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Photosynthesis and photosynthetic electron transport in the soft coral *Sarcophyton spp*

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ABSTRACT: Blue-diode Pulse Amplitude Fluorometry (PAM) and rapid light curves, fitted using the Waiting-in-Line equation, were used to measure photosynthetic electron transport rate (ETR) of the common soft coral *Sarcophyton spp* (Alcyoniidae, Cnidaria). Optimum irradiance (E_{opt}) of newly-collected material was 353 ± 28.9 μmol quanta $m^{-2}s^{-1}$ (≈18% sunlight, 400–700 nm), ½ optimum irradiance ($E_{1/2-ETR}$) ≈82.0 μmol quanta $m^{-2}s^{-1}$ with substantial photoinhibition at higher irradiances. Maximum ETR (ETR_{max}) = 25.1 ± 1.28 μmol e^- $m^{-2}s^{-1}$ (surface area basis) or 86.8 ± 4.44 μmol e^- g^{-1} Chl *a* s^{-1} (Chl *a* basis). Due to strong midday inhibition total daily photosynthesis would be greater on cloudy days, during the overcast wet season and high tides. Open aquarium-grown material had E_{opt} ≈300 μmol quanta $m^{-2}s^{-1}$; ETR_{max} ≈21 μmol e^- $m^{-2}s^{-1}$ or ≈50 μmol e^- g^{-1} Chl *a* s^{-1}. Chl *a* was ≈300 (field material) vs. ≈450 (aquaculture aquarium) mg m^{-2} but with no change in Chl c_2/a ratio (0.521 ± 0.0181). Based on light/dark O_2 electrode methods in aquaria Gross Photosynthesis (P_g) = 21.9 ± 6.85 μmol O_2 g^{-1} Chl *a* s^{-1}, P_{net} = 15.1 ± 6.38 μmol O_2 g^{-1} Chl *a* s^{-1}; respiration −6.81 ± 2.23 μmol O_2 g^{-1} Chl *a* s^{-1}; P/R ratio = 3.72 ± 1.86 at an irradiance 192 ± 20.3 μmol quanta $m^{-2}s^{-1}$. The culturable dinoflagellate zooxanthellae material might not necessarily be representative of the resident zooxanthellae population. Their PAM characteristics were similar to the soft coral (E_{opt} ≈219 μmol quanta $m^{-2}s^{-1}$, ETR_{max} ≈138 μmol e^- g^{-1} Chl *a* s^{-1}), but the Chl c_2/a ratio (≈0.16) was very different.

KEYWORDS: *Sarcophyton*, soft coral, photosynthetic electron transport, optimum irradiance, photoinhibition

INTRODUCTION

Sarcophyton spp (Alcyoniidae, Alcyonacea, Anthozoa, Cnidaria) are common soft coral (octocoral) inhabitants of Indo-Pacific reef flats [1] (Fig. 1). There are ≈40 species. Like scleractinian corals they are photosynthetic using dinoflagellate zooxanthellae (*Capnella gaboensis*, [2]; various species including *Sarcophyton*, [3, 4]). There is some data on their growth rate [5]. Soft corals such as *Sarcophyton* often take over degraded reef flats [3, 6].

Sarcophyton species acquire their zooxanthellae (*Symbiodinium*) at the primary polyp stage, not as oocytes [7, 8] and so the symbiont in question would seem likely to vary considerably from species to species even though the *Symbiodinium* of Soft Corals of the West Pacific, belong to a single clade [1]. As in hard corals, the soft-coral-zooxanthellae symbiosis is fragile: bleaching events of *Sarcophyton* occur in Thailand and elsewhere [9–13]. In later studies we found that a cryptic green algal symbiont (Zoochlorellae) is also present in *Sarcophyton* (Fig. 2) as they are in the case of the sea anemone, *Anthopleura elegantissima* [14].

Little information is available on soft coral photosynthesis (*Capnella gaboensis* [2, 3]; *Sarcophyton* and several other species, [15]. Most hard corals behave very much like "sun plants" with some photoinhibition at high irradiances, but their geometry and absorbances are different to vascular plants [16–19]. The *Symbiodinium* symbiont has Chl c_2 which has an absorbance peak at about 630 nm *in vivo* and has Chl $a + c_2$+ peridinin [20–22].

Here we look at photosynthetic electron transport using PAM fluorometry methods [13, 16, 19, 23] in *Sarcophyton spp* growing on a reef flat in the Patong beach area of Phuket Island on the Andaman seacoast of Thailand, colonies grown in an aquaculture facility and cultured zooxanthellae from *Sarcophyton*. Some photosynthesis measurements were also made on *Sarcophyton* grown at the aquarium facility using O_2-electrode light/dark bottle experiments.

MATERIALS AND METHODS

Experimental material

Sarcophyton spp grow on a degraded reef flat in the Patong beach area of Phuket Island on the Andaman seacoast of Thailand (7°53′18″ N 98°16′24″ E). The reef flat is degraded from effluent from hotels and guest houses and from building work runoff and ex-

Fig. 1 Field-collected *Sarcophyton spp* colony with its polyps extended. The colony is about 15 cm in diameter. In this picture the polyps are emergent but when withdrawn the colony has a leather-like appearance. The thallus is about 5 mm thick and is partially calcified.

