

Incongruence between the distribution of a common coral reef sponge and photosynthesis

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ABSTRACT: Symbiosis between coral reef fauna and microorganisms drives the growth, maintenance and diversity of coral reef habitats. Sponges, a key faunal component of coral reefs, form complex symbiotic relationships with microorganisms which may supply over half of their nutritional requirements through photosynthesis. The habitat distribution of the coral reef sponge *Rhopaloeides odorabile* correlates with light availability, suggestive of photosynthesis. The present study directly investigated the hypothesis that habitat distribution of *R. odorabile* is correlated with photosynthesis. Results of photorespirometry trials of 30 ind. exposed to light intensities between 0 and 900 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ showed no evidence of photosynthesis. Furthermore, no photopigments were present in these sponges and no cyanobacteria could be detected within the tissue. These results did not vary between sponges collected from nutrient rich inner- and mid-shelf reefs, or from oligotrophic outer-shelf reefs. These findings demonstrate that *R. odorabile* is not a phototrophic sponge and that habitat distributions clearly correlated with light can be due to factors other than photosynthesis.

KEY WORDS: Symbiosis · Photosynthesis · Porifera · *Rhopaloeides odorabile* · Great Barrier Reef · Cyanobacteria

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INTRODUCTION

Animal–plant and animal–microbial symbioses are common in tropical benthic communities and facilitate the growth, maintenance and diversity on coral reefs through photosynthetic productivity, mineral recycling and the production of carbonate skeletons (Smith & Douglas 1987). Many sponge species form symbioses with microorganisms, including filamentous cyanobacteria, zooxanthellae, diatoms and unicellular algae (reviewed by Sarà et al. 1998). In addition, sponges have complex and highly specific interactions with a consortium of bacteria that contribute to their nutrition, including the assimilation of dissolved organic carbon and nitrogen (DOC and

DON) and photosynthetically derived carbon (reviewed in Taylor et al. 2007, Bell 2008). Cyanobacteria are the most common photosynthetic symbionts of sponges on the Great Barrier Reef (GBR) (Wilkinson 1978), in some cases providing phototrophic sponges with at least 50% of their nutrition as fixed carbon from photosynthesis (Wilkinson 1983, reviewed in Taylor et al. 2007, Erwin & Thacker 2008). Other benefits of sponge–microbe associations include nitrification, methane oxidation, sulfate reduction, UV protection, enhanced boring and growth rates, secondary metabolite production and dehalogenation processes (reviewed in Taylor et al. 2007).

On coastal coral reefs of the GBR, the majority of sponges are heterotrophic, whilst those found on off-

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shore reefs are predominantly phototrophic or mixotrophic (Wilkinson & Trott 1985, Wilkinson 1987, Wilkinson & Cheshire 1989). It has been suggested that the prevalence of heterotrophic sponges on coastal reef habitats is due to increased suspended sediments, higher nutrient levels and reduced light availability associated with land-based runoff (Wilkinson & Cheshire 1989, Cleary & de Voogd 2007). In contrast, clear water leads to a dominance of phototrophic sponges on offshore reefs where the higher light intensities provide the carbon required for growth and survival (Wilkinson & Trott 1985, Cleary & de Voogd 2007).

