

SUPPLEMENTARY METHODS

Survey site, sampling and manipulations.

We collected 0.5-1.5 cm wide and 2-5 mm thick specimens of the colonial didemnid ascidians *Trididemnum paracyclops*, *Diplosoma virens*, and *Diplosoma similis* from within dense patches of dead branched corals cemented together by coralline algae on the reef flat off Heron Island (23°26'S, 151°55'E), Great Barrier Reef, Australia. Several cm²-large and up to 2-3 cm thick colonies of the didemnid *Lissoclinum patella* were collected at 5-7 m depth in shaded parts of the outer reef slope. All specimens were transported in seawater to the Heron Island Research Station, where they were identified^{S1} and kept in a shaded aquarium with a continuous supply of fresh seawater (26-28°C). Maximal irradiance in the aquaria at noon was ~200 μmol photons m⁻² s⁻¹. Several colonies of each species were investigated. For further investigation, 0.2-1 mm thin sections were prepared by cutting vertically through colonies with a razorblade. Care was taken to avoid smearing or expulsion of symbionts or epizoic cells when preparing the slices, and only intact thin sections were used for experiments. Mechanical handling and slicing was done with a colony immersed in ample seawater to avoid deleterious acidification due to release of acid vacuoles in the ascidian tissue. Slices were transferred to small Petri dishes with fresh seawater and were photographed with a digital camera (Coolpix 4500, Nikon) before subsequent microscopy or fluorescence imaging measurements. High maximal quantum yields of photosynthesis were measured in the samples (see below) indicating no inhibitory effect on the cyanobacteria due to the cutting of the ascidians.

Genetic analysis.

Scrapings of biofilm areas with *Acaryochloris*-like cells were used as an inoculum for enrichment under far-red light (>690 nm). Cultures of the Chl-*d* containing organism isolated from the biofilms were kept as described previously^{S2}. DNA was obtained by standard chloroform-phenol extraction and ethanol precipitation. The light-harvesting complex gene, *pcbC* (AY552463) and 16S rRNA (AY163573) genes were identified by PCR techniques using degenerate primers that were specific for *A. marina*^{S3}: The forward: 5'-AGCCGTCCAAACTGATTTACC -3' (1878 – 1899 bp of *pcb*'s) and reverse 5'-GAAAGTGTGGAATAAACCG-3' (2230 – 2212 bp of *pcb*'s) to yield a 302 bp *pcbC* product by PCR; The forward: 5'-GCAACGCGAAGAACCTTACC-3' (921 – 940 bp of 16S rRNA) and reverse 5'-ACCTGCGATTACTAGCGATTCC-3' (1311 - 1290 bp of 16S rRNA) to yield a 392 bp product by PCR.

Microscopy and spectroscopy.

By combining digital microphotography and microspectrometry, we were able to link spectral absorbance and fluorescence characteristics to specific morphotypes. Thin tissue sections were carefully transferred to a microscope slide covered with seawater and sealed with a cover slip. The preparations were inspected with a bright field and epi-fluorescence microscope (Olympus, Japan). Defined sub-areas within sections were characterized by microscopic measurements of absorbance and fluorescence spectra as previously described^{S4} using a PC-controlled spectrometer (USB2000, Ocean Optics, USA) mounted on one of the microscope oculars. The same areas were documented by digital photography with a camera (Coolpix 4500, Nikon, Japan) mounted on the microscope via an adapter (Optem, USA). Additional microscopy and spectroscopy was done on samples from an *A. marina* culture, which was grown as described previously^{S2}.

Light penetration in ascidian tissue.

A fiber-optic scalar irradiance microprobe connected to a sensitive CCD spectrometer^{S5} was used for light measurements in intact specimens of *D. virens*, fixed inside a small flow channel with aerated seawater. Collimated light from a halogen lamp (Schott, KL1500, Germany) was used for illumination. It was necessary to carefully make a small cut with the tip of a 29G hypodermic needle in the tough epidermal cell layer of the test before inserting the microprobe into the tissue using a micromanipulator. The microprobe was inserted at a zenith angle of 135° relative to the vertically incident light. Additional irradiance measurements were done directly below ascidians where the epizoid biofilm had been carefully removed. All spectra were normalised to the downwelling irradiance at the tissue surface.

Active fluorescence measurements.

The photosynthetic performance of specific areas inside or below the ascidians was determined by imaging of active fluorescence using a new pulse-amplitude modulated imaging system (Imaging-PAM, Walz GmbH, Germany), which is described in detail elsewhere^{S6,S7}. For this, a Petri dish containing an ascidian tissue slice in seawater was placed under the imaging system camera. After focusing of the camera, the sample was allowed to dark-adapt for 10-15 minutes before further measurements. The imaging system monitors pulse-amplitude-modulated chlorophyll fluorescence, thus tolerating ambient background light. It features a special LED-Array light source, which besides weak blue measuring light (peak 470 nm) also provides strong blue actinic light and saturation pulses. The pulse-modulated measuring light was sufficiently weak ($<0.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) to be considered non-actinic during assessment of the minimal fluorescence yield, F_o , of the dark-adapted sample. Using the saturation-pulse-method^{S8}, images of the maximal quantum yield of PSII photosynthetic energy conversion, $(\Phi_{II})_{\text{max}} = (F_m - F_o)/F_m$ and of the effective quantum yield of PSII, $\Phi_{II} = (F_m' - F)/F_m'$, were measured at defined levels of actinic light, PAR . Relative rates of PSII-driven electron transport were calculated as: $rETR = PAR \cdot \Phi_{II}$.

The LED-Array also contains red (650 nm) and near-infrared (NIR, 780 nm) LEDs for imaging the light absorption by photosynthetic pigments, here approximated from the ratio of red to NIR remission, R/NIR , as the absorptivity, $ABS = 1 - (R/NIR)$. While this approximation originally was derived for green leaves, where the PS II action of 470 nm and 650 nm photons is similar due to the presence of Chl *b*, it can also be applied for *Prochloron*, which also contains Chl *b*, and to *Acaryochloris*, which displays almost identical PS II action at 470 and 650 nm^{S10} due to the presence of Chl *d*. The combined information on quantum yield, absorptivity and incident light enabled us to calculate images of relative photosynthetic activity at defined levels of actinic light as: $PS = 0.5 \cdot rETR \cdot ABS$. Plots