

Abbreviations: **$a^*_{\text{chl}\,a}$, specific absorption coefficient of chl *a*; Ddn, diadinoxanthin; Dtn, diatoxanthin; GBR, Great Barrier Reef; GFP, green fluorescent protein; K_d, light attenuation coefficient of downwelling irradiance**

The success of scleractinian corals in tropical seas is largely attributed to their relationship with photosynthetic unicellular dinoflagellates (zooxanthellae) of the genus *Symbiodinium* (Muscatine and Porter 1977) residing within the gastrodermal cells of the coral host (Muscatine and Cernichiari 1969) where they excrete large quantities of photosynthate. This coral–algal symbiosis drives the construction of the reef framework presenting a multitude of habitats for a diverse range of organisms (Reaka-Kudla 1997, Hoegh-Guldberg 1999). As symbiont photosynthesis is of paramount importance for coral metabolism and growth, mechanisms for adjusting internal irradiances to a near-optimal range for *Symbiodinium* cells can have adaptive significance for the symbiosis by optimizing energy acquisition across variable

[1]Received 27 January 2010. Accepted 14 January 2011.
[2]Author for correspondence: e-mail p.kaniewska@uq.edu.au.

environmental gradients (Falkowski and Raven 1997, Hoogenboom et al. 2008), but detailed studies of such mechanisms are lacking.

Corals are distributed across a wide range of light habitats from high-light environments on shallow-water reef flats (Hoegh-Guldberg and Jones 1999, Jones and Hoegh-Guldberg 2001) to low-light environments in deep waters (>50–100 m; Fricke et al. 1987, Kaiser et al. 1993, Mass et al. 2007), shaded caves (Anthony and Hoegh-Guldberg 2003, Kaniewska et al. 2008), and turbid water (Anthony and Fabricius 2000). There is substantial information on ambient light levels across coral habitats (e.g., Falkowski et al. 1990, Anthony and Hoegh-Guldberg 2003), but little is known about the actual levels of irradiance that reach the symbiotic dinoflagellates within coral tissue. A few laboratory studies have reported irradiance levels within coral tissues (Kühl et al. 1995, Magnusson et al. 2007) or in the skeleton underneath (Shibata and Haxo 1969, Fine et al. 2005, Magnusson et al. 2007), and differences in light attenuation between coral species have been detected (Magnusson et al. 2007). However, to date, no estimates have been made of irradiance levels reaching *Symbiodinium* populations in hospite.

Both the host and the symbiotic dinoflagellates can modify their optical characteristics at the tissue and cellular levels to optimize light harvesting. The symbiont density in the host tissue is maintained by the coral host through growth control and low-level expulsion of symbiont cells from the gastroderm (Hoegh-Guldberg et al. 1987, Falkowski et al. 1993, Weis 2008). Common photoacclimatory responses at the cellular level involve modulating chl levels, adjusting xanthophylls and other carotenoids in the photosynthetic unit (Dustan 1979, Bjorkman 1981, Iglesias-Prieto and Trench 1994), which can affect self-shading within coral tissues. *Symbiodinium* cells can both change in density and adjust the amount of photosynthetic pigments with irradiance fluctuations (Falkowski and Dubinsky 1981, Iglesias-Prieto and Trench 1997, Titlyanov et al. 2002). Shading within coral tissues can also occur due to light absorption by fluorescent and nonfluorescent host pigments (Salih et al. 2000, Dove et al. 2001, 2008, D'Angelo et al. 2008) or the production of mycosporine-like amino acids (MAAs) that provide protection against UV radiation (Shick et al. 1999). Other mechanisms that modulate the light exposure of corals include polyp contraction/expansion (Levy et al. 2003), tissue retraction (Brown et al. 2002), and light-scattering properties arising from polyp/skeletal structures (Enriquez et al. 2005). All these mechanisms modulate the ambient irradiance levels available to *Symbiodinium* in hospite.

At the environmental scale, scleractinian corals display extensive interspecific variation in colony morphology across coral families, ranging from highly complex branching structures to simple hemispherical and encrusting forms (Veron 1995). Phenotypic plasticity in colony morphology is also common among corals (Veron and Pichon 1982, Willis 1985, Bruno and Edmunds 1997), where within-species variation in structure is predominantly dictated by environmental gradients of ambient light and water flow conditions (Sebens et al. 1997, Vermeij and Bak 2002, Anthony et al. 2005, Kaandorp et al. 2005). In coral species where colony architecture is influenced by light, changes in colony morphology may function to maintain favorable within-colony irradiance levels as external light levels vary (Muko et al. 2000, Anthony et al. 2005, Kaniewska et al. 2008). Some coral species develop more ''open'' geometries in low-light environments, in particular in deep water (Willis 1985, Titlyanov and Titlyanova 2002, Anthony et al. 2005), which can increase light capture. In high-light habitats, it is common for corals to adopt a colony morphology with closely spaced branches/plates to reduce the amount of tissue surface area subjected to supra-optimal irradiance that potentially causes photoinhibition (Anthony et al. 2005, Kaniewska et al. 2008).

These features of reef-building corals highlight the close coevolution that has occurred between corals and *Symbiodinium*. It is critical for phototrophs to adjust their light-harvesting capacity and efficiency of their photosynthetic apparatus to the local light environment. Phenotypic plasticity in corals at the colony and within-tissue levels can modulate light capture and help optimize photosynthetic energy acquisition (Hoogenboom et al. 2008). Scleractinian corals have a high diversity of adaptive solutions for the morphological regulation of absorption and scattering of light at different levels of organization ranging from chloroplast, cell, tissue-coral skeleton to colony scales. Given the diversity of morphological solutions for light modulation both at the colony level and at the subtissue and cellular levels through changes in pigmentation, it is important to understand the relative contribution of both colony and tissue-scale variability in reef-building corals. Here, we test the hypothesis that similar internal light regimes at the scale of the symbiont cells can be achieved by different morphological and biochemical light management strategies in two coral species of contrasting colony morphology. Specifically, we test whether microscale properties of tissues, such as pigmentation and tissue thickness, can provide light management in a massive coral species, which is comparable to environmental-scale light modulation by colony architecture in a branching coral species.

MATERIALS AND METHODS

Field data and coral collection. To investigate the relative contribution of morphological mechanisms (at colony and tissue scales) for modulating light levels reaching *Symbiodinium* cells, we used two species with contrasting colony morphology: *S. pistillata* (purple variety), a branching species displaying high intraspecific morphological plasticity (Veron 1995) with small

polyp size (1 mm ± 0.04) (mean ± SE); and *L. corymbosa*, a common species that has a massive colony morphology and large fleshy polyps (45.2 mm ± 2.39). The purple variety of *S. pistillata* was chosen as it forms symbiosis with one symbiont type as opposed to the brown morph, which forms symbiosis with many different subclades on Heron Island (Sampayo et al. 2007, 2008).

Light measurements were conducted in situ at midday (11:00–14:00) on cloudless days, at 5 m below lowest astronomical tides, for seven colonies of *S. pistillata* (~30 cm diameter, ~25–30 mm branch spacing, ~80 mm colony height) and six colonies of *L. corymbosa* (~30–40 cm diameter), at Harry's Bommie, Heron Island (23°27.625′ S, 151°55.759′ E), southern Great Barrier Reef (GBR), Australia. To account for irradiance variation across colony surfaces, six irradiance points were used on each colony: three points (0, 1, and 2 cm from the top) on the top part of the colony and three (0, 1, and 2 cm from the top) irradiance points on the side part of the colony (see Fig. 1). The depth of the measurements into the coral colonies was determined by the depth of the *L. corymbosa* polyp structure (corallite), which is 2 cm (this is less than the depth of the branches of *S. pistillata*), so that a comparison between the two species could be made.

Downwelling quantum irradiance within the photosynthetically active range (PAR, 400–700 nm) was measured at each point within the colonies using a small calibrated cosine corrected fiber optic quantum sensor (2 mm diameter; Diving-F1; Walz, Effeltrich, Germany) attached to a submersible fluorometer (Diving-PAM, Walz). Measurements of incident downwelling irradiance were first taken at the tip of the coral branch/polyp with the sensor held horizontally, to provide estimates of ambient irradiance as a reference. Consecutive measurements were taken with increasing distance from the tip following the orientation of the polyps on the side of the branch of *S. pistillata*/or the contour of the *L. corymbosa* polyp (Fig. 1). In this way, the light profile would represent a ''polyp view'' of the incident light at various parts of the branch (Anthony et al. 2005), assuming that polyp light capture is similar to a cosine collector. The vertical attenuation coefficient (K_d) for downwelling irradiance was calculated using point measurements of coral surface irradiance values for the two coral species and according to the relationship described by Kirk (1981):

$$K_d = [1/z_2 - z_1] \ln(E_d z_1 / E_d z_2) \quad (1)$$

where $E_d z_1$ and $E_d z_2$ are downwelling irradiances at depths z_1 and z_2 at the coral surface.

To establish coral surface irradiance levels within the complex branching *S. pistillata* colonies, additional irradiance profiles (10 profiles per colony) were determined for 10 *S. pistillata* colonies (~30 cm diameter, 25–30 mm branch spacing, ~80 mm colony height), based on light measurements conducted on top and side sections of the colony. Again, measurements of incident downwelling irradiance were first read at the tip of the coral branch with the sensor held horizontally, to provide estimates of ambient irradiance as a reference. Consecutive measurements with increasing distance from the tip were following the orientation of the polyps on the side of the branch. These irradiance measurements were used to estimate light attenuation by fitting the function to the data using nonlinear regression:

$$I_{(d)} = I_{(0)}\, a \times d^{-(b)} \quad (2)$$

where $I_{(d)}$ is irradiance at depth d, $I_{(0)}$ is ambient downwelling irradiance, and a and b are estimated attenuation coefficients.

After light profile measurements, two branches from each *S. pistillata* colony and two polyps from each *L. corymbosa* colony were collected and used for scalar irradiance microprobe measurements. To account for within-colony variation, one branch/polyp from the top part of the colony and one branch/polyp from the side part of the colony were collected.