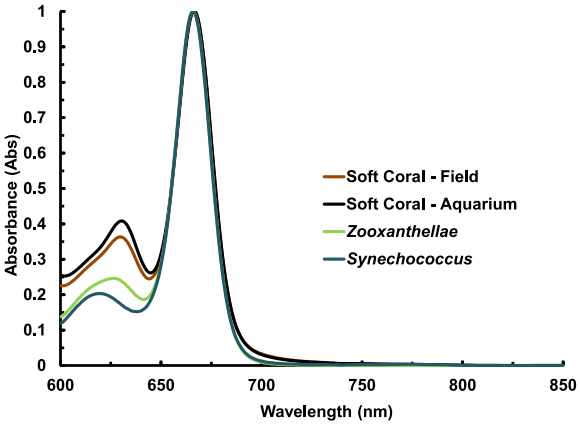

Fig. 2 Chlorophyll content in DMSO solvent of *Sarcophyton* (Field & Aquarium material, $n = 8$), *Zooxanthellae* and *Synechococcus* standardized onto the Chl *a* peak. The Chl *a*-peak is at 666 nm and the Chl c_2-peak at 631 nm. The Chl c_2/a ratio is higher in aquarium (0.718 ± 0.046) vs. field (0.571 ± 0.038) *Sarcophyton* colonies, much lower in the zooxanthellae (0.189 ± 0.0029) and Chl c_2 is absent in *Synechococcus*.

periences runoff problems as outlined in Okinawa, Japan [6]. Only a few scleractinian corals (*Porites spp.*, *Favites spp.* and *Leptastrea spp*: [24]) and the soft corals *Sarcophyton* and *Sinularia spp* were left growing on the reef flat with no seagrasses. *Sarcophyton spp* occupied about 1.6% of the surface area of the reef flat (Dummee unpublished). Field material was collected by Vipawee Dummee and Sukin Chooklin. Cultured specimens were grown for two months in the plastic-roof outdoor aquaculture facility at the Sapanhin Aquaculture Laboratory, Faculty of Technology and Environment, Prince of Songkla University Phuket Campus. Soft corals are very sensitive to handling and so experimental handling needs to be minimised [5]. Dr. Thanongsak Chanmethakul (Phuket Rajabhat University, Phuket) supervises the Sapanhin aquarium facility. The cyanobacterium *Synechococcus R-2* PCC7942 grown in modified BG-11 [25] was originally from the Pasteur Culture Collection, Paris, France was used as a source of Chlorophyll *a* (Chl *a*).

Culture conditions

The *Sarcophyton spp* could be kept in a shallow water open-air roofed aquaculture aquarium facility in clean seawater (Sapanhin Aquaculture Laboratory). The light intensity was about 100 to 400 µmol photons $m^{-2}s^{-1}$ (PPFD, 400–700 nm) measured using a MQ-200 Quantum Meter, Apogee Instruments, Logan, Utah, USA. Zooxanthellae cultures were grown in a culture room at PSU-Phuket (100 to 150 µmol photons $m^{-2}s^{-1}$ PPFD, 400–700 nm) in enriched f/2 seawater provided with silicate [26]. The algal cultures were grown in 250 and 500 ml conical flasks which were shaken each day. Cool white or warm-white fluorescent tubes were used (Cool Daylight, Philips, The Netherlands) and the temperature was 25 to 30 °C.

Chemicals

DMSO (Dimethylsulphoxide, dimethyl sulfoxide, $(CH_3)_2SO$) was from WINNEX (Thailand) Co. Ltd., Bangkok, Thailand. Acetone $(CH_3)_2CO$ 99.5 AR/ACS was from LOBA Chemie PVT. Ltd., Mumbai, India. 90% acetone and 100% DMSO were neutralised with Mg carbonate.

Isolation of zooxanthellae

Sarcophyton was too rubbery in texture for successful brushing of the soft coral to collect zooxanthellae (cf. [7]). Samples of the soft coral were cleaned with cotton gauze and then a sterile hypodermic needle was inserted to extract cells which were then incubated in sterile f/2 seawater [26]. The zooxanthellae cells grew in loose filaments and clumps rather than as motile cells in the sterile seawater unlike the experience of Barneah et al [7] (Fig. S1). A readily grown green algal zoochlorellae was also present but its presence was not obvious spectroscopically in solvent extracts from the soft coral (Fig. 2) [14].

Glass fibre disks of cultured zooxanthellae were prepared by filtering cell suspensions onto glass fibre disks (Whatman GF/C, Whatman International, Maidstone, England, UK) in a 16.2-mm internal diameter (206.12×10^{-6} m^2) Millipore filter apparatus to create

a uniform disk of cells: the Chl *a* content per unit area could then be calculated as mg Chl *a* m^{-2}.

Scanning dual beam spectrophotometer

A Shimadzu UV-1601, UV-Visible Spectrophotometer, Shimadzu Corporation, Kyoto, Japan was used for routine spectrophotometric scans of chlorophylls from the soft coral and from the zooxanthellae. The bandwidth was set at 1 nm.

Routine Chlorophyll determinations

Chlorophyll extracts for both the soft coral and for the zooxanthellae were done in 6 or 8 replicates. Pieces of soft coral cut with a 9.7 mm diameter cork borer were used for chlorophyll determinations giving chlorophyll content on both a thallus surface area basis. Both *Sarcophyton* and the cultured zooxanthellae were "recalcitrant" material for chlorophyll determination. For *Sarcophyton* discs grinding in solvent with sand in a mortar & pestle was required. For the zooxanthellae, 100 to 150 ml of the algae were centrifuged at 5000 rpm (3914 rcf) for 5 min and the supernatant removed (Hermle Z323K, Hermle Labortechnik, Wehingen, Germany) or glass fibre disks impregnated with the alga were used. The best extractant for both the soft coral and the zooxanthellae extractant was a 1:1 mixture of acetone and DMSO [22]. No heating was required for the soft coral but heating at 55 °C was necessary for the cultured zooxanthellae. Solvent extracts were cleared of cell debris by centrifugation [22, 27, 28].