Rhopaloeides odorabile is a common reef-associated sponge that colonises both inshore and offshore reefs (Wilkinson & Cheshire 1989, Bannister et al. 2010) and displays distinct morphs between locations (Thompson et al. 1987). On coastal reefs, *R. odorabile* are slender and show partial surface depigmentation, whereas on offshore reefs *R. odorabile* are large and have dark reddish-brown surface pigmentation, characteristic of sponges harbouring symbiotic cyanobacteria (Giano et al. 1977, Wilkinson 1980). Furthermore, the culturable microbial community of *R. odorabile* has been isolated and includes a unique cyanobacterium within the order Oscillatoriales (Webster & Hill 2001), as well as other microbial symbionts (Webster et al. 2001a,b). The cyanobacterium isolated from *R. odorabile* could not be enumerated, was never detected during extensive transmission electron microscopy (TEM) analysis and its role within *R. odorabile* remains unclear (Webster & Hill 2001). The differential depth distribution of *R. odorabile* on exposed fore-reef slopes, offshore reefs (between 5 and 15 m) and coastal reefs (<10 m) (Bannister et al. 2010) strongly suggests that a symbiotic relationship with cyanobacteria, or other potential photosymbionts, may restrict the distribution and abundance of *R. odorabile* through its reliance on a photosynthetic energy source. While this is a plausible hypothesis, there is no confirmation that light and photosynthesis play a critical role in determining the distribution of *R. odorabile*, or that photosynthesis even occurs.

Given that *Rhopaloeides odorabile* is one of the most studied sponge species, and in many cases is a model for sponge biology and ecology on the GBR, the present study aims to determine the role of light, by way of photosynthesis, as a factor affecting the distribution of *R. odorabile* across the continental shelf of the central GBR. It specifically addresses the issue of whether *R. odorabile* individuals across inner-, mid- and outer-shelf reef locations possess photosymbionts and whether their presence/absence can explain the apparent light-driven distribution of *R. odorabile* between shelf locations.

MATERIALS AND METHODS

Collection of specimens. In January 2005, sponge explants were cut from 10 *Rhopaloeides odorabile* individuals at each of 3 reefs along a GBR shelf gradient: Pith Reef (outer-shelf reef), Rib Reef (mid-shelf reef) and Pelorus Island (inner-shelf reef). Five sponge explants were also cut from 5 *Carteriospongia foliascens* individuals, a known phototrophic sponge (Bergquist et al. 1988), at Rib Reef to use as a positive control for oxygen evolution measurements. At each location these explants were placed into plastic moulded recovery cages (Aqua-Tech) anchored to the sea floor at 9 m (the same depth at which these sponges were collected). Explants were left in these cages for 7 wk to allow their damaged surfaces to heal and recover (Louden et al. 2007).

In March 2007, 7 wk after excising sponge explants, individual explants were collected and transported back to the aquarium facilities at James Cook University in aerated seawater. Sponges were placed into separate 30 l aquaria (5 sponges aquarium⁻¹) and acclimated at the same conditions for 3 d prior to conducting oxygen evolution measurements. Each aquarium had a mesh rack mounted on the bottom to maintain adequate water flow around the sponge explants and circulation was provided by a power-head pump (Aqua-clear 402). Seawater in the aquarium system was collected from the Australian Institute of Marine Science, Cape Cleveland, 50 km south of Townsville, and was filtered to 10 µm. Bio-filtration, foam fractionators and sand filters were used to maintain water quality. Each aquarium was illuminated on a 10 h light:14 h dark regime using 400 W metal halide lamps (Eye) at approximately 500 µmol photons m⁻² s⁻¹, representative of near-natural light regimes (Fabricius & Klumpp 1995, Cheshire et al. 1997).

Photophysiology. To quantify photosynthesis within the tissues of *Rhopaloeides odorabile*, oxygen evolution was measured using a set of 6 closed, recirculating perspex respirometry (flow) chambers (2.7 l) fitted with calibrated Clark-type oxygen electrodes (Cheshire Systems). For a detailed description of the respirometry system see Hoogenboom et al. (2006). Two suspended 400 W metal halide lamps (Eye) were used to adjust the light intensity over the respirometry chambers, exposing the sponges to 12 discrete light levels for 25 min periods (0, 15, 40, 60, 100, 120, 200, 290, 420, 530, 710 and 900 µmol photons m⁻² s⁻¹). Measuring across this range of light levels allows a good characterisation of the shape of the photosynthesis-irradiance response (Hoogenboom et al. 2006), and is representative of light levels experienced on the GBR at the depths where *R. odorabile* are commonly found (Fabricius & Klumpp 1995, Cheshire et al. 1997). Dis-

crete light levels were measured using a LI-192S LICOR probe connected to a LI-1000 data logger. During each 25 min period, oxygen concentrations were recorded every 30 s onto a central data logger (CR10X Campbell Scientific), and chambers were flushed with new seawater for 5 min after each 25 min recording interval to maintain oxygen saturation above 85% and prevent oxygen supersaturation.