Scalar irradiance microprobe measurements. Microscale measurements of scalar irradiance within tissue layers of *S. pistillata* and *L. corymbosa* were performed under constant artificial lighting (275 W metal halide; Aquamedic, Bissendorf, Germany) using methods and equipment described in Magnusson et al. (2007) and Kühl (2005). The microprobe was mounted in a manually operated micromanipulator and was carefully inserted to a depth of 2 mm below the coral tissue surface for both species, without touching the skeleton beneath and ensuring that there still was a tissue layer between the sensor and the skeleton for both coral species. Prior to insertion of the microprobe, a hole was carefully drilled into the tissue with a 0.5 mm diameter carbide drill. To compare scalar irradiance microprobe measurements between the two species, the depth of the measurements was determined by the depth (in relation to the coral tissue surface) of the *S. pistillata* tissue thickness (measured on a cross-section of the coral with a ruler), which is a little more than 2 mm, as it is less than the *L. corymbosa* tissue thickness. The spectral attenuation coefficient of scalar irradiance $K_0(\lambda)$ in the coral tissues of the two species was calculated in units of mm^{-1} (Kühl and Jørgensen 1994, Kühl 2005):

$$K_0(\lambda) = -\ln(E_0(\lambda)_1 / E_0(\lambda)_2)/(z_2 - z_1) \quad (3)$$

where $E_0(\lambda)_1$ and $E_0(\lambda)_2$ are spectral scalar irradiances at depths (mm) z_1 and z_2 within the tissue layer. After measurements, the samples were snap frozen in liquid N_2 and stored at −80°C for subsequent pigment analysis.

Mapping of within-colony light distributions. The light distribution across branches in a whole *S. pistillata* colony was estimated from measured light attenuation profiles (see above) for top and side branches, as derived from nonlinear regression. Monte Carlo simulation was used to generate a tissue surface light distribution across a coral branch using the estimated best-fit parameters and a variance–covariance matrix for the light attenuation profiles of both top and side branches. This step was iterated 100 times by randomly selecting parameter values according to a normal distribution in Excel (ver. 2007; Microsoft Inc., Redmond, WA, USA) and PopTools (ver. 3.2.3) (Hood 2010). We used the daily maximum irradiance (mean ± SD) measured at 5 m at Harry's Bommie, Heron Island, as recorded with underwater data loggers (see Kaniewska et al. 2008), as the input variable. Each simulation generated a tissue surface light distribution across the coral branch, and the results from 100 simulations then represented the light distribution at the coral tissue surface for a *S. pistillata* colony.

The assumptions for the Monte Carlo simulations were that the residuals were normally distributed and that the ratio of direct to diffuse light remained constant over the entire branch surface. We used the maximum rather than the average daily ambient irradiance because corals in shallow water are more likely to adjust to the highest and potentially most damaging irradiances, where the costs of photoinhibition can be substantial (Hoogenboom et al. 2006, 2009). Maximal daily irradiance exposure can last up to 5 $h \cdot d^{-1}$, which is almost half the daylight period. Light distribution across the surface of the coral colony was determined for *L. corymbosa* as described above, but using PAR attenuation parameters derived from point irradiance measured at top and side polyps of *L. corymbosa* colonies, in the Monte Carlo simulations. The mode of the simulated PAR distribution and a chi-squared (χ^2) test of light distributions for the two species were used to determine the difference in within-colony surface light distributions between *S. pistillata* and *L. corymbosa*. Light distributions within tissue

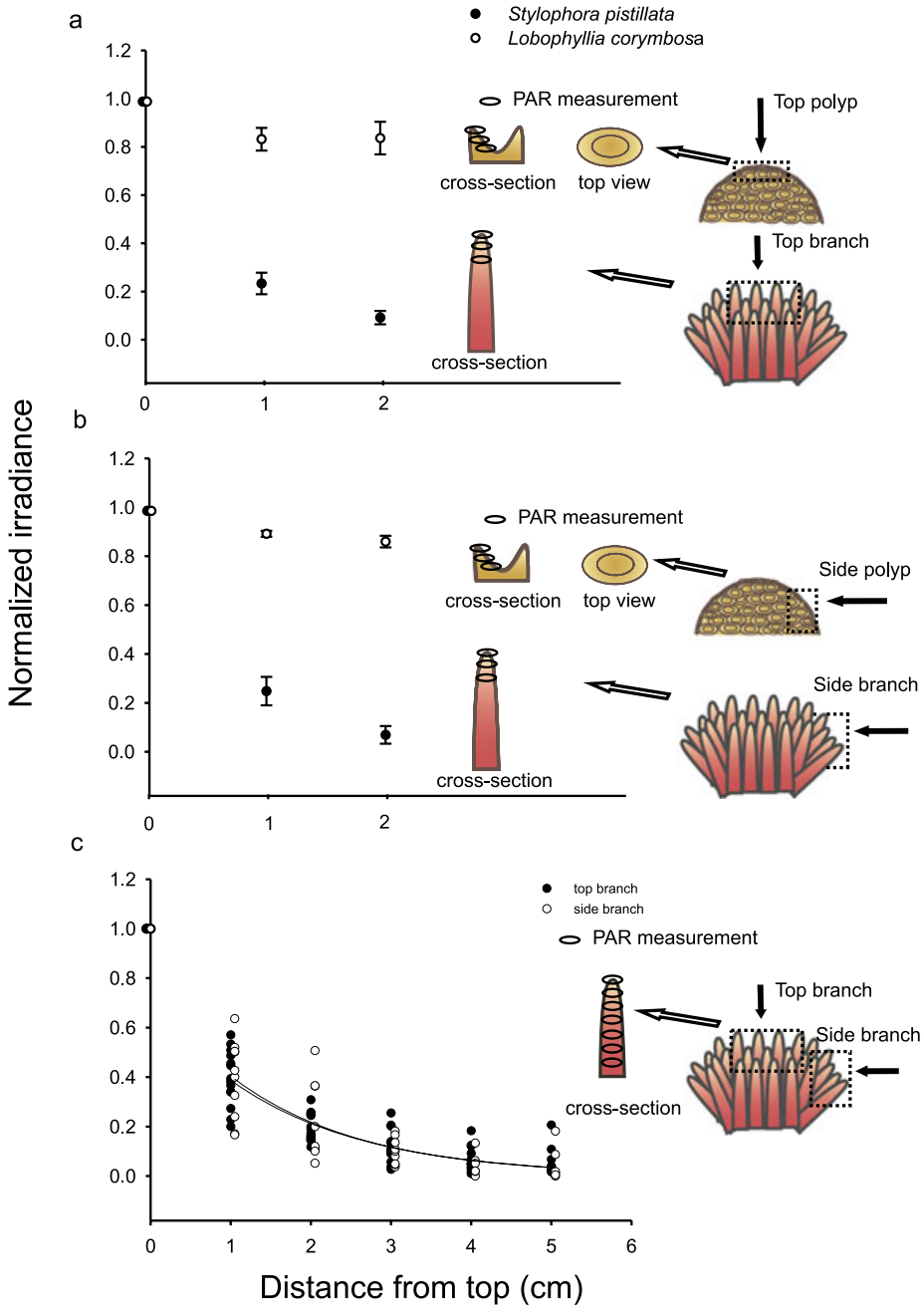

FIG. 1. Normalized irradiance (PAR, 400–700 nm; percentage of incident downwelling irradiance) on coral surface at 1 and 2 cm away from the tip/edge of the branch/polyp in the reef-building corals *Stylophora pistillata* (solid circles) and *Lobophyllia corymbosa* (open circles) at 5 m at Harry's Bommie, Heron Island, southern Great Barrier Reef. Irradiance at positions on (a) top of colonies and (b) at points along the side of colonies. (c) Within-colony light attenuation profiles for top (solid circles) [$I_{(d)} = I_{(0)}$ $(0.707 \pm 0.065) \times d^{-(0.601\pm0.057)}$, $r^2 = 0.75$, $P < 0.001$] and side parts (open circles) [$I_{(d)} = I_{(0)}$ $(0.730 \pm 0.131) \times d^{-(0.620\pm0.121)}$, $r^2 = 0.59$, $P < 0.001$] of *S. pistillata* colonies, where $I_{(d)}$ is irradiance at depth d and $I_{(0)}$ is ambient downwelling irradiance. Irradiance was normalized to the average irradiance at the top of each colony. Illustrations depict positions on the coral branch/polyp and positions in the colony where PAR measurements were taken. Error bars represent the standard error of the mean.

layers (2 mm into the coral tissues) across a colony were simulated for both species using the PAR attenuation parameters within the tissue determined from scalar irradiance microprobe measurements, and by converting the simulated whole colony surface light distributions derived above to within-tissue light distribution, where randomly selected parameters for all functions were used simultaneously. The mode of the simulated PAR distribution and a χ^2 test of light distributions for the two species were used to determine differences in the light distribution within tissue layers between *S. pistillata* and *L. corymbosa*.

In situ coral reflected light spectrum. Reflected light was measured underwater at 5 m at noon on a cloudless day, using a battery-driven USB2000 spectrometer (Ocean Optics,

Dunedin, FL, USA; bandwidth 200–850 nm, in a custom-made underwater housing) with an attached fiber-optic probe (core diameter of 200 μm). The measurements were converted to a proxy for coral tissue absorbance (D) as log $[1/R(\lambda)]$ (Shibata 1969). Here, $R(\lambda)$ is the reflectance calculated as (I/I_0), where I is the reflected spectrum measured from the coral tissue at a chosen point on the coral branch and I_0 is the reflected spectrum from a white diffusing reference surface made out of polytetrafluoroethylene (PTFE) at the same distance and angle. Reflected light was measured for each of the seven *S. pistillata* and six *L. corymbosa* colonies with 15 repetitions per colony.

Symbiodinium *identity.* The genotype of *Symbiodinium* in *L. corymbosa* colonies used in this study was identified by denaturing-gel electrophoresis (DGGE) using methods described in Sampayo et al. (2007, 2008), where the internal transcriber space 2 (ITS2) region of nuclear ribosomal DNA was amplified using primer sequences of LaJeunesse et al. (2003). Profiles were compared with symbiont profiles generated by Sampayo et al. (2007, 2008, 2009). Prominent DGGE bands were excised, sequenced, and identified as described in Sampayo et al. (2008). The genotype of *Symbiodinium* in each of the seven *S. pistillata* colonies used in this study has previously been identified by Sampayo et al. (2008).