In solvent determinations of Chls were made using DMSO or 90% acetone [22, 27, 28]. Frigaard et al [29] was used as the source of standard reference spectra to check that the chlorophyll from the soft coral was a mixture of Chl *a* and c_2. Acetone/DMSO extractants were then diluted to 5% in DMSO or 90% acetone for assay. The equations for 90% acetone in water were used as the benchmark for chlorophyll assays [2, 21, 22, 28, 29]. There was some question about the long term stability of chlorophylls in DMSO which has been favourably resolved experimentally [30]. Routine scans from 600 to 850 nm were used. No photosynthetic bacteria (BChl *a*) were present (Fig. 2).

PAM fluorometry

We used a blue-diode (445 nm) Junior PAM (Pulse Amplitude Modulation Fluorometry) portable chlorophyll fluorometer (Gademann Instruments, WÃijrzburg, Germany). The PAM parameters (Y, rETR, qP, NPQ) were automatically calculated using the WINCONTROL software (v2.08 and v2.13; Heinz Walz Gmbh, Effeltrich, Germany) using the standard default settings for rapid light curves (absorptance factor, AbtF = 0.84) and a PSI/PSII allocation factor of 0.5, assuming that a photon is equally likely to activate PSII or PSI [31, 32] to calculate the relative photosynthetic electron transport rate (rETR). The full protocol has been described previously [33]. The absorptance of the soft coral and zooxanthellae cell suspensions filtered on glass fibres disks was measured using a RAT (Reflectance-Absorptance-Transmission) device [34]. The experimental absorptance (Abt_{465nm}) was used to calculate the actual ETR from rETR.

The Waiting-in-Line equation is a good model of ETR vs. Irradiance,

$$ETR = \frac{ETR_{max} \times E}{E_{opt}} e^{1-E/E_{opt}}, \quad (1)$$

where ETR is the photosynthetic electron transport rate (μmol e^- $m^{-2}s^{-1}$), E is the irradiance (μmol photon $m^{-2}s^{-1}$ 400–700 nm PPFD), E_{opt} is the optimum irradiance and ETR_{max} is the maximum photosynthetic electron transport rate. The maximum photosynthetic efficiency (α_0) is the initial slope of the curve at E = 0 ($\alpha_0 = ETR_{max} \times e/E_{opt}$). The Waiting-in-Line model includes photoinhibition at high irradiance. The errors of the fitted parameters can be calculated by sums of squares of differentials and matrix inversion. The PAM measures photosynthetic ETR on a surface area basis as μmol e^- $m^{-2}s^{-1}$. If the Chl *a* content of the material being used has a known Chl *a* per unit surface area the ETR can be converted to μmol e^- g^{-1} Chl *a* s^{-1} [33].

Non-photochemical quenching (NPQ) is often interpreted as a measure of the degree of stress upon plants but caution is necessary [32]. We usually found that NPQ vs. irradiance (E) could be fitted to a simple exponential saturation curve of the form $NPQ = NPQ_{max} \times (1 - e^{-kE})$, where NPQ_{max} is the maximum NPQ and k is an exponential constant. The shape of the curve can be described by quoting the maximum NPQ (NPQ_{max}) and the irradiance at which ½ of the NPQ_{max} is achieved ($\ln 2/k = E_{1/2-NPQ}$). The complex NPQ equation [31, 32] often generates very low NPQ values and division by zero errors at high irradiances. NPQ vs Irradiance curves might not fit a simple saturation curve very well in algae [33]. It is thus important not to over-interpret NPQ data: typically NPQ_{max} has a very low value in algae compared to vascular plants [31–33].

Oxygen electrode experiments

Classic light/dark bottle experiments were used to estimate photosynthesis and respiration of small *Sarcophyton* colonies (2 to 3 cm diameter) growing on cement cylinder blocks (54 mm diameter × 45 mm = 33 cm^3) at the Sapanhin Aquaculture Laboratory by S Chooklin. The colonies had been growing for about 2 months. Incubations were in plastic containers (420 ml nominal) with an O-ring seal in dark (aluminium foil) or light in the aquarium tank. The volume (allowing for the volume of the cement substrate) was 385 ml. Incubations were run in the same 3 × 1 × 1 m tanks in which the colonies had been grown

at 29 °C and 30‰ salinity. Standard incubations were for 3 h. Dissolved O_2 was measured using a LAQUA DO210 oxygen electrode (HORIBA (Thailand) Co. Ltd., Bangkok, Thailand) using 0.5% Sodium dithionite as zero (methylene blue indicator). O_2 solubility tables used for 100% saturation [35]. Oxygen was measured before (≈5 to 7 mg O_2 l^{-1}, air saturation 6.50 mg l^{-1}) and after incubations: in the light experiments the [O_2] increased by no more than ≈150% and in the dark the O_2 degreased by no more than −20%. The PAR was 192 ± 20 µmol quanta (400–700 nm) $m^{-2}s^{-1}$. Oxygen fluxes were calculated as mol O_2 s^{-1}, then using Chl *a* assays, converted into mol O_2 g^{-1} Chl *a* s^{-1}.