Oxygen evolution measurements were conducted over a period of 7 consecutive days, with 5 sponges measured each day. Temperature was maintained constant at 27 to 28°C by suspending the respirometry chambers in a 200 l water jacket connected to a thermostat regulated water chiller (Carrier Systems). On each day, 1 control explant (*Carteriospongia foliascens*) and 4 *Rhopaloeides odorabile* explants were used as collected from either of the inner-, mid- or outer-shelf treatments and randomly selected using a random-number table. One respirometry chamber was left empty during each experiment to control for photosynthesis and respiration of microorganisms within the water. Chambers were regularly cleaned to prevent biofilm formation (Hoogenboom et al. 2006). Whole explants were weighed immediately following each daily experiment to obtain wet weights (fresh weight, FW), and oxygen flux values were subsequently normalised by sponge weight ($\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ h}^{-1}$). Surface tissue samples (1 cm^3) were collected from each sponge explant and frozen at -40°C for later quantification of photopigments.

Photopigment extraction. To extract photopigments (chlorophyll *a*, c_1 and c_2 and phycoerythrin), whole tissue samples were prepared following methods modified from Larkum et al. (1987). To extract water-soluble pigments (phycobiliproteins), tissue samples were frozen and thawed several times in ice-cold buffer (0.2 M phosphate, pH 7.4). Subsequently, to extract chlorophyll, tissue samples used for phycobiliprotein extractions were freeze-dried to remove excess water, crushed using liquid nitrogen and a mortar and pestle and then placed into cold 90% acetone for 24 h. Both acetone and water-soluble extracts were centrifuged at $1000 \times g$ to obtain a clear supernatant. The supernatant from each extract was decanted and then scanned separately at wavelengths between 350 and 750 nm using an Agilent 8453 UV-visible spectrophotometer.

Cyanobacteria determination in *Rhopaloeides odorabile*. To enumerate quantitatively the presence of cyanobacteria within *R. odorabile*, sponge sections for TEM were excised from the pinacoderm to a depth of 0.5 cm into the mesohyl for all sponges sampled from Davies Reef ($n = 6$; mid-shelf reef) and Pelorus Island ($n = 4$; inner-shelf reef). Samples were cut into small pieces (ca. 1 mm diameter) and fixed in 0.1 M sodium cacodylate buffer (pH 7.4) prepared in artificial seawater

(ASW) and containing 2.5% (v/v) glutaraldehyde for 20 h. Fixed tissue samples were removed, placed in fresh 0.1 M sodium cacodylate buffer and stored at 4°C until further processing. Fixed tissue samples were placed in a 1% (w/v) osmium tetroxide solution (prepared in 0.2 M potassium phosphate buffer, pH 7.4) for 3.5 h and subsequently dehydrated in a graded ethanol series (15, 35, 55, 75, 85 and 95% (v/v) ethanol). Tissue was embedded in Spurr's resin, sectioned with an ultramicrotome and stained with 2% (w/v) uranyl acetate followed by 0.2% (w/v) lead citrate. Sections were mounted on 200-mesh copper grids coated with carbon and Formvar. Samples were visualised by TEM (Jeol 2000 FX). Between 18 and 25 fields of view from each sponge sampled were assessed for the presence of cyanobacteria.