Population density and pigment content of Symbiodinium. The cell density and pigment content of *Symbiodinium* were measured by removing tissue from coral fragments by air-brushing frozen fragments in 5 mL 0.06 M phosphate buffer pH 6.65. The homogenate was centrifuged (Sigma 3K15, Osterode am Harz, Germany) at 4,000*g* for 5 min. The supernatant was removed and transferred into a tube to be used for host pigment quantification. The remaining dinoflagellate pellet was resuspended in filtered seawater (0.45 μm) and separated into aliquots that were used for pigment quantification and symbiont cell counts. *Symbiodinium* pigment quantification aliquots were centrifuged at 4,000*g* for 5 min, the supernatant was removed, and 1 mL 100% cold methanol was added to the pellet. The solution was sonicated on ice-cold water for 10 min and then centrifuged at 4,000*g* for 5 min. The supernatant was collected and transferred into a tube. This process was repeated until complete pigment extraction was achieved (when the final supernatant was clear). The total final extracted solution was filtered (0.45 μm) and used for pigment separation in a Shimadzu SCL-10 HPLC linked to a Shimadzu SPD-M10A photodiode array detector (Shimadzu, Kyoto, Japan), using the column and method described in Zapata et al. (2000) with solutions A (methanol:acetonitrile:aquose pyridine, 50:25:25 v:v:v) and B1 (methanol:acetonitrile: acetone, 20:60:20 v:v:v). Standards for methanol-extracted pigments (chl *a*, chl c_2, peridinin *a*, peridinin *b*, β carotene, diatoxanthin [Dtn], diadinoxanthin [Ddn]; Dove et al. 2006) were used for quantifying pigments and normalized on a per-cell basis. *Symbiodinium* cell counts were estimated using eight randomly selected replicates counted using a hemocytometer (Boeco, Hamburg, Germany) on a Zeiss standard microscope (Zeiss, Oberkochen, Germany). *Symbiodinium* counts were normalized to coral surface area in cm^2, as obtained by dipping coral fragments into paraffin wax following the method of Stimson and Kinzie (1991).

Total water-soluble protein content. To determine total water-soluble protein content, the supernatant from the air-brushed tissue was used. The supernatant was analyzed in a Shimadzu UV 2450 spectrophotometer recording absorbance values at 235 and 280 nm. Total water-soluble protein content was determined using the equations of Whitaker and Granum (1980).

Estimation of chl a*–specific absorption coefficient.* To calculate the chl *a*–specific absorption coefficient, which is a proxy for the light absorption efficiency of chl *a* (Morel and Bricaud 1981, Kiefer and Mitchell 1983, Enriquez et al. 2005), branches/polyps were collected using a hammer and chisel from a natural gradient of bleached and nonbleached parts of *S. pistillata* (17 colonies) and *L. corymbosa* (17 colonies), at 5 m at Harry's Bommie, Heron Island.

The reflected light spectrum of the intact coral fragments was measured with a S2000 spectrometer (Ocean Optics; bandwidth of 350–900 nm) equipped with two optical fibers with a core diameter of 1 mm. Coral fragments were placed in a black nonreflecting container with seawater and illuminated with a 150W OSEKE Halogen lamp (Osram Sylvania, Danvers, MA, USA). Reflected light spectrum was measured with the optical fiber held at a constant distance of 1 cm above the coral surface, positioned at an angle of 45° relative to the vertically incident light to avoid self-shading. Both optical fibers took measurements simultaneously, one pointing to a white diffusing reference surface and the other to the coral sample. The reflectance, $R(\lambda)$ was calculated as (I/I_0), where I is the reflected spectrum measured from the coral tissue at a chosen point on the coral branch, and I_0 is the reflected spectrum measured at the same distance and angle from a white diffusing reference surface made out of PTFE. The measurements were converted to a proxy for coral tissue absorbance as log $[1/R(\lambda)]$ (Shibata 1969) as described above. Following measurements, samples were snap frozen and stored at −80°C until processed.

Tissue was removed from coral fragments using the procedure described above. The extracted tissue was centrifuged (Sigma 3K15) at 4,000*g* for 10 min at 4°C, the supernatant was removed, and the pellet was resuspended to a final concentration of 90% acetone and kept in darkness at 4°C for 24 h for photosynthetic pigment extraction. Sample extracts were assayed on a Shimadzu UV 2450 spectrophotometer (Shimadzu), taking absorbance readings at 630 and 663 nm. Chl *a* concentrations were determined using the equations of Jeffrey and Humphrey (1975). *Symbiodinium* cell counts and coral fragment surface area were determined as described above. Chl *a* concentrations were standardized to coral fragment surface area.

The chl *a*–specific absorption coefficient $a^*_{\text{chl}\,a}$ was calculated using the relationship $a^*_{\text{chl}\,a} = (D/\rho)\ \ln 10$ (Enriquez et al. 2005), where D is the absorbance value at 675 nm for the intact coral fragment and ρ is the chl *a* content ($\text{mg} \cdot \text{m}^{-2}$) of the extracted *Symbiodinium*.

Data analysis. All data were tested for normality and homogeneity of variance, and when assumptions were violated, the data were transformed prior to analysis. Nonparametric equivalents of tests were used in cases where assumptions were violated despite transformations. Two-way analysis of variance (ANOVA) was used to determine the effect of species and colony area on irradiance within the coral tissue, *Symbiodinium* density and pigmentation, and total host water soluble protein. A Kruskal–Wallis test was used to determine the effect of species and colony area on external coral surface irradiance. Nonlinear regression equations were fitted to the within-colony light profiles of *S. pistillata* and the specific absorption coefficient ($a^*_{\text{chl}\,a}$) data. Chi-squared tests of estimated PAR distribution at the coral surface and within the coral tissue were used to determine differences between the two coral species. All statistical analyses were performed using STATISTICA 7.0 (Statsoft Inc., Tulsa, OK, USA).

RESULTS

Coral surface irradiance. Light was 10–20 times more attenuated at the level of the colony coral surface in *S. pistillata* (K_d top: $1.99 \pm 0.23\ \text{cm}^{-1}$; K_d side: $2.33 \pm 0.33\ \text{cm}^{-1}$) than in *L. corymbosa* (K_d top:

0.18 ± 0.06 cm^{-1}; K_d side: 0.12 ± 0.01 cm^{-1}) (Kruskal–Wallis test, $H_{1,24} = 17.28$, $P < 0.001$). No differences were detected between the top and side parts of colonies in both species (Kruskal–Wallis test, $H_{1,24} = 0.003$, $P = 0.954$). Within the first cm away from the tip of the branch, the proportion of incident downwelling irradiance in branching *S. pistillata* was 25%, that is, 3- to 4-fold less than in massive *L. corymbosa* (87%) at the first cm away from the polyp edge, where any self-shading could only occur at the fleshy polyp structure level. A similar trend was determined at 2 cm away from the branch tip in *S. pistillata* where the proportion of incident ambient downwelling irradiance was 10%, that is, 8- to 9-fold less than light at the coral surface at 2 cm away from the polyp edge in *L. corymbosa*, where there was 85% of the incident ambient irradiance (Fig. 1, a and b). Consistent with the point irradiance measurements for *S. pistillata* reported above, within-colony light attenuation profiles, and the estimated light attenuation function, which provided more detail on the coral surface irradiance for the more complex branching colony morphology in *S. pistillata*, showed similar light attenuation coefficients for top and side branches (Fig. 1c). A detailed description of the mapped tissue surface irradiances is presented below.

Within-tissue scalar irradiance spectra. Attenuation was most pronounced at the absorption maxima of chl *a* (675 nm) and *c* (~635 nm) and in regions of combined chl and carotenoid absorption (400–550 nm). The light attenuation as described by the spectral attenuation coefficient of scalar irradiance [$K_0(\lambda)$] was 2- to 3-fold greater in the outer 2 mm of *L. corymbosa* (top: 2.39 ± 0.41; side: 2.21 ± 0.19 mm^{-1}) tissues than in *S. pistillata* tissues (top: 0.88 ± 0.11; side: 0.93 ± 0.11 mm^{-1}) (two-way ANOVA, $F_{1,24} = 40.77$, $P < 0.001$), while there was no difference in tissue light attenuation between top and side parts of colonies within species (two-way ANOVA, $F_{1,24} = 0.06$, $P = 0.805$) and no significant interaction between species and location within colony (two-way ANOVA, $F_{1,24} = 0.11$, $P = 0.739$) (Fig. 2). At 2 mm depth within the coral tissues, the percentage of incident downwelling irradiance was 10-fold less for *L. corymbosa* (3.06 ± 2.09%) than in *S. pistillata* (21.6 ± 4.66%), in the top part of colonies. In the side part of colonies at 2 mm depth within the coral tissues, the percentage of incident downwelling irradiance was 6-fold less for *L. corymbosa* (1.72 ± 0.65%) than in *S. pistillata* (17.04 ± 3.49%) (Fig. 2).

Light distribution at different spatial scales. Simulated light distributions across whole colonies of the two coral species underscored the differences in colony surface irradiances, showing a 3-fold difference in the amount of PAR at the whole colony coral surface of *S. pistillata* as compared to *L. corymbosa*. An illustration mapping theses tissue surface irradiances for both coral species is depicted in Figure 3a. Despite this pronounced difference at the colony scale, light levels (PAR) 2 mm into the tissues were similar between the two species due to differential optical properties within the tissues.

For *S. pistillata*, the frequency distribution of 14 irradiance ranges (25–800 μmol photons · m^{-2} · s^{-1}) at the coral surface level showed a shift toward lower irradiance, where the modal range was 50–150 μmol photons · m^{-2} · s^{-1}. *L. corymbosa*, on the other hand, displayed a shift toward higher irradiance, where the modal range was 400–600 μmol photons · m^{-2} · s^{-1} (Fig. 3a). These differences were confirmed by a chi-squared test of within-colony surface light distributions between the two species (chi-squared test, $x^2 = 27.296$, $P = 0.018$).

In contrast, the whole colony within-tissue (2 mm into the coral tissue) frequency distribution of 10 irradiance ranges (5–175 μmol photons · m^{-2} · s^{-1}) revealed similar irradiance levels for both *S. pistillata* and *L. corymbosa*, where both species had a modal value of 30 μmol photons · m^{-2} · s^{-1} (Fig. 3b). No difference was observed in the within-tissue light distributions between the two species (chi-squared test, $x^2 = 0.286$, $P = 1.000$).