Statistics

Cochran and Snedecor [36] was used as the statistical reference text. All data are quoted as means ± 95% confidence limits. Errors of the fitted parameters of curves were calculated by matrix inversion. The EXCEL files for the Waiting-in-Line model for fitting photosynthesis curves and the chlorophyll calculator are available upon request.

RESULTS

Fig. 1 shows a field-collected *Sarcophyton spp* colony with its polyps extended. The colony is about 15 cm in diameter. When the polyps are withdrawn the colony has a leather-like appearance. The thallus is about 5 mm thick and is partially calcified. Handling needs to be minimised. Fig. S1 shows a photomicrograph of dinoflagellate zooxanthellae cells withdrawn from a *Sarcophyton* colony using a sterile hypodermic needle and gown in enriched f/2 seawater. The cells are about 5 µm in diameter. In culture, these cells formed fragile filamentous chains that disintegrated easily. The cells are dense and embedded in a thick layer of mucus and do not form a suspension of motile cells. The mucoid nature of the culture is probably the reason why it was so difficult to extract chlorophyll from them using 90% acetone.

Fig. 2 shows the spectral properties in DMSO solvent of field-collected *Sarcophyton*, aquarium-grown *Sarcophyton*, zooxanthellae cultures and the cyanobacterium *Synechococcus* (as a zero Chl c_2 blank). The curves are the means of the scans used to determine the chlorophyll contents of the field-collected *Sarcophyton* colony used for the PAM experiments shown in Figs. 3 & 4 ($n = 16$), and the aquarium-grown material (Figs. 5 & 6). The Chl *a*-peak was at 666 nm and the Chl c_2-peak at 631 nm. The peak at 631 nm shows that *Sarcophyton* zooxanthellae dinoflagellates *in situ* contain large amounts of Chl c_2. Fig. 2 shows the means of the 16 scans obtained from DMSO extracts using a cork borer of the animal and standardized onto the red peak for Chl *a* at 666 nm. The putative Chl c_2 peak conforms to the diagnostic scans for Chl c_2 [29] and the spectrum stripping scans in Ritchie et al [22, 27].

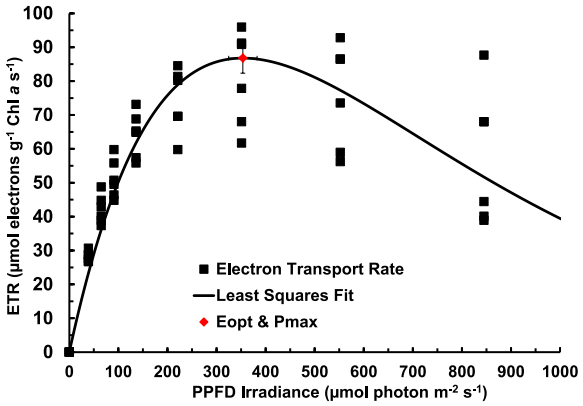

Fig. 3 Photosynthesis of *Sarcophyton* expressed on a Chl *a* basis. The optimum irradiance of newly collected material is about 350 µmol quanta $m^{-2}s^{-1}$ (18% sunlight).

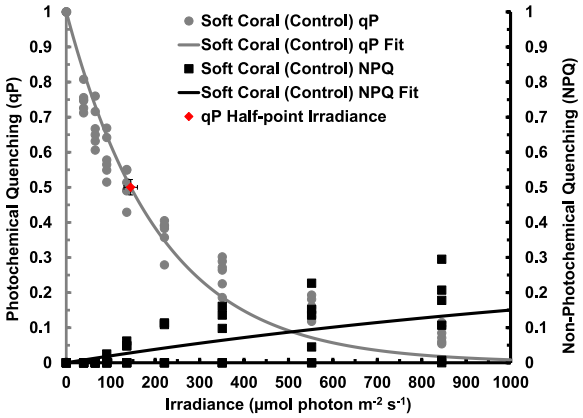

Fig. 4 Photochemical and Non-Photochemical Quenching (NPQ) in *Sarcophyton*. qP follows a simple, well resolved, exponential decay curve with an intercept at 1. NPQ is very low and the kinetics are not well resolved.

The Chl c_2/a ratio is sometimes higher in the aquarium material than in the field material (Fig. 2) but based on 4 field-collected and 4 aquarium-kept colonies the overall mean was 0.521 ± 0.0181 ($n = 8, 64$) (range 0.718 to 0.328 for 8 colonies) and lower in the cultured zooxanthellae (varied from 0.13 to 0.19). A Photomicrograph of the putative zooxanthellae culture is shown (Fig. S1).

PAM methods can be used very successfully to measure photosynthesis of a *Sarcophyton* colony based on 6 rapid light curves performed on the colony with 9 different irradiances (Fig. 3) ($n = 6, 54$). The overall Chl *a* content of cork borer cuttings of three colonies was 301 ± 35 mg m^{-2}. PAM curves were done on whole colonies by gently placing the contact PAM probe on several locations on an intact colony rather than on cut pieces. Fig. 3 is in XYY format. Yield decreases exponentially as the irradiance increases

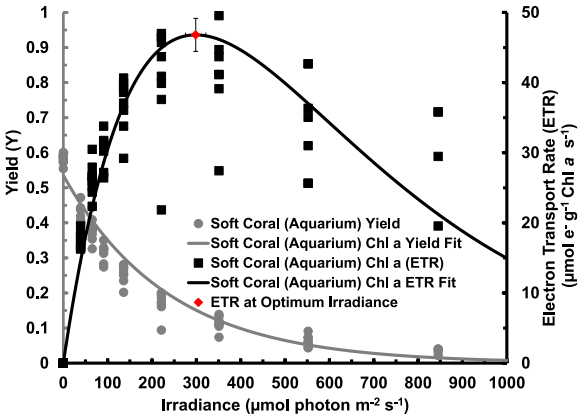

Fig. 5 Photosynthesis of aquarium-grown *Sarcophyton* expressed on a Chl *a* basis. The optimum irradiance is about 300 µmol quanta $m^{-2}s^{-1}$ (14% sunlight). The ETR_{max} is lower on a chlorophyll *a* basis than the field material.

