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Direct and diffuse light propagation through coral tissue

Daniel Wangpraseurt^{*a} and Michael Kühl^{a,b,c}

^aPlant Functional Biology and Climate Change Cluster, University of Technology Sydney, New South Wales 2007, Australia; ^bMarine Biological Section, Department of Biology, University of Copenhagen, DK-3000 Helsingør, Denmark; ^cSingapore Centre on Environmental Life Sciences Engineering, School of Biological Sciences, Nanyang Technological University, Singapore

ABSTRACT

This study describes the propagation of direct and diffuse light through coral tissue and how changes in the directional quality of light affect photosynthesis. Scalar irradiance microsensors were used *in vivo* to measure tissue light propagation of incident collimated and diffuse irradiance. O₂ microsensors were used to estimate changes in local O₂ evolution. The results show that the directional quality of incident irradiance affects both coral optics and photosynthesis. Collimated irradiance is enhanced at the coral surface while diffuse irradiance is enhanced at the coral skeleton. Coral O₂ evolution is enhanced under collimated compared to diffuse light. It is concluded that the directional quality of light is an important and hitherto ignored parameter in coral photosynthesis.

Keywords: coral tissue optics, marine optics, coral reefs, photosynthesis, bio-optics, diffuse light, microsensors

*daniel.wangpraseurt@uts.edu.au; phone +614 52423237; fax +612 9514 4079; c3.uts.edu.au

1. INTRODUCTION

Corals are marine animals, composed of an aragonite skeleton and a biological tissue that contains photosynthesizing symbiotic microalgae known as *Symbiodinium*. The symbiotic interaction between the coral animal and *Symbiodinium* forms the basis for one of the most diverse biotopes on Earth, the coral reef ecosystem. Solar irradiance is critical for the life of corals as it stimulates photosynthesis by the microalgae, which in turn provides the coral animal with O₂ and essential sugars needed for coral animal metabolism. Coral photobiology has been intensively studied over decades and much is known about light absorption and chemical energy conversion underlying light-harvesting [3-6]. However, the optics of corals has received little attention, albeit insight into coral light transport is fundamental to understanding coral photosynthesis and quantum efficiency.

Coral light transport is modulated by the optical properties of coral tissue and skeleton. The optical properties of corals have similar characteristics to human skin and plant leaves, and thus other highly scattering media [1, 2]. Incident irradiance can be trapped by the coral tissue and efficiently scattered, leading to lateral light transfer over the coral and enhanced photosynthesis by the microalgae [3, 4]. The aragonite skeleton can be strongly backscattering, redirecting diffused photons back into the overlying tissue, again enhancing local scalar irradiance and photosynthesis [5, 6]. However, coral optics are likely to be highly variable and depend upon the coral species and the local light conditions of the marine environment the corals are adapted to [6].

The underwater irradiance regime varies in intensity and spectral quality. Studies have investigated in detail how such variations affect coral photophysiology [e.g., 7, 8, 9]. However, solar radiation perceived by corals varies also in its directional quality, i.e. the degree of light collimation [10] and this aspect has hitherto been neglected. On a temporal scale, the degree of light collimation will vary, e.g. depending on the solar zenith, cloud cover and tidal height [11]. On a spatial scale, water depth strongly affects the directional quality of light, where the diffuse character increases with water depth. Within a given water depth, the directional quality of incident irradiance varies with the reflective properties of the surrounding environment [11]. Backscattering of diffuse light by coral reef sediment can be an important component of diffusely scattered light and contribute about 50 % to the local tissue surface scalar irradiance [Wangpraseurt et al.

unpubl., 12]. Given the high variability in the angular distribution of underwater incident irradiance it is of interest to understand how light direction affects coral optics and photosynthesis.

In this report, we present first measurements of coral optics studied under collimated and diffuse irradiance. We investigate coral light fields and the transport of collimated and diffuse irradiance *in vivo* using light microsensors. We also study the effects of light direction on coral photosynthesis.

2. METHODS

2.1 Corals

Small fragments (< 5cm in diameter) of the brain coral *Platygyra spp.* were collected from shallow waters (<3 m depths) on the reef flat of the Heron Island lagoon, Great Barrier Reef, Australia (152°06'E, 20°29'S). Samples were maintained in outdoor aquaria supplied with a continuous supply of natural seawater and shaded conditions at Heron Island Research station.

2.2 Experimental set-up

Coral fragments were placed in a custom-made black acrylic flow chamber supplied with seawater (salinity 33‰, temperature 25°C) at a flow velocity of $\sim 3 \text{ cm s}^{-1}$. Fiber-optic scalar irradiance microprobes with a spherical light-diffusing tip [diameter $\sim 80 \text{ }\mu\text{m}$; [13]] were used to map coral light microenvironments [3]. The fiber-optic probes were mounted on a PC-controlled motorized micromanipulator for automatic profiling (Pyro-Science GmbH, Germany), at an angle of 45° relative to the vertically incident light [to avoid self-shading, see [13]]. The micromanipulator was fixed onto a heavy-duty vibration-free metal stand. Scalar irradiance spectra were recorded with the microprobes connected to a PC-controlled fiber-optic spectrometer controlled by the manufacturer's software (USB2000+ and Spectrasuite, Ocean Optics, USA). Positioning of the microprobe was facilitated by the manufacturer's software (Profix, Pyro-Science GmbH, Germany). The complete set-up was covered with black cloth to avoid external light entering the system.

Vertically incident collimated illumination at a downwelling quantum irradiance (400-700 nm) of $150 \text{ }\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was provided by a fiber-optic tungsten-halogen lamp (KL-2500, Schott GmbH, Germany), equipped with a heat filter and a collimating lens. For diffuse illumination, a light-diffusing sheet was placed on top of the flow chamber in close proximity to the water surface. The diffusing sheet was of clear polymer doped with TRIMM [Transparent Refractive Index Matched Microparticles; [14]]. Ultimately, the diffusing sheet would be placed directly in water so as to minimize Fresnel reflection of low-angle light at the air-water interface. However, this was not feasible as the diffuser interfered with the flow regime surrounding the coral and also complicated the positioning of the microsensor on the coral surface.

2.3 *In vivo* light measurements under direct and diffuse illumination

To measure scalar irradiance at the tissue surface of corals under identical quantities of diffuse and collimated illumination, the incident light regime was carefully calibrated prior to each measurement. The light microsensor was positioned at a 45° angle in the center of the vertically incident collimated light beam ($\sim 3 \text{ cm}$ below the water surface), above a black light-absorbing surface (black rubber) and the incident spectral scalar irradiance was recorded. The light regime was then changed to diffuse light and the scalar irradiance was recorded for the change in total output by adjusting the aperture of the light source until the spectra of incident collimated and diffuse irradiance closely overlapped.

After the calibration procedure, tissue surface scalar irradiance was measured at the surface of the coenosarc tissue, i.e. the tissue connecting two polyps. For light measurements within the coral tissue, a micro-incision was performed as described previously [see [3]]. Upon incision, the microsensor was carefully positioned at the skeleton surface using the micromanipulator and the automatic profiling function of the motor. Profiling was then performed upwards from the skeleton surface into the overlying tissue in steps of $50 \text{ }\mu\text{m}$ logging the average of 10 spectra at each measuring depth until reaching the tissue surface. At each measuring depth the angular distribution of the incident irradiance was rapidly changed from collimated to diffuse by adding the diffuser and re-arranging the aperture as performed during light calibrations.

The spectral attenuation coefficient of scalar irradiance was calculated over the entire coral tissue thickness ($200 \text{ }\mu\text{m}$) as:

$$K = \ln(E_1/E_2)/(z_2 - z_1) \quad (1)$$

where E_1 and E_2 are the scalar irradiance at depths z_1 and z_2 , respectively [see [15]].

2.4 O₂ microsensor measurements

We used Clark-type O₂ microelectrodes with a tip size of 25 μm , a 90% response time of <0.5 s and a stirring sensitivity of ~1% (Unisense A/S Aarhus, Denmark) to measure coral photosynthesis. Gross photosynthesis, i.e. total O₂ evolution was estimated using the light-dark shift technique [for details see [16]] with the sensor placed at the coral tissue surface. Net photosynthesis, i.e. total O₂ evolution minus light respiration, was estimated by determining O₂ profiles from the coral surface upwards into the water column through the diffusive boundary layer. Areal rates of net photosynthesis were calculated from oxygen profiles using Fick's first law of diffusion $J = D_0 \times (dC/dz)$ using with an O₂ molecular diffusion coefficient of $2.24 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ (calculated for in situ temperatures of 25°C and salinity of 33‰) and where dC/dz is the slope of the linear O₂ concentration profile in diffusive boundary layer of the coral [17]

3. RESULTS

3.1. Distribution of collimated and diffuse incident irradiance in coral tissue:

The angular distribution of incident irradiance affected the photon scalar irradiance (450-700 nm) at the coral tissue surface (Figure 1). E_0 (450-700 nm) at the tissue surface was ~12% (± 2.5 SE) higher when illuminated with collimated light as compared to diffuse light. This enhancement was fairly consistent over the spectral range of photosynthetically active radiation (PAR), i.e., between 450-750 nm (Figure 2). In contrast, above the coral skeleton (i.e. 200 μm within the tissue) there was a trend towards enhanced E_0 (mean = $9\% \pm 16\%$) when illuminated with diffuse light (Figure 1). The enhancement of diffuse over collimated light at the coral skeleton was especially pronounced at wavelengths overlapping with the chlorophyll *a* absorption maxima of coral microalgae, that is around 675 nm and 450 nm (Figure 2).

There were no clear differences in the spectral attenuation coefficient of scalar irradiance, K for the entire coral tissue (0-200 μm) when illuminated with collimated or diffuse irradiance (Figure 3).

Figure 1. Effect of direct vs diffuse illumination on coral tissue scalar irradiance (E_0 ; 450-700 nm). 0 μm and 200 μm tissue depth relate to the coral tissue surface and the skeleton surface, respectively. Bars towards the right and left side of the figure show enhanced E_0 due to collimated and diffuse illumination, respectively ($n=3$).

Figure 2. Scalar irradiance ratio of collimated : diffuse incident irradiance measured at the coral tissue surface (solid black line) and directly above the coral skeleton (solid grey line). The dotted black line indicates the scenario where incident collimated and diffuse irradiance do not affect E_0 .

Figure 3. Spectral attenuation coefficient K over the 200 μm thick coenosarc tissue. Solid red line shows incident collimated irradiance and solid black line represents incident diffuse irradiance. For clarity, only upper or lower SEM (both as grey dotted lines) are shown.

3.2. Effects of angular distribution of light on coral photosynthesis:

The angular distribution of incident illumination affected coral O_2 evolution (Figure 4). Average gross photosynthetic rates (in $\text{nmol O}_2 \text{ cm}^{-3} \text{ s}^{-1}$) at the coral surface were about twice as high when illuminated with incident direct vs diffuse illumination (18.3 ± 1.2 SEM and 41.3 ± 7.4 SEM, respectively). Likewise, the net photosynthetic flux (i.e. gross photosynthesis- light respiration) was enhanced by $>20\%$ when the coral was illuminated with collimated compared to diffuse irradiance (Figure 4).

Figure 4. Effect of direct vs diffuse illumination on coral photosynthesis. The incident downwelling irradiance (either collimated or diffuse) was $320 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (A) local gross photosynthesis at the tissue surface ($n=6$). (B) Net photosynthesis at the tissue surface ($n=3$).

4. DISCUSSION

We here show that the directional quality of incident irradiance affects both coral optics and photosynthesis. Direct light is enhanced at the coral surface while diffuse light is enhanced at the coral skeleton. Coral photosynthesis is enhanced under direct compared to diffuse light.

Collimated light transport through tissue has an enhanced forward character compared to diffuse light, as Fresnel reflection at the tissue surface and at optical boundaries within the tissue increases with the angle of incidence [e.g., 18]. Therefore, light at oblique angles is preferentially backscattered, leading to an enhanced penetration depth of direct over diffuse light. Enhanced tissue penetration depth under collimated light follows that more light reaches the coral skeleton. The coral skeleton can be a strong backscatterer [19], and the enhanced tissue surface scalar irradiance (Fig. 1) could thus be partly explained by greater skeleton backscattering of collimated light back into the overlying tissue. Additionally, enhanced reflection of diffuse light over direct light at the tissue surface might contribute in the relative enhancement of direct light observed here.

In contrast, measurements above the coral skeleton showed that diffuse light was enhanced over direct light (Fig. 1). This effect was pronounced at wavelengths overlapping with the light absorption maxima of *Symbiodinium* [20], while wavelengths that are largely transmitted by *Symbiodinium* (e.g. in the green) were of similar intensity under direct and diffuse irradiance (Fig. 2). Similarly, studies on terrestrial leaves found an enhanced absorption of collimated light for wavelengths strongly absorbed by plant chloroplasts. It is suggested that the greater absorption of collimated light is caused by specific leaf anatomy and cellular arrangement [19]. Therefore, the light distribution measured here, could suggest that collimated light is preferentially absorbed by *Symbiodinium* cells.

This hypothesis is supported by the clear enhancement of both net and gross photosynthesis during direct illumination. It is likely that photosynthesis was not only enhanced at the coral surface (Fig. 4a) but also throughout the entire tissue as enhanced tissue light penetration could be beneficial for photosynthesis by symbionts harbored within deeper tissue layers. The enhanced photosynthesis under collimated light is an important finding in coral photobiology and highlights that the role of the directional quality of light in corals should be studied in further detail.

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