Symbiodinium *identification.* The two coral species harbored different *Symbiodinium* symbionts. Seven purple *S. pistillata* colonies were previously shown to harbor the c8/a symbiont type (Sampayo et al. 2008), and there were no within-colony differences in *Symbiodinium* genotype for the *S. pistillata* purple variety in waters surrounding Heron Island (Sampayo et al. 2007). The six *L. corymbosa* colonies, however, were found to harbor a different *Symbiodinium* strain. The sequence identity was found to be c3k when compared to known *Symbiodinium* ITS2 sequences on GenBank (http://www.ncbi.nih.gov).

Coral reflectance and the specific absorption coefficient of chl a. In situ spectral reflectance measurements suggested that the two coral species harbored different pigments within their tissues (Fig. 4a). Differences in tissue pigment composition were more apparent in the in situ absorbance spectra (Fig. 4b), revealing absorbance peaks in the red part of the spectrum, representing the symbiont photopigments chl *a* and the accessory antenna pigment chl c_2, at 675 and 639 nm, respectively. Absorbance was also apparent in regions of combined chl and carotenoid absorption, and host green fluorescent protein (GFP)–like pigments (400–580 nm) (Fig. 4, a and b). In the UV-absorbing region (280–400 nm) of the spectrum, there was also evidence for the presence of UV-absorbing compounds (e.g., MAAs) in both coral species. Reflectance spectra measurements done in the lab (Fig. 4, c and d) reveal some differences compared to the in situ reflected light spectra (Fig. 4, a and b). The main difference was in the red region ~700 nm where the in situ reflected light spectra showed higher absorbance. Overall changes in the specific absorption coefficient of chl *a*, $a^*_{\text{chl}\,a}$,

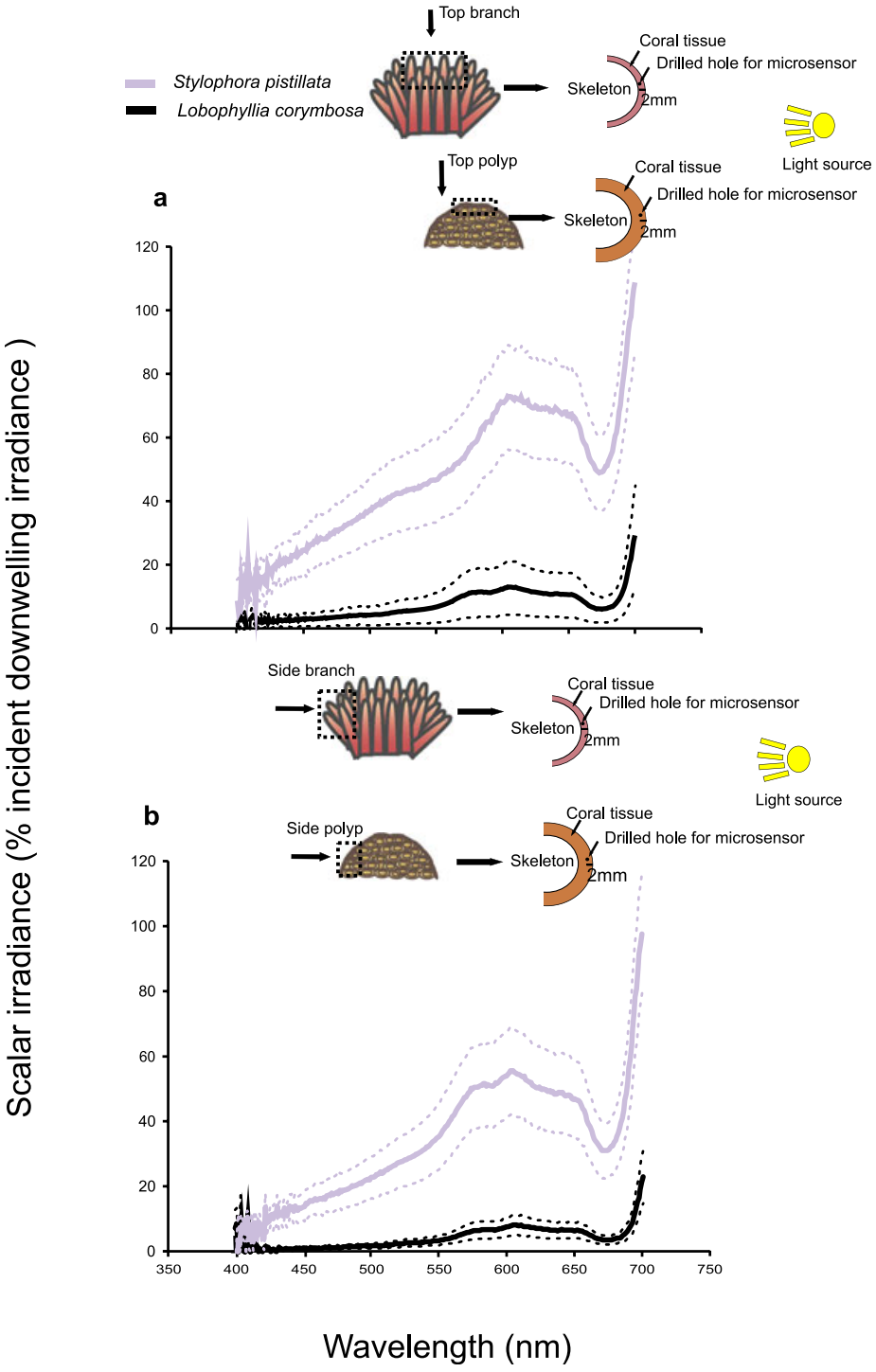

FIG. 2. Spectral scalar irradiance (percentage of incident downwelling irradiance) measured 2 mm into the coral tissue for the reef-building corals, *Stylophora pistillata* (gray line) and *Lobophyllia corymbosa* (black line) at the top of colonies (a) and sides of colonies (b). Data were normalized to the incident downwelling irradiance at the coral surface, and curves represent an average of multiple measurements ($n = 7$ for *S. pistillata* and $n = 6$ for *L. corymbosa*); dotted lines indicate the standard error of the mean. Illustrations depict positions on the coral branch/polyp and positions in the colony where PAR measurements were taken.

with increasing chl *a* concentration showed similar trends for both species, that is, a decrease in $a^*_{\text{chl}\,a}$, with increasing chl *a* content. Specifically, the parameters explaining the relationship between $a^*_{\text{chl}\,a}$ and chl *a* concentration were not significantly different between species (Fig. 4c).

Population density and pigmentation of Symbiodinium. Side parts of *S. pistillata* colonies harbored 45%

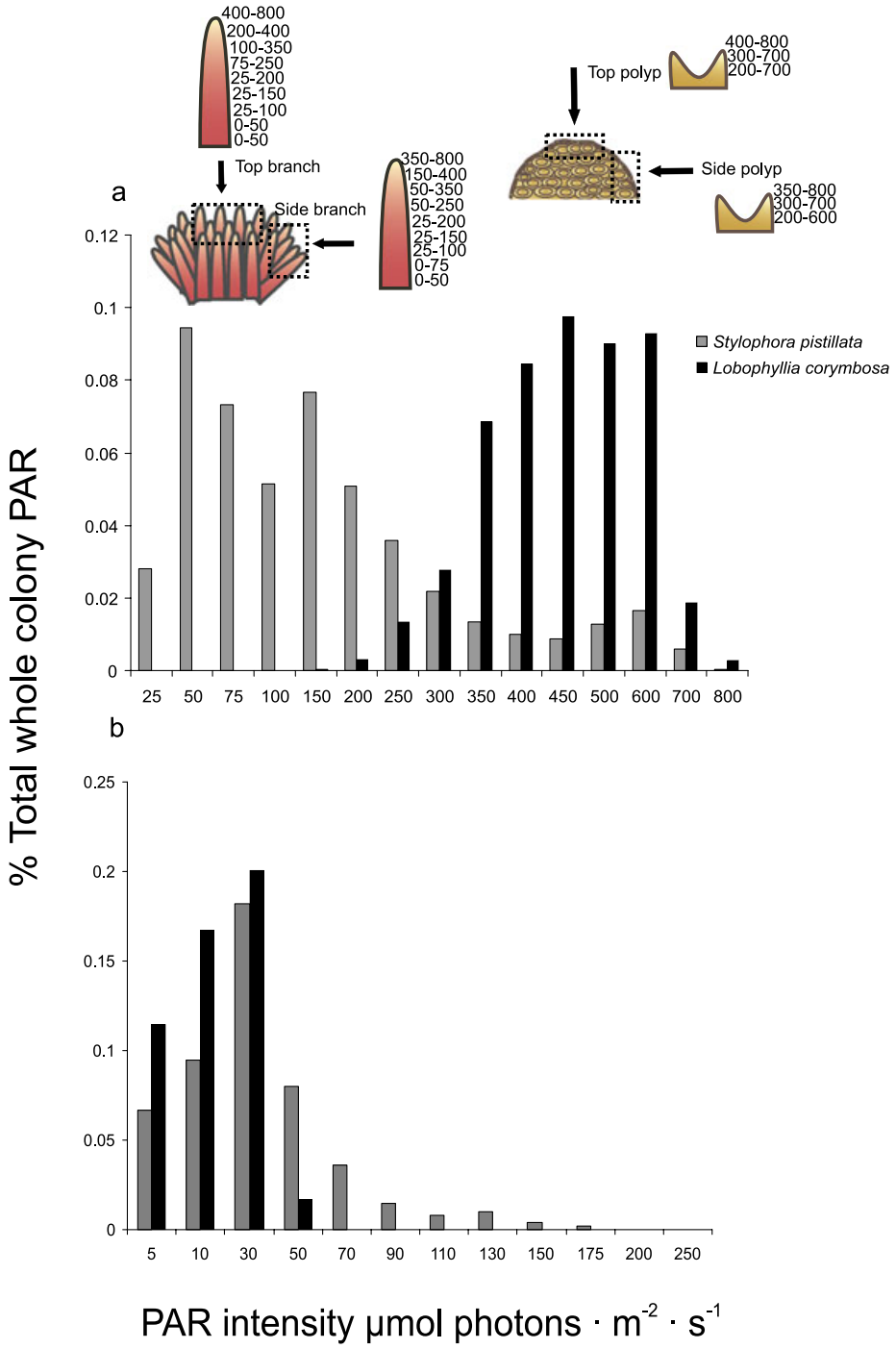

FIG. 3. (a) Whole colony PAR distribution for external coral surface irradiance in *Stylophora pistillata* (gray) and *Lobophyllia corymbosa* (black) at Heron Island. (b) Whole colony PAR distribution, 2 mm into the coral tissue for *S. pistillata* (gray) and *L. corymbosa* (black). The light distributions were obtained from 100 Monte Carlo iterations using the mean daily maximum irradiance as input value and estimated best-fit function parameters for tissue surface light attenuation profiles and within-tissue attenuation coefficients. The light distributions are given as percentages across irradiance categories of the total whole colony PAR level. Illustrations depict a distribution map of irradiance categories across the coral branch/polyp and positions in the colony for each species.