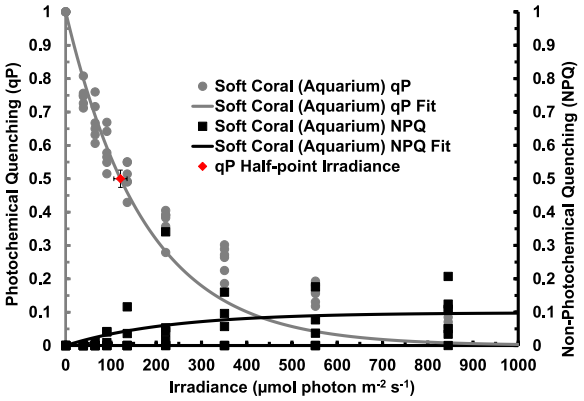

Fig. 6 Photochemical and Non-Photochemical Quenching (NPQ) in aquarium-grown *Sarcophyton*. qP follows a simple, well resolved, exponential decay curve with an intercept at 1. NPQ is very low but the kinetics were better resolved than in the field material.

($Y_{max} = 0.5356 \pm 0.0200$, $E_{1/2-Ymax} = 164 \pm 15.0$ µmol quanta $m^{-2}s^{-1}$, $r = 0.9827$, $n = 6, 54$). The optimum irradiance (E_{opt}) of newly collected material was about 353 ± 28.9 µmol quanta $m^{-2}s^{-1}$ (≈18% sunlight) with a ½ optimum irradiance ($E_{1/2-ETR}$) of 82.0 ± 6.7 µmol quanta $m^{-2}s^{-1}$, the maximum ETR (ETR_{max}) on a surface area basis was 25.1 ± 1.28 µmol e^- $m^{-2}s^{-1}$ equivalent to 86.8 ± 4.44 µmol e^- g^{-1} Chl *a* s^{-1} when expressed on a Chl *a* basis (Fig. 3), $r = 0.9212$, $n = 54$. The theoretical photosynthetic efficiency (Alpha, α_0) at zero irradiance on a surface area basis was 0.193 ± 0.0186 e^- $photon^{-1}$ and 0.667 ± 0.0643 e^- g^{-1} Chl *a* $photon^{-1}$ m^2 on a Chl *a* basis. The qP and NPQ results are shown in Fig. 4: photochemical quenching (qP) follows a simple exponential curve with an intercept at 1, the theoretical maximum NPQ

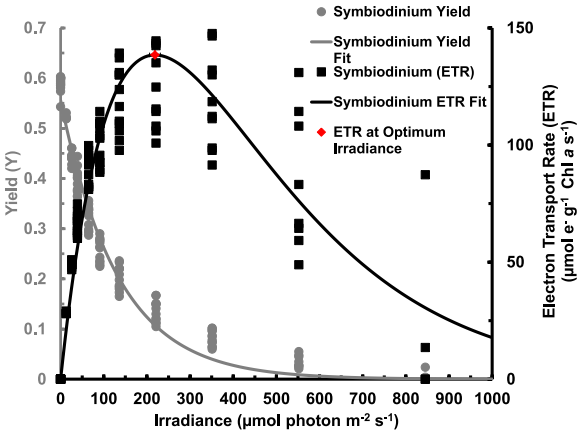

Fig. 7 Yield & ETR of Zooxanthellae of *Sarcophyton* in XYY format. The ETR optimum (E_{opt}) is lower than found in the soft coral animal and ETR_{max} on a Chl *a* basis is higher.

was 0.317 ± 0.989 and so the kinetics were not well resolved. On the other hand, qP was well resolved with a half-saturation point ($E_{1/2-qP}$) at 145 ± 15.2 µmol quanta $m^{-2}s^{-1}$, $r = 0.9843$, $n = 54$.

Fig. 5 shows Yield and ETR of aquarium-grown *Sarcophyton* expressed on a Chl *a* basis. Aquarium grown material had more Chl *a* per unit surface than the field material (453 ± 78 mg m^{-2}, $n = 8$), but no difference in Yield ($Y_{max} = 0.536 \pm 0.0225$; $E_{1/2-Ymax} = 164 \pm 17.5$ µmol quanta $m^{-2}s^{-1}$, $r = 0.9688$). The optimum irradiance was $E_{opt} = 299 \pm 22.9$ µmol quanta $m^{-2}s^{-1}$. ETR_{max} on a surface area basis was 21.2 ± 1.07 µmol e^- $m^{-2}s^{-1}$ (slightly below the field material) and Alpha (α_0) $= 0.193 \pm 0.0177$ e^- $photon^{-1}$ (not different to the Field material). The ETR_{max} is lower on a chlorophyll *a* basis than the field material (ETR_{max} $= 46.8 \pm 2.36$ µmol e^- g^{-1} Chl *a* s^{-1}; Alpha $\alpha_0 = 0.426 \pm 0.0391$ e^- g^{-1} Chl *a* $photon^{-1}$ m^2, $r = 0.8918$, $n = 75$). The E_{opt} value is about 14% sunlight. Fig. 6 shows qP and NPQ in aquarium-grown *Sarcophyton*. qP again follows a simple, well resolved, exponential decay curve with an intercept at 1. NPQ is very low but its kinetics are slightly better resolved than in the case of the field material (Fig. 4) ($E_{1/2-qP} = 159 \pm 189$ µmol quanta $m^{-2}s^{-1}$; $NPQ_{max} = 0.098 \pm 0.050$; $r = 0.4276$, $n = 75$).