Statistical analysis. To quantify the dependence of net photosynthesis rate on irradiance, the hyperbolic tangent model was fitted to photosynthesis data. The model fits were conducted using a non-linear estimation routine in the software package Statistica (StatSoft 1999). The hyperbolic tangent model (our Eq. 1, Jassby & Platt 1976) was chosen, because it generally provides the best fit (highest r^2 values) to photosynthesis versus irradiance (P - I) data (Chalker 1981) in the absence of photoinhibition beyond the irradiance at which saturation (I_k) occurs.

$$P_n = P_{\max} \tanh(I/I_k) - R_{\text{dark}} \quad (1)$$

where P_n is the hourly rate of photosynthesis ($\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ h}^{-1}$), R_{dark} is the rate of respiration in darkness ($\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ h}^{-1}$), P_{\max} is the maximum rate of photosynthesis ($\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ h}^{-1}$), I is irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) and I_k is the sub-saturation irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Where the hyperbolic tangent model did not adequately capture the variation in the photosynthesis data (i.e. if photosynthesis did not increase asymptotically with increasing irradiance), simple linear regressions were also fitted to the oxygen evolution data to detect if there was any light-dependent change in respiration activity of inner-, mid- and outer-shelf reef sponge explants.

RESULTS AND DISCUSSION

Rhopaloeides odorabile does not display typical photosynthetic-irradiance responses of sponges containing photosymbionts (Fig. 1) and the shelf location of the reef from which individual sponges originated did not influence this result. Even for sponges collected from oligotrophic, outer-shelf locations, we did not observe any positive net photosynthesis (Fig. 1). Although a cyanobacterium has previously been isolated from *R. odorabile* tissue and identified by 16S rRNA

sequence analysis (Webster & Hill 2001), the abundance of cyanobacteria within tissue sections was not empirically determined. In the present study, extensive analysis of TEM micrographs from replicate sponges at 2 shelf locations did not identify cyanobacteria in either the pincode or mesohyl matrix (Fig. 2). Furthermore, molecular analysis of >85 000 sequences of *R. odorabile* symbionts showed that cyanobacteria comprise <0.71% of the sequenced microbial community (Webster et al. 2010).

Several studies have linked the photosynthetic activity of marine sponges to the presence of cyanobacteria within the tissues (Wilkinson 1983, Cheshire et al. 1997, Erwin & Thacker 2008). Nevertheless, even if present in *Rhopaloeides odorabile*, photosymbionts do not provide this species with carbon generated through photosynthesis. This is unequivocally demonstrated by the absence of a relationship between net oxygen exchange rate and light intensity ranging between 0 and 900 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Fig. 1) for *R. odorabile* individuals from inner- ($r^2 = 0.11$, $n = 10$, $p = 0.274$), mid- ($r^2 = 0.28$, $n = 10$, $p = 0.060$) and outer-shelf reef locations ($r^2 = 0.18$, $n = 10$, $p = 0.154$). In contrast, *Carteriospongia foliascens*, a sponge with multiple species of cyanobacterial symbionts (Steindler et al. 2005), displayed the typical *P-I* response curve that was well characterised by the photosynthesis model ($r^2 > 0.98$; Fig. 1). In fact, this species showed strong photosynthetic activity with a maximum rate of net photosynthesis that was more than 4 times greater than the rate of respiration in darkness (Table 1), corresponding well to other studies investigating *P-I* responses for other phototrophic coral reef sponges