denser *Symbiodinium* populations than the top parts of *S. pistillata* colonies and *L. corymbosa* side and top parts of colonies (Tables 1 and 2). No difference in *Symbiodinium* density was seen between top and side parts of *L. corymbosa* colonies. While the chl *a* and chl c_2 content per cell did not show variation, other photosynthetic pigments differed between species (Tables 1 and 2). The symbionts in *L. corymbosa* had

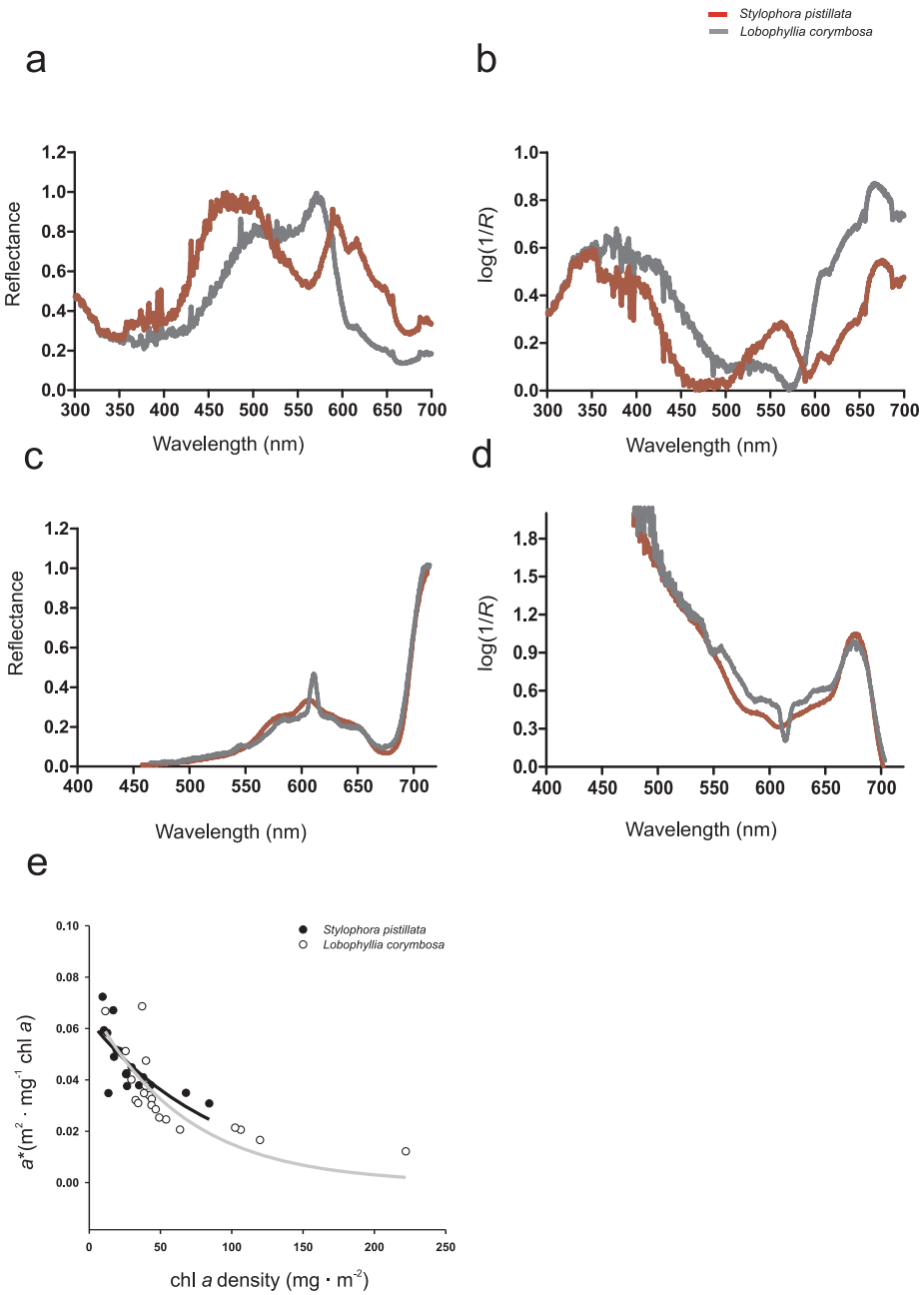

FIG. 4. Underwater measurements of spectral reflectance (a) and derived absorbance spectra (b) at Harry's Bommie, Heron Island, southern Great Barrier Reef (5 m), of (red line) *Stylophora pistillata* ($n = 7$) and (gray line) *Lobophyllia corymbosa* ($n = 6$); curves represent an average of multiple measurements. Laboratory measurements of spectral reflectance (c) and derived absorbance spectra (d) for *S. pistillata* (red line; 37.9 mg chl $a \cdot m^{-2}$) and *L. corymbosa* (gray line; 39.8 mg chl $a \cdot m^{-2}$). (e) The specific absorption coefficient of chl *a* ($a^*_{\text{chl}\,a}$) as a function of chl *a* concentration in the reef-building corals *S. pistillata* (solid circles) ($a^*_{\text{chl}\,a} = 0.063 \pm 0.005 \times \text{chl } a^{-0.011 \pm 0.003}$, $r^2 = 0.51$, $P = 0.001$) and *L. corymbosa* (open circles) ($a^*_{\text{chl}\,a} = 0.071 \pm 0.010 \times \text{chl } a^{-0.015 \pm 0.004}$, $r^2 = 0.61$, $P < 0.001$).

2.5-fold more peridinin *a* per cell than in *S. pistillata*. There was 2.3-fold more peridinin *b* in *L. corymbosa* symbiont cells than in *S. pistillata* symbionts cells. Highest β-carotene concentrations were observed in *L. corymbosa* colonies; here the symbiont cells had 2.3-fold more β-carotene than in *S. pistillata* symbiont cells. The xanthophyll pool (Dtn and Ddn) was 2.2-fold higher in *L. corymbosa* symbiont cells than in *S. pistillata* symbiont cells. Symbiont cells in *L. corymbosa* also had a 40% higher conversion of Ddn to Dtn than in *S. pistillata* symbiont cells (Tables 1 and 2).

Total water-soluble protein of the coral host. The tissue of *L. corymbosa* had twice the amount of water-soluble protein than the tissue of *S. pistillata* (Tables 1 and 2).

TABLE 1. Means (±SE) of photoacclimatory properties of *Symbiodinium* cells and the coral host for top and side parts of colonies of *Lobophyllia corymbosa* ($n = 6$) and *Stylophora pistillata* ($n = 7$).

Photoacclimatory properties in *Symbiodinium* cells/coral host	*S. pistillata* mean ± SE				*L. corymbosa* mean ± SE			
	Top		Side		Top		Side	
Symbiodinium								
cell no · cm^{-2}	7.708E+05	5.513E+04	1.119E+06	1.355E+05	6.271E+05	1.157E+05	7.022E+05	6.918E+04
ng chl a · $cell^{-1}$	16.741	4.351	17.411	4.086	18.332	5.346	21.254	5.808
ng chl c_2 · $cell^{-1}$	4.902	1.234	5.154	1.247	9.522	3.623	9.684	3.455
ng peridinin a · $cell^{-1}$	7.625	2.025	8.547	2.193	19.053	7.152	18.920	7.074
ng peridinin b · $cell^{-1}$	3.294	0.790	3.301	0.776	7.436	2.532	7.319	2.661
ng β carotene · $cell^{-1}$	0.486	0.125	0.545	0.134	1.179	0.441	1.250	0.552
ng (Dtn + Ddn) · $cell^{-1}$	2.363	0.584	2.533	0.653	5.295	1.756	5.479	1.690
Dtn/Dtn + Ddn	0.135	0.012	0.124	0.013	0.183	0.030	0.178	0.037
Coral host								
mg water-soluble protein · cm^{-2}	0.447	0.068	0.449	0.039	0.766	0.146	0.954	0.228

Dtn, diatoxanthin; Ddn, diadinoxanthin.

TABLE 2. Summary of factorial analysis of variance results for photoacclimatory properties in *Symbiodinium* and coral host, with species (*Stylophora pistillata* and *Lobophyllia corymbosa*) and colony area (top and side) as sources of variance.

Photoacclimatory properties in	Source of variance	Species			Colony area			Species × colony area		
		df	F	P	df	F	P	df	F	P
Symbiodinium	cell no · cm^{-2}	1	7.734	**0.011**	1	4.414	**0.047**	1	1.837	ns
	Total	22			22			22		
	ng chl a · $cell^{-1}$	1	0.314	ns	1	0.137	ns	1	0.054	ns
	Total	22			22			22		
	chl c_2 · $cell^{-1}$	1	3.375	ns	1	0.007	ns	1	0.001	ns
	Total	22			22			22		
	peridinin a · $cell^{-1}$	1	4.954	**0.036**	1	0.007	ns	1	0.012	ns
	Total	22			22			22		
	peridinin b · $cell^{-1}$	1	5.184	**0.032**	1	0.001	ns	1	0.001	ns
	Total	22			22			22		
	β carotene · $cell^{-1}$	1	5.003	**0.037**	1	0.043	ns	1	0.001	ns
	Total	20			20			20		
	(Dtn + Ddn) · $cell^{-1}$	1	5.836	**0.024**	1	0.021	ns	1	0.001	ns
	Total	22			22			22		
	Dtn/Dtn + Ddn	1	4.550	**0.044**	1	0.111	ns	1	0.012	ns
	Total	22			22			22		
Coral host	mg water-soluble protein · cm^{-2}	1	10.908	**0.003**	1	0.457	ns	1	0.280	ns
	Total	22			22			22		

Significant results are highlighted in bold. Dtn, diatoxanthin; Ddn, diadinoxanthin.