Fig. 7 shows the results of a rapid light curve PAM experiment on the zooxanthellae culture. The ETR_{opt} and ETR_{max} are comparable to the field collected soft coral (Fig. 3) and the aquarium-grown soft coral (Fig. 5) but the zooxanthellae are identifiably dinoflagellates (Fig. S1) but the species has not yet been verified. The batch of cells used for Figs. 7 & 8 had a Chl c_2/a ratio of 0.135 ± 0.0053 ($n = 6$) and so was very different to the soft coral colonies. Yield decreased exponentially as the irra-

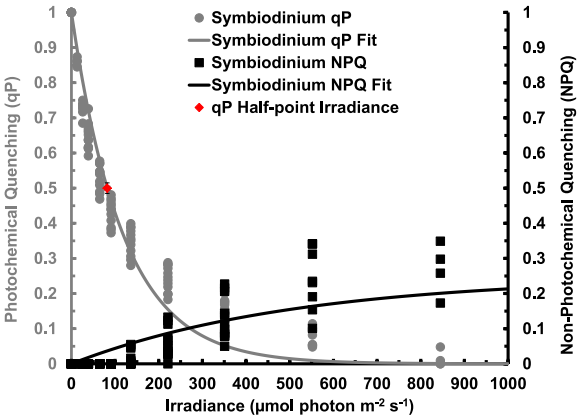

Fig. 8 Photochemical and Non-Photochemical Quenching (NPQ) in XYY format. qP follows a simple exponential decay curve. NPQ in zooxanthellae is very low but like the aquarium-grown soft corals more clearly follows a simple saturation curve than the field collected soft coral.

diance increased (Y_{max} = 0.556 ± 0.0136, $E_{1/2–Ymax}$ = 92.3 ± 5.35 µmol quanta $m^{-2}s^{-1}$, r = 0.9861, n = 108). The E_{opt} of the cultured cells in the laboratory was about 219 ± 12.6 µmol quanta $m^{-2}s^{-1}$ (≈10% sunlight) with an $E_{1/2–ETR}$ of 50.7 ± 2.91 µmol quanta $m^{-2}s^{-1}$, ETR_{max} was 138 ± 4.96 µmol e^- g^{-1} Chl *a* s^{-1} when expressed on a Chl *a* basis, r = 0.9283. The theoretical photosynthetic efficiency at zero irradiance on a Chl *a* basis was 1.72 ± 0.116 e^- g^{-1} Chl *a* photon^{-1} m^2. Fig. 8 shows qP and NPQ. The qP fits a simple exponential decay function with an origin at 1 and was well resolved with a half-saturation point ($E_{1/2–qP}$) at 82.1 ± 5.71 µmol quanta $m^{-2}s^{-1}$, r = 0.9888. The NPQ theoretical maximum NPQ (NPQ_{max}) was as low as found *in situ*: NPQ_{max} = 0.249 ± 0.111 and as in the case of the *in vivo* study of the soft corals its kinetics with increasing irradiance was poorly resolved.

Light/dark bottle oxygen electrode experiments (n = 10) were also run on *Sarcophyton* colonies grown on cement cylinders over course of 4 days (4 in mornings 09:00 to 12:00 h and one on morning and afternoon 13:30 to 16:30 in the aquarium facility). The overall Gross Photosynthetic rate (P_g) was 21.9 ± 6.85 µmol O_2 g^{-1} Chl *a* s^{-1}, the P_{net} was 15.1 ± 6.38 µmol O_2 g^{-1} Chl *a* s^{-1}; respiration −6.81 ± 2.23 µmol O_2 g^{-1} Chl *a* s^{-1} with a P/R ratio of 3.72 ± 1.86. The average irradiance was 192 ± 20.3 µmol quanta (400–700 nm) $m^{-2}s^{-1}$, very similar to the optimum irradiance found in the PAM experiments. P_g is closest to ETR measurements and so the approximate ETR found was about 88 ± 27 µmol e^- g^{-1} Chl *a* s^{-1}. This is a good match to estimates of ETR_{max} measured by PAM methods (Figs. 3 & 5).

DISCUSSION

PAM methods can be used very successfully to measure photosynthetic electron transport of a *Sarcophyton* colony based on rapid light curves [3, 13, 31, 32], however, this study was limited by us using a contact PAM probe on the colonies. As we have found frequently in studies of algal-based photosynthetic systems [31–33] we found that NPQ had a low NPQ_{max} with poorly resolved saturation kinetics and so as pointed out in the Methods we have chosen not to try to over-intepret our NPQ results (Figs, 4, 6 and 8). Fabricius and Klumpp [15] using oxygen-electrodes were able to measure both emergent and contracted colonies and were able to show that photosynthesis was lower in the contracted colonies. Farrant et al [2] working on *Capnella gaboensis* colonies was able to measure photosynthesis using O_2-electrodes and ^{14}C on undisturbed emergent colonies. Pupier et al [4] used non-radioactive ^{13}C methods on various soft corals and used long incubation times on undisturbed specimens.