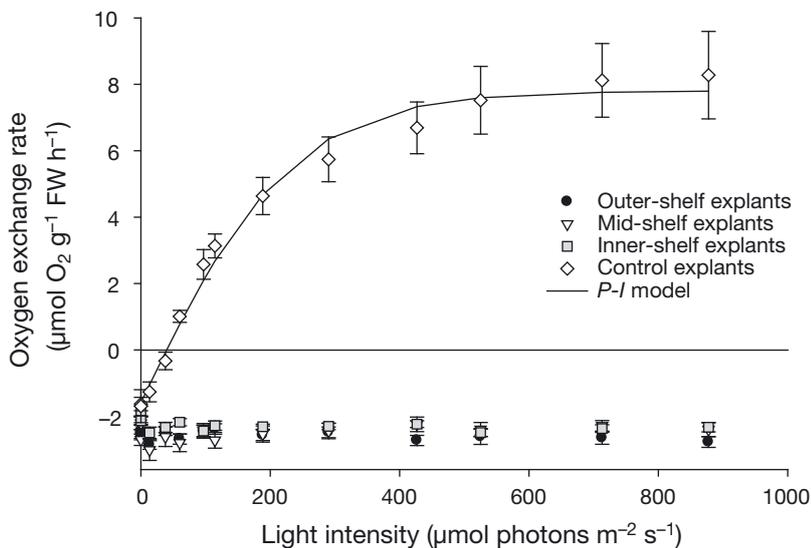


Fig. 1. *Rhopaloeides odorabile* and *Carteriospongia foliascens*. Photosynthesis versus irradiance (*P-I*) curves for *R. odorabile* from inner-, mid- and outer-shelf locations (each, $n = 10$) and the control sponge *C. foliascens* ($n = 5$). Error bars represent SE of the mean. FW: fresh weight

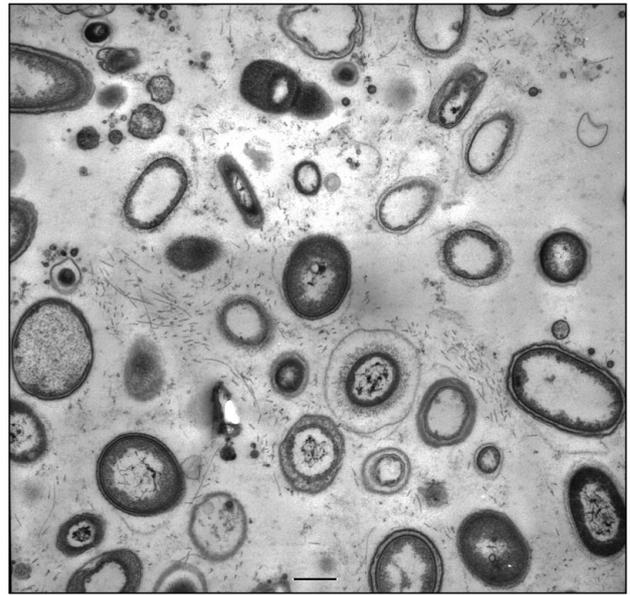


Fig. 2. *Rhopaloeides odorabile*. Transmission electron micrograph of *R. odorabile* depicting the bacterial community within the mesohyl from the upper 0.5 cm of sponge tissue. Scale bar = 0.4 μm

(Wilkinson 1983, Cheshire & Wilkinson 1991, Erwin & Thacker 2008).

The results of the present study also demonstrate that photosynthetic pigments, including chlorophylls and red and blue phycobiliproteins, are not present within the surface tissues of *Rhopaloeides odorabile* (Fig. 3). Surprisingly, in light of the differences in surface coloration for sponges collected from different locations, absorbance profiles of sponges from inner-, mid- and outer-shelf reefs were not strongly different. These results highlight the fact that sponge-microbe symbioses are highly complex. Indeed, only a handful of studies have elucidated the key roles of microbial consortia to sponge physiology, which include UV protection, enhanced boring and growth rates, metabolite production, and most importantly, nutrient and carbon acquisition (reviewed in Taylor et al. 2007, Erwin & Thacker 2008). Nevertheless, the latter does not appear to be the case for the common and abundant reef-associated sponge *R. odorabile*, which suggests that (1) the cyanobacterium is an inhabitant of the surrounding seawater inadvertently filtered by the sponge; (2) the sponge-cyanobacterial association is commensal as is the case for some other coral reef sponge-cyanobacterial asso-

Table 1. *Carteriospongia foliascens*. Non-linear estimations of steady-state sub-saturation irradiance (I_k) and steady-state maximum rate of gross photosynthesis (P_{\max}) for the control sponge. R_{dark} : rate of respiration in darkness; FW: fresh weight