DISCUSSION

Our results demonstrate that different coral species can use a variety of light modification strategies and still achieve similar internal irradiances available for the photosynthetic dinoflagellates in the coral tissues. It may be that different combinations of colony morphology, pigmentation, and tissue thicknesses allow reef-building corals to successfully inhabit a wide range of environmental light levels by achieving favorable light levels for their symbionts within their tissues. We show that measures of external light fields do not reflect the internal irradiance available for the photosynthetic symbionts. Here, the comparison of two coral species with contrasting colony morphology, exposed to identical external light fields, showed that despite 3-fold differences in irradiance levels at the colony surface, the light conditions within the first 2 mm of tissues were similar for the two species. This is due to different mechanisms and strategies of light management at a combination of colony and tissue scales. Tissue characteristics in *L. corymbosa* provided light modulation at the tissue scale, attenuating light within the first 2 mm of tissue to a mere 3% of the tissue surface irradiance. It is common for massive species to have greater tissue thickness compared to branching species (Loya et al. 2001), and it has been suggested that these coral species may have a greater self-shading capacity in their tissues compared to thin branching species (Hoegh-Guldberg 1999, Loya et al. 2001). The results of this study support this suggestion, as the tissue light attenuation in the massive *L. corymbosa* was 2- to

3-fold higher than in the thin-tissued branching *S. pistillata*. In *S. pistillata*, on the other hand, colony architecture provided environmental-scale light control. Here, self-shading from branches within the first 2 cm of light penetration into the colonies reduced within-colony light levels to only 10% of the ambient irradiance level.

Colony- and tissue-scale light distribution. Light is considered to be within a favorable range for phototrophs when photon capture by light-harvesting pigments equals the rate at which photochemical charge separations are processed, thus minimizing build-up of active oxygen species (Falkowski and Raven 1997). On the basis of measures of irradiance at the onset of photosynthesis saturation (E_k) from photosynthesis-irradiance curves of *Symbiodinium* both in culture and in hospite (irradiance measured at coral surface), Anthony et al. (2005) estimated the theoretical favorable surface irradiance range for corals to be 150–370 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}. A theoretical lower irradiance limit for reef distribution has been suggested to be 50 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} (Kleypas et al. 1999). In this study, the light distribution mode of the whole colony coral surface for *S. pistillata* was 50–150 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}, which is at the lower end of the suggested favorable irradiance levels for *Symbiodinium*, and it was similar to that found for the foliose coral *Turbinaria mesenterina*, which had a modal within-colony coral surface irradiance range of 100–150 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} (Anthony et al. 2005). *Turbinaria* have often been found to harbor a c1 (symbiont type, van Oppen et al. 2001) symbiont strain (Ulstrup et al. 2006), which is different from the strains found in *S. pistillata* and *L. corymbosa*. In contrast, the light distribution of the whole colony coral surface for *L. corymbosa* revealed a modal range of 400–500 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} due to its dome shape and minimal structural self-shading. This level is above the suggested favorable irradiance levels reported in Anthony et al. (2005), suggesting that if these irradiance levels were reaching populations of *Symbiodinium* within the *L. corymbosa* tissue, then *Symbiodinium* would possibly be subject to regular photoinhibition in this species (Anthony et al. 2005).

Here, the two corals are using different strategies, that is, self-shading due to branching morphology (*S. pistillata*) versus self-shading due to tissue thickness and tissue properties (*L. corymbosa*), to obtain the same within-tissue light fields. In both corals, the modal irradiance within the first 2 mm of the tissue was 30 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}, which is potentially not favorable as measured under field (referring to light levels reaching coral surface) and laboratory culture situations (Iglesias-Prieto and Trench 1994, 1997, Anthony et al. 2005). These relatively low-light levels found in both coral species under the relatively high ambient irradiances at 5 m may help explain that photoinhibition occurs in *Symbiodinium* cultures under high irradiances (Shick et al. 1995, Brown et al. 1999), while evidence for photoinhibition in hospite is more inconclusive (Hoogenboom et al. 2006). This trend may be due to the fact that when a coral colony is exposed to high irradiances, it is likely that *Symbiodinium* experiences a much more shaded environment inside the coral tissues less subject to photoinhibition.

Besides tissue thickness, other properties such as host pigmentation (Salih et al. 2000, Dove et al. 2008) and the properties of the skeleton (amplifying low light via scattering; Enriquez et al. 2005), the extent of which will depend on tissue pigmentation, are likely to play key roles in modulating the light field within the coral tissues. In the current study, light amplification due to skeletal properties is less likely as these were fully pigmented corals, and strong light attenuation occurs before light reaches the skeleton, where such amplification might occur. Irradiance levels within the coral tissue have not previously been correlated with irradiance levels measured at the coral surface in the field (Kühl et al. 1995, Magnusson et al. 2007). Our results indicate that by observing irradiances reaching the photosynthetic *Symbiodinium* populations, as opposed to coral surface light levels, we are able to detect new patterns and strategies of light management in the coral–algal complex, indicating that light measurements at this scale are important to assess photoacclimation states.

Symbiodinium *identity and light absorption efficiency.* Despite the stronger light attenuation within the coral tissues of *L. corymbosa* as compared to *S. pistillata,* the light absorption efficiency by the main light-harvesting pigment chl *a*, as quantified by the specific chl *a* absorption coefficient ($a^*_{\text{chl}\,a}$) (Enriquez et al. 2005), was similar for both species (Fig. 4). This implies that other properties of the coral tissue/*Symbiodinium* are likely to play a more important role in the light attenuation within the tissues. In addition, the similarity in internal light environments and light absorption efficiencies of symbionts within the tissues of both corals is also interesting as the two species harbor different strains of *Symbiodinium* (c3k and c8/a) (symbiont type, LaJeunesse 2001). Several recent studies (e.g., Rowan and Knowlton 1995, Toller et al. 2001, Sampayo et al. 2007) have assumed that the light environment experienced by algal symbionts is similar to the external light environment, suggesting that variation in *Symbiodinium* strains with depth is a function of varying external light environments. Our results show that the external light climate does not reflect the irradiances that *Symbiodinium* populations may experience, and this should be considered when correlations with changes in external light fields are made.

Within-tissue light modulation—coral host. The differences in tissue-light attenuation between *L. corymbosa* and *S. pistillata* can further be explained by host tissue properties. The reflectance spectra

conducted in the field revealed that the tissues of *L. corymbosa* (thicker tissue, large fleshy polyps) and *S. pistillata* (thin tissue, small polyps) had disparate tissue compositions, with differential absorbance between species at 380–450, 560, 639, and 675 nm. Considering that *Symbiodinium* is distributed in gastrodermal cells, a significant amount of light attenuation can occur in the epithelium before reaching the photosynthetic unit. *L. corymbosa* had higher levels of total water-soluble proteins per unit surface area, in part due to greater tissue thickness. This level would influence the amount of PAR and UV light reaching *Symbiodinium* deep within the gastroderm, through absorption of light by proteins in the tissue. Many massive species have thicker tissues compared to branching species (Loya et al. 2001), and corals with thick tissues like *L. corymbosa* are also able to change light levels through tissue movements such as tissue extension and retraction (Brown et al. 2002, Levy et al. 2003). In addition, there are also differences in diurnal polyp extension patterns for different corals, where there is a trend for corals with small polyps such a branching *S. pistillata* to have fully extended polyps all day, while massive fleshy species such as *L. corymbosa* tend to have their polyps only extended during the night (Levy et al. 2006). Polyp extension in *S. pistillata* during the day may help optimize light levels for the symbiont cells within, while in *L. corymbosa* this behavioral host tissue property would not affect light levels within the coral as the polyps only extend at night. Our scalar microprobe measurements reflect all the coral tissue between the tissue surface and 2 mm into the tissues. However, it would not have measured the potential behavioral effects of *S. pistillata* polyp extension, and the effect of these host properties on within colony/tissue light levels should be investigated in future studies. It is likely that a range of mechanisms are used for adjusting light levels reaching the major symbiont populations within the coral tissues.

GFP-like host pigments are another tissue property that can potentially absorb excessive irradiance and protect the photosynthetic unit from overexcitation (Salih et al. 2000, Dove et al. 2001, 2008, Dove 2004). GFP-like host proteins can also play a significant role in absorbing excessive irradiance as cell densities diminish and coral skeletal light amplification may occur (Dove et al. 2008). One of the differences between the purple and brown *S. pistillata* morphs on Heron Island is that the purple *S. pistillata* contains more GFP-like proteins and has thicker coral tissue (data not shown). Although not quantified in this study, it is possible that *L. corymbosa* may have higher amounts of host pigments, which would aid in light attenuation within the coral tissue.

Within-tissue light modulation—Symbiodinium. Differences in the pigmentation of *Symbiodinium* cells are also likely to contribute to greater within-tissue light attenuation in *L. corymbosa* compared to *S. pistillata*, as these light-absorbing compounds modify the within-tissue light environment. Symbiont cell density can be regulated as a consequence of coral growth and maintenance (Hoegh-Guldberg et al. 1987, Falkowski et al. 1993, Weis 2008). Such regulation of cell density can also be used in photoacclimation, and it is common to find higher *Symbiodinium* densities in coral surfaces exposed to low-light levels (e.g., Falkowski and Dubinsky 1981, Titlyanov et al. 2001), which may explain why side branches of *S. pistillata* in our study had the highest cell density, as these would have received less light than the top branches and all areas of the *L. corymbosa* colony surface. Although the population densities of *Symbiodinium* were highest in side branches of *S. pistillata*, *Symbiodinium* cells in *L. corymbosa* had greater concentrations of most accessory photosynthetic pigments, while chl *a* and chl c_2 reached similar levels in both species. It is worth noting that in the purple *S. pistillata*, symbiont densities are much lower, while accessory pigments are higher than those found in the brown morph of *S. pistillata* in the southern GBR (data not shown). Accessory pigments such as β-carotene, and other carotenoids, absorb excess light energy (Baroli et al. 2003), and high-light exposure can induce carotenoid production in corals (Chaumont and Thepenier 1995). An increase in β-carotene in *L. corymbosa* could provide protection against potentially harmful radiation levels and increase light attenuation within the coral tissue.