The E_{opt} of newly collected *Sarcophyton* was about 353 ± 28.9 µmol quanta $m^{-2}s^{-1}$ or about 18% sunlight (Fig. 3) (comparable to Fabricius and Klumpp [15]). Substantial photoinhibition of ETR set in at higher irradiances (Figs. 3 & 5) (cf. [2]). These light curves imply that optimum conditions for photosynthetic electron transport in the field are in the early morning and late afternoons with strong midday inhibition on bright sunny days [33]. *Sarcophyton* is not a "sun plant". Overall, daily photosynthesis would be greater on cloudy days such as during the generally overcast wet season and during high tides. The soft coral would be able to modulate its photosynthetic properties behaviourally (Fig. 1 , a colony with fully extended polyps) to some extent [15, 17, 19]. Other PAM studies such as Lichtenberg et al [17] and Wangpraseurt et al [19] used setups which allowed measurement of photosynthesis on colonies with their polyps fully extended. The optical properties of soft corals and corals in general are rather different to leaf systems of vascular plants, mainly because of light scattering [19]. Coral bleaching events are generally thought to be the result of unfortuitous co-occurrence of high irradiances and high temperatures [6, 9–12]. Most PAM studies have focused upon coral and soft coral bleaching phenomena: fewer studies have been made of the effects of spectral quality upon photosynthesis of zooxanthellae [16, 37]: this in an important gap in our knowledge of photosynthesis in animals with symbiotic symbionts [20] and their peculiar optical properties and behavioural responses also need to be taken into account [18, 19].

The PAM machine worked well on both the field-collected and aquarium-grown soft corals (Figs. 3, 4, 5 & 6) and the cultured zooxanthellae filtered onto glass fibre disks (Figs. 7 & 8). The E_{opt} and ETR_{max} of *Sarcophyton* and the cultured zooxanthellae appear to

be comparable, at least on a Chl *a* basis (Figs. 3 & 5 vs. Fig. 7) and have an optimum irradiance similar to that found in zooxanthellae of hard corals with substantial photoinhibition at higher irradiances: but most corals behave more like sun plants (like water lilies) with little photoinhibition at high irradiances (*Acropora aspera* & other corals, the clam *Triacna maxima*, an anemone, *Hetereactis sp*: [16]; *Montastrea curta*: [17]). Most of the several soft coral species studied by Fabricius and Klumpp [15] were not sun plants, saturating at or below 300 μmol photons $m^{-2}s^{-1}$. The maximum ^{14}C fixation rate of *Capnella gaboensis* found by Farrant et al [2] was 65.6 μmol C mg^{-1} Chl *a* h^{-1} or 18.2 μmol C g^{-1} Chl *a* s^{-1}. Considering as a rough estimate, 4 e^- from water are produced per carbon fixed, this is equivalent to an ETR of ≈80 μmol e^- g^{-1} Chl *a* s^{-1}, which is comparable to the ETR_{max} found in the present study for *Sarcophyton* (Figs. 3 & 5). Our O_2 electrode measurements of the Gross Photosynthetic oxygen evolution rate (P_g) of *Sarcophyton* are closely comparable (P_g ≈ 22 μmol O_2 g^{-1} Chl *a* s^{-1} (≡ ETR ≈ 90 μmol e^- g^{-1} Chl *a* s^{-1}) but have a large relative error (±≈30%).

A very unexpected finding in the present study was that field-collected and aquarium-kept *Sarcophyton* had a very high (but highly variable: range ≈ 0.4 to 0.7) Chl c_2/a ratio of ≈0.52; but the cultured zooxanthellae had a quite different Chl c_2/a ratio of ≈0.16 (overall from this study and our previous study, Ritchie et al [22]). The *Sarcophyton* colonies were also different in appearance to the cultures of zooxanthellae: the collected colonies were brown or orange-brown in appearance (Fig. 1) but variability between different colonies ruled out clear-cut Chl c_2/a ratio adaptation in the aquarium-grown material (Fig. 2) [18, 19]. The culturable zooxanthellae had a different in solvent spectrum (Fig. 2). The cultured zooxanthellae are definitely dinoflagellates (Fig. S1): since zooxanthellae are acquired post-settlement the occurrence of more than one symbiont is possible [7, 8]. Similarly, the Chl b/a ratio of the Lamp Post dwelling green alga were comparable between field collected material and the *Trentepohlia* alga when grown in the laboratory [33].

Sarcophyton had an easily grown chlorophyte endosymbiont (Chl $a+b$) present in very low abundance (Fig. 2). The organism is a very small (≈4 μm) immotile green alga closely similar in appearance to *Elliptochloris marina* (Letsch) found in the sea anemone, *Anthopleura elegantissima* (Brant) [14, 38]. Formulae for mixed phytoplankton populations (Chl $a + b + c$) [21] soft coral solvent extracts gave apparent Chl *b* contents that were not significantly different from zero or so low they were only marginally above zero. The green algal symbiont is not likely to make substantial contributions to photosynthesis of *Sarcophyton in situ* (cf. *Elliptochloris*, contributes about 1/3 to 1/2 of the photosynthesis of *Anthopleura*). The green algal symbiont grew easily in culture: some endosymbionts are very difficult to grow *in vitro* [4, 15].

The Chl *a* content was lower in field-grown (Chl *a* ≈300 mg m^{-2}) vs. aquarium-grown soft coral (≈450 mg m^{-2}) but without a clear-cut change in Chl c_2/a ratio. Chl *a* values of 300 to 450 mg m^{-2} are comparable to those found in *Capnella gaboensis* (≈30 to 50 μg Chl *a* cm^{-2}, [2]). The absorptance was ≈95% and so the soft coral adsorbed almost all incident light whereas algal impregnated filter disks typically had absorptances of 60–70%, hence the higher apparent photosynthetic efficiency on a Chl *a* basis for the zooxanthellae on the filter disks (field material ≈0.67, aquarium-grown material ≈0.43 vs. zooxanthellae ≈1.7 e^- g^{-1} Chl *a* $photon^{-1}$ m^2). The zooxanthellae *in situ* in the soft coral seem to be different to those we found for the zooxanthellae we isolated in culture but there are self-shading issues and zooxanthellae are likely to be arranged in a very specific orientation *in situ* [19] creating a package effect [39]. The zooxanthellae culture had a different Chl c_2/a ratio than the soft coral animal (Fig. 2). Despite six separate attempts, no other dinoflagellate zooxanthellae from *Sarcophyton* was successfully cultured (Fig. S1) notably no motile species. The culturability of endosymbionts varies greatly [4, 15]. Some dinoflagellates have atypical pigmentation because of the composite endosymbiotic origin of their chloroplasts [40]. Myxotrophy appears to be widespread in soft corals [15]: the green alga found as a minor resident in *Sarcophyton* could well be photoheterotrophic *in hospite*.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2023.017. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Appendix A. Supplementary data

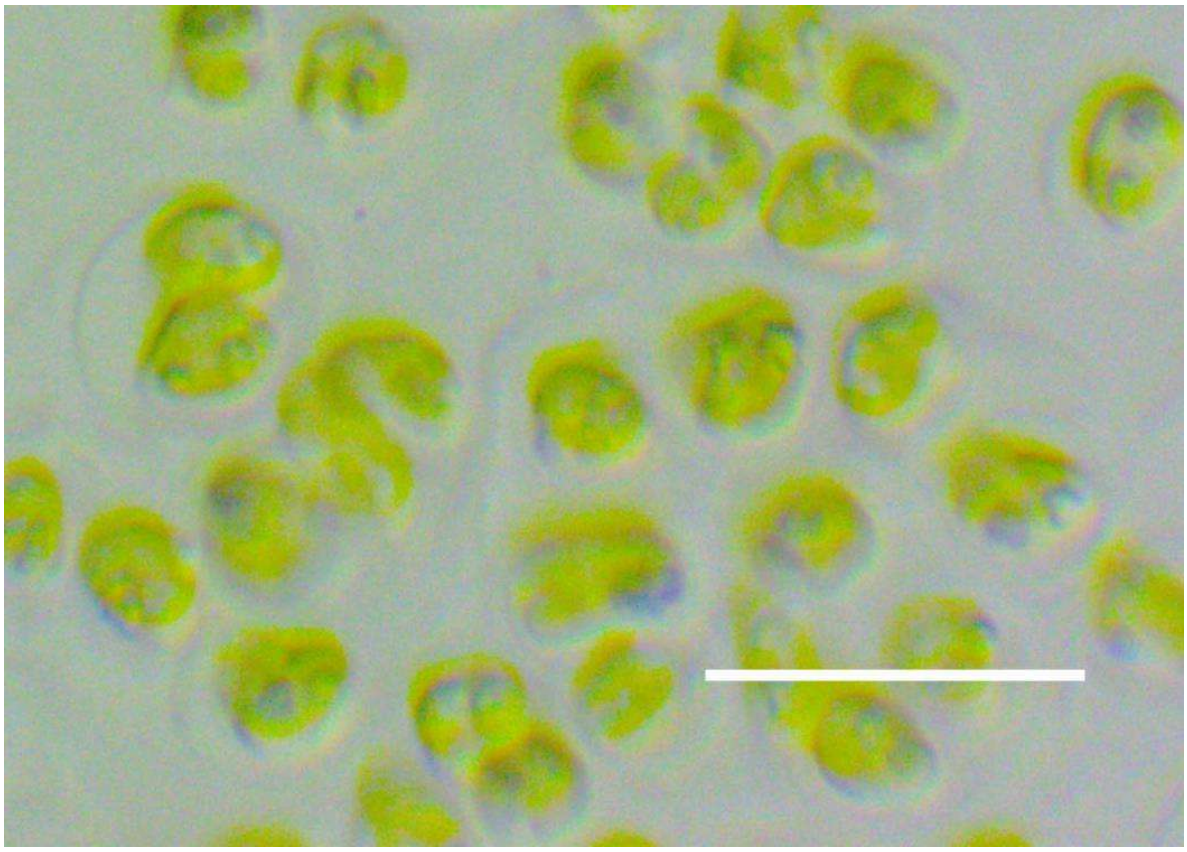

Fig. S1 Dinoflagellate zooxanthellae cells grown from *Sarcophyton* colony using a sterile hypodermic needle gown in enriched f/2 seawater. Magnification 1000 × , scale is 10 μm (HumaScope Advanced LED: HUMAN Gesellschaft fÃijr Biochemica und Diagnostica GmbH Max-Planck-Ring 21, 65205 Wiesbaden Germany). In culture, the cells form loose filamentous chains that disintegrate easily. The mucus layer around the cells is apparent.