	Estimate	SE	p
P_{\max} ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	9.386	0.304	<0.001
I_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	233.691	12.447	<0.001
R_{dark} ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	1.582	7.012	<0.001

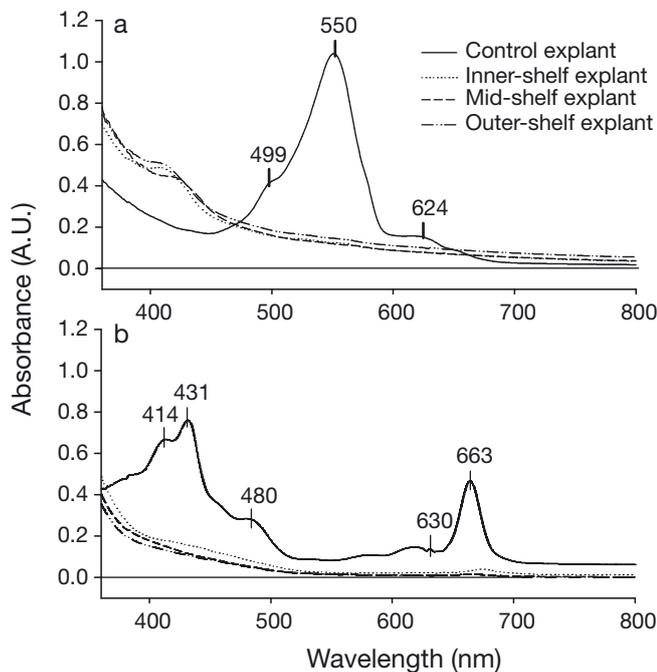


Fig. 3. *Rhopaloeides odorabile* and *Carteriospongia foliascens*. (a) Mean absorbance spectra of water-soluble pigments for inner-, mid- and outer-shelf reef explants (each, $n = 10$) of *R. odorabile* and for explants of the control sponge *C. foliascens* ($n = 5$). Pigments expressed between 500 and 600 nm wavelength are characteristically red phycobiliproteins (phycocerythrin; Jeffery & Hallegraff 1990). (b) Mean absorbance spectra of acetone-extracted pigments for inner-, mid- and outer-shelf reef explants (each, $n = 10$) of *R. odorabile* and for explants of *C. foliascens* ($n = 5$). Chlorophyll *a* is at 664 nm and c_1 and c_2 at 630 nm (Jeffery & Humphrey 1975)

ciations (e.g. Thacker 2005, López-Legentil et al. 2008); or (3) cyanobacteria may exhibit temporally variable relationships with *R. odorabile*.

As *Rhopaloeides odorabile* does not acquire additional energy through photosynthesis, energy and nutritional requirements are obtained through filter feeding. Therefore, it remains unclear what role, if any, light plays in determining the depth distribution of *R. odorabile*, which has highest abundance within the phototrophic zone (8 to 12 m depth; Bannister et al.

2010). One explanation for this pattern may be that the depth distributions across shelf locations are regulated by light availability and larval settlement behaviours. Larvae of *R. odorabile* display positive phototactic behaviours prior to settlement, coinciding with a preference to settle on light-exposed surfaces (Whalan et al. 2008). Alternatively, changes in food availability associated with light may be driving the observed differential depth distributions of *R. odorabile*, since food availability can be linked to the distribution patterns of coral reef sponges (e.g. Lesser 2006). Furthermore, suspended sediments (turbidity) correlated with light availability (Anthony et al. 2004) may affect depth distributions on inner-shelf reefs.

In conclusion, *Rhopaloeides odorabile* does not possess active or beneficial photosymbionts, or they are not present in sufficient numbers for light-driven photosynthesis to be detected. Since several environmental factors can be correlated with light availability, distribution patterns strongly associated with light cannot automatically be assumed to invoke a phototrophic mode of nutrition.

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