Xanthophyll de-epoxidation, that is, the conversion of Ddn to Dtn upon light absorption, can alleviate high-light stress through dissipation of absorbed radiation to heat (Brown et al. 1999, Muller et al. 2001, Baroli et al. 2004). Both the xanthophyll pool (Dtn + Ddn) and de-epoxidation (Dtn/Dtn + Ddn) were greater in *L. corymbosa*, thus adding to increased light attenuation within the coral tissue. A correlation between an increase in de-epoxidation and greater nonphotochemical quenching has been shown (Ulstrup et al. 2008). This finding is consistent with a possible greater need for nonphotochemical quenching in the uppermost layer of the thick *L. corymbosa* tissue, where a high-light environment may be present for those symbiont cells as opposed to symbiont cells distributed at a 2 mm depth and deeper, where light attenuation by a range of mechanisms has already reduced the potential risk of excess excitation energy. An increase in accessory pigments, such as peridinin *a* and peridinin *b*, usually occurs as an acclimation to lower light levels (Falkowski and Dubinsky 1981, Iglesias-Prieto and Trench 1994, Fitt and Cook 2001, Titlyanov et al. 2001). We determined that these pigments reached highest concentrations in *L. corymbosa* perhaps representing the more shaded layers of the *L. corymbosa* tissue and reflecting the higher frequency of lower irradiances (<10 μmol photons $\cdot$ $m^{-2} \cdot s^{-1}$) (Fig. 3b) within the tissues of *L. corymbosa*.

CONCLUSION

In summary, this study demonstrates that coral shape and tissue characteristics at different spatial scales modify the light environment experienced by endosymbiotic *Symbiodinium* in corals. Our results suggest that such modulation may form part of the photoacclimation strategy in the symbiosis. By measuring actual irradiance levels reaching some of the photosynthetic *Symbiodinium*, we demonstrate that the external light environment is a poor proxy for internal irradiances experienced by the symbiotic dinoflagellate and that internal mechanisms of light attenuation must be accounted for to assess photoacclimation potential. It remains to be determined if the differential light modulation by shape versus tissue characteristics applies to many other coral species with varying colony morphology. Future studies may also use microsensor photosynthesis measurements or combined microscale O_2 and variable chl fluorescence measurements (Schreiber et al. 1996, Kühl 2005, Kühl and Polerecky 2008) to resolve photoacclimatory states and activity patterns at high spatial resolution across colonies and in different tissues. This step may then help us understand to what extent the light attenuation within the colony and the tissue affects the variation of areas exposed for light collection and the total photosynthetic production of a coral colony.

This work was supported by funding from the Australian Research Council, the Danish Natural Science Research Council (M. K.), and the University of Queensland. We thank Dr. E. Sampayo for technical advice and we would like to thank Dr. P. Campbell, Dr. S. Enriquez, and three anonymous reviewers for insightful comments on drafts of the manuscript. This is a contribution from the ARC Centre of Excellence for Coral Reef Studies.

Anthony, K. R. N. & Fabricius, K. E. 2000. Shifting roles of heterotrophy and autotrophy in coral energetics under varying turbidity. *J. Exp. Mar. Biol. Ecol.* 252:221–53.

Anthony, K. R. N. & Hoegh-Guldberg, O. 2003. Variation in coral photosynthesis, respiration and growth characteristics in contrasting light microhabitats: an analogue to plants in forest gaps and understoreys? *Funct. Ecol.* 17:246–59.

Anthony, K. R. N., Hoogenboom, M. O. & Connolly, S. R. 2005. Adaptive variation in coral geometry and the optimization of internal colony light climates. *Funct. Ecol.* 19:17–26.

Baroli, I., Do, A. D., Yamane, T. & Niyogi, K. K. 2003. Zexanthin accumulation in the absence of a functional xanthophyll cycle protects *Chlamydomonas reinhardtii* from photooxidative stress. *Plant Cell* 15:992–1008.

Baroli, I., Gutman, B. L., Ledford, H. K., Shin, J. W., Chin, B. L., Havaux, M. & Niyogi, K. K. 2004. Photo-oxidative stress in the xanthophyll-deficient mutant of *Chlamydomonas*. *J. Biol. Chem.* 279:6337–44.

Bjorkman, O. 1981. Responses to different quantum flux densities. *In* Lange, O. L., Nobel, P. S., Osmond, C. B. & Ziegler, H. [Eds.] *Encyclopedia of Plant Physiology*. Springer-Verlag, Berlin, pp. 57–107.

Brown, B. E., Ambarsari, I., Warner, M. E., Fitt, W. K., Dunne, R. P., Gibb, S. W. & Cummings, D. G. 1999. Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. *Coral Reefs* 18:99–105.

Brown, B. E., Downs, C. A., Dunne, R. P. & Gibb, S. W. 2002. Exploring the basis of thermotolerance in the reef coral *Goniastra aspera*. *Mar. Ecol. Prog. Ser.* 242:119–29.

Bruno, J. F. & Edmunds, P. J. 1997. Clonal variation for phenotypic plasticity in the coral *Madracis mirabilis*. *Ecology* 78:2177–90.

Chaumont, D. & Thepenier, C. 1995. Carotenoid content in growing cells of *Haematococcus pluvalis* during a sunlight cycle. *J. Appl. Phycol.* 7:529–37.

D'Angelo, C., Denzel, A., Vogt, A., Matz, M. V., Oswald, F., Salih, A., Nienhaus, G. U. & Wiedenmann, J. 2008. Blue light regulation of host pigment in reef-building corals. *Mar. Ecol. Prog. Ser.* 364:97–106.

Dove, S. 2004. Scleractinian corals with photoprotective host pigments are hypersensitive to thermal bleaching. *Mar. Ecol. Prog. Ser.* 272:99–116.

Dove, S., Hoegh-Guldberg, O. & Ranganathan, S. 2001. Major colour patterns of reef-building corals are due to a family of GFP-like proteins. *Coral Reefs* 19:197–204.

Dove, S., Lovell, C., Fine, M., Deckenback, J., Hoegh-Guldberg, O., Iglesias-Prieto, R. & Anthony, K. R. N. 2008. Host pigments: potential facilitators of photosynthesis in coral symbioses. *Plant Cell Environ.* 31:1523–33.

Dove, S., Oritz, J. C., Enriquez, S., Fine, M., Fisher, P., Iglesias-Prieto, R., Thornhill, D. & Hoegh-Guldberg, O. 2006. Response of holosymbiont pigments from the scleractinian coral *Montipora monasteriata* to short term heat stress. *Limnol. Oceanogr.* 51:1149–58.

Dustan, P. 1979. Distribution of zooxanthellae and photosynthetic chloroplast pigments of the reef-building coral *Montastrea annularis* (Ellis and Solander) in relation to depth on a West Indian coral reef. *Bull. Mar. Sci.* 29:79–95.

Enriquez, S., Mendez, E. R. & Iglesias-Prieto, R. 2005. Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnol. Oceanogr.* 50:1025–32.

Falkowski, P. G. & Dubinsky, Z. 1981. Light-shade adaptation of *Stylophora pistillata*, a hermatypic coral from the Gulf of Eliat. *Nature* 289:172–4.

Falkowski, P. G., Dubinsky, Z., Muscatine, L. & McCloskey, L. 1993. Population control in symbiotic corals. *Bioscience* 43:606–11.

Falkowski, P. G., Jokiel, P. L. & Kinzie, R. A. 1990. Irradiance and corals. *In* Dubinsky, Z. [Ed.] *Ecosystems of the World: Coral Reefs*. Elsevier, Amsterdam, pp. 89–107.

Falkowski, P. G. & Raven, J. A. 1997. *Aquatic Photosynthesis*. Blackwell Science, Malden, Massachusetts, pp. 33–64.

Fine, M., Meroz, E. B. & Hoegh-Guldberg, O. 2005. Tolerance of endolithic algae to elevated temperature and light in the coral *Montipora monasteriata* from the southern Great Barrier Reef. *J. Exp. Biol.* 280:75–81.

Fitt, W. K. & Cook, C. B. 2001. Photoacclimation and the effect of the symbiotic environment on the photosynthetic response of the symbiotic dinoflagellates in the tropical marine hydroid *Myrionema amboinense*. *J. Exp. Mar. Biol. Ecol.* 256:15–31.

Fricke, H. W., Vareschi, E. & Schlichter, D. 1987. Photoecology of the coral *Leptoseris fragilis* in the Red Sea twilight zone (an experimental study by submersible). *Oecologia* 73:371–81.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* 50: 839–66.

Hoegh-Guldberg, O. & Jones, R. J. 1999. Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. *Mar. Ecol. Prog. Ser.* 183:73–86.

Hoegh-Guldberg, O., McCloskey, L. R. & Muscatine, L. 1987. Expulsion of zooxanthellae by symbiotic cnidarians from the Red Sea. *Coral Reefs* 5:201–4.

Hood, G. M. 2010. *PopTools Version 3.2.3*. Available at: http://www.poptools.org (release date, September 2010).

Hoogenboom, M. O., Connolly, S. R. & Anthony, K. R. N. 2006. Energetic cost of photoinhibition in corals. *Mar. Ecol. Prog. Ser.* 313:1–12.

Hoogenboom, M. O., Connolly, S. R. & Anthony, K. R. N. 2008. Interactions between morphological and physiological plasticity optimize energy acquisition in corals. *Ecology* 89: 1144–54.

Hoogenboom, M. O., Connolly, S. R. & Anthony, K. R. N. 2009. Effects of photoacclimation on the light niche of corals: a process-based approach. *Mar. Biol.* 156:2493–503.

Iglesias-Prieto, R. & Trench, P. K. 1994. Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. *Mar. Ecol. Prog. Ser.* 113:163–75.

Iglesias-Prieto, R. & Trench, P. K. 1997. Acclimation and adaptation to irradiance in symbiotic dinofagellates. II. Response of chlorophyll-protein complexes to different photon-flux densities. *Mar. Biol.* 130:23–33.

Jeffrey, S. W. & Humphrey, G. F. 1975. New spectrometric equation for determining chlorophyll a, b and c2 on higher plants, algae and natural phytoplankton. *Biochem. Physiol. Aflanz.* 167:191–4.

Jones, R. J. & Hoegh-Guldberg, O. 2001. Diurnal changes in photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: photoprotection, photoactivation and the relationship to coral bleaching. *Plant Cell Environ.* 24:89–99.

Kaandorp, J. A. P., Sloot, M. A., Merks, R. M. H., Bak, R. P. M., Vermeij, M. J. A. & Maier, C. 2005. Morphogenesis of the branching reef coral *Madracis mirabilis*. *Proc. R. Soc. Lond. B* 272:127–33.

Kaiser, P., Schlichter, D. & Fricke, H. W. 1993. Influence of light on algal symbionts of the deep water coral *Leptoseris fragilis*. *Mar. Biol.* 17:45–52.

Kaniewska, P., Anthony, K. R. N. & Hoegh-Guldberg, O. 2008. Variation in colony geometry modulates internal light levels in branching corals, *Acropora humilis* and *Stylophora pistillata*. *Mar. Biol.* 155:649–60.

Kiefer, D. & Mitchell, B. G. 1983. A simple steady state description of phytoplankton growth based on absorption cross-section and quantum efficiency. *Limnol. Oceanogr.* 28:770–7.

Kirk, J. T. O. 1981. Monte Carlo study of the nature of the underwater light field and the relationship between optical properties of turbid yellow waters. *Mar. Freshw. Res.* 32:517–32.

Kleypas, J. A., McManus, J. W. & Menez, L. A. B. 1999. Environmental limits to coral reef development: where do we draw the line? *Am. Zool.* 39:146–59.

Kühl, M. 2005. Optical microsensors for analysis of microbial communities. *Methods Enzymol.* 397:166–99.

Kühl, M., Cohen, Y., Daalsgard, T., Jørgensen, B. B. & Revsbech, N. P. 1995. Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. *Mar. Ecol. Prog. Ser.* 117:159–72.

Kühl, M. & Jørgensen, B. B. 1994. The light field of microbenthic communities: radiance distribution and microscale optics of sandy coastal sediments. *Limnol. Oceanogr.* 39:1368–98.

Kühl, M. & Polerecky, L. 2008. Functional and structural imaging of phototrophic microbial communities and symbioses. *Aquat. Microb. Ecol.* 53:99–118.

LaJeunesse, T. C. 2001. Investigating the biodiversity, ecology and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search for a ''species'' level marker. *J. Phycol.* 37:866–80.

LaJeunesse, T. C., Loh, W. K. W., van Woesik, R., Hoegh-Guldberg, O., Schmidt, G. W. & Fitt, W. K. 2003. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnol. Oceanogr.* 48:2046–54.

Levy, O., Dubinsky, Z. & Achituv, Y. 2003. Photobehavior of stony corals: responses to light spectra and intensity. *J. Exp. Biol.* 206:4041–9.

Levy, O., Dubinsky, Z., Achituv, Y. & Erez, J. 2006. Diurnal polyp expansion behavior in stony corals may enhance carbon availability for symbionts photosynthesis. *J. Exp. Mar. Biol. Ecol.* 333:1–11.

Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H. & van Woesik, R. 2001. Coral bleaching: the winners and losers. *Ecol. Lett.* 4:122–31.

Magnusson, S. H., Fine, M. & Kühl, M. 2007. Light microclimate of endolithic phototrophs in the scleractinian corals *Monitpora monasteriata* and *Porites cylindrica*. *Mar. Ecol. Prog. Ser.* 332:119–28.

Mass, T., Einbinder, S., Brokovich, E., Shashar, N., Vago, R., Erez, J. & Dubinsky, Z. 2007. Photoacclimation of *Stylophora pistillata* to light extremes: metabolism and calcification. *Mar. Ecol. Prog. Ser.* 334:93–102.

Morel, A. & Bricaud, A. 1981. Theoretical results concerning light absorption in a discrete medium, and application to specific absorption of phytoplankton. *Deep-Sea Res.* 28:1375–93.

Muko, S., Kawasaki, K. & Sakai, K. 2000. Morphological plasticity in the coral *Porites sillimaniani* and its adaptive significance. *Bull. Mar. Sci.* 66:225–39.

Muller, P., Xiao-Ping, L. & Niyogi, K. K. 2001. Non-photochemical quenching: a response to excess light energy. *Plant Physiol.* 125:1558–66.

Muscatine, L. & Cernichiari, E. 1969. Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biol. Bull.* 137:506–23.

Muscatine, L. & Porter, J. W. 1977. Reef corals: mutualistic symbiosis adapted to nutrient-poor environments. *Bioscience* 27: 454–60.

van Oppen, M., Palstra, F. P., Piquet, A. M. T. & Miller, D. J. 2001. Patterns of coral-dinoflagellate associations in Acropora: significance of local availability and physiology of *Symbiodinium* strains and host-symbiont selectivity. *Proc. R. Soc. Lond. B* 268:1759–67.

Reaka-Kudla, M. L. 1997. The global biodiversity of coral reefs: a comparison with rainforests. *In* Reaka-Kudla, M. L., Wilson, D. E. & Wilson, E. O. [Eds.] *Biodiversity II. Understanding and Protecting Our Biological Resources*. Joseph Henry Press, Washington, D.C., pp. 83–108.

Rowan, R. & Knowlton, N. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc. Natl. Acad. Sci. U. S. A.* 92:2850–3.

Salih, A., Larkum, A., Cox, G., Kühl, M. & Hoegh-Guldberg, O. 2000. Fluorescent pigments in corals are photoprotective. *Nature* 408:850–3.

Sampayo, E. M., Dove, S. & LaJeunesse, T. C. 2009. Cohesive molecular genetic data delineate species diversity in the dinoflagellate genus *Symbiodinium*. *Mol. Ecol.* 18:500–19.

Sampayo, E. M., Franceschinis, L., Hoegh-Guldberg, O. & Dove, S. 2007. Niche partitioning of closely related symbiotic dinoflagellates. *Mol. Ecol.* 16:3721–33.

Sampayo, E. M., Ridgeway, T., Bongaerts, P. & Hoegh-Guldberg, O. 2008. Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proc. Natl. Acad. Sci. U. S. A.* 105:10444–9.

Schreiber, U., Kühl, M., Klimant, I. & Reising, H. 1996. Measurement of chlorophyll fluorescence within leaves using a modified PAM fluorometer with a fiber-optic microprobe. *Photosynth. Res.* 47:103–9.

Sebens, K. P., Witting, J. & Helmuth, B. 1997. Effects of water flow and branch spacing on particle capture by the reef coral *Madracis mirabilis* (Duchassaing and Michelotti). *J. Exp. Mar. Biol. Ecol.* 211:1–28.

Shibata, K. 1969. Pigments and UV absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant Cell Physiol.* 10:325–35.

Shibata, K. & Haxo, F. T. 1969. Light transmission and spectral distribution through epi- and endozoic algal layers in the brain coral, *Favia*. *Biol. Bull.* 136:461–8.

Shick, J. M., Lesser, M. P., Dunlap, W. C., Stochaj, W. R., Chalker, B. E. & Won, J. W. 1995. Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microphthalma*. *Mar. Biol.* 122:41–51.

Shick, J. M., Romaine-Lioud, S., Ferrier-Pages, C. & Gattuso, J. P. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. *Limnol. Oceanogr.* 44:1667–82.

Stimson, J. & Kinzie, R. A. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *J. Exp. Mar. Biol. Ecol.* 153:63–74.

Titlyanov, E. A. & Titlyanova, T. V. 2002. Reef-building corals-symbiotic autotrophic organisms: 2. Pathways and mechanisms of adaptation to light. *Russ. J. Mar. Biol.* 28(Suppl. 1): S16–31.

Titlyanov, E. A., Titlyanova, T. V., van Woesik, R. & Yamazato, K. 2002. Acclimation of the hermatypic coral *Stylophora pistillata* to bright light. *Russ. J. Mar. Biol.* 28(Suppl. 1):S41–6.

Titlyanov, E. A., Titlyanova, T. V., Yamazato, K. & van Woesik, R. 2001. Photo-acclimation dynamics of the coral *Stylophora pistillata* to low and extremely low light. *J. Exp. Mar. Biol. Ecol.* 263:211–25.

Toller, W. W., Rowan, R. & Knowlton, N. 2001. Zooxanthellae of the *Montastraea annularis* species complex: patterns of distribution of four taxa of *Symbiodinium* on different reefs and across depths. *Biol. Bull.* 201:348–59.

Ulstrup, K. E., Berkelmans, R., Ralph, P. J. & van Oppen, M. J. H. 2006. Variation in bleaching sensitivity of two coral species across a latitudinal gradient on the Great Barrier Reef: the role of zooxanthellae. *Mar. Ecol. Prog. Ser.* 314:135–48.

Ulstrup, K. E., Hill, R., van Oppen, M. J. H., Larkum, A. W. D. & Ralph, P. J. 2008. Seasonal variation in the photo-physiology of homogeneous and heterogenous *Symbiodinium* consortia in two scleractinian corals. *Mar. Ecol. Prog. Ser.* 361:139–50.

Vermeij, M. J. A. & Bak, R. P. M. 2002. How are coral populations structured by light? Marine light regimes and the distribution of Madracis. *Mar. Ecol. Prog. Ser.* 233:105–16.

Veron, J. E. N. 1995. *Corals in Time and Space*. Cornell University Press, Ithaca, New York, pp. 14–8, 241.

Veron, J. E. N. & Pichon, M. 1982. *Scleractinia of Eastern Australia*. Australian National University Press, Canberra, 159 pp.

Weis, V. M. 2008. Cellular mechanisms of cnidarian bleaching: stress causes the collapse of symbiosis. *J. Exp. Biol.* 211:3059–66.

Whitaker, J. R. & Granum, P. E. 1980. An absolute method for protein determination based on differences in absorbance at 235 and 280 nm. *Anal. Biochem.* 109:156–9.

Willis, B. L. 1985. Phenotypic plasticity versus phenotypic stability in the reef corals *Turbinaria mesenterina* and *Pavona cactus*. *Proc. Fifth Int. Coral Reef Symp.* 4:107–12.

Zapata, M., Rodriquez, F. & Garrido, J. L. 2000. Separation of chlorophylls and carotenoids from marine phytoplankton: a new HPLC method using a reversed-phase C8 column and pyridine-containing mobile phases. *Mar. Ecol. Prog. Ser.* 195:29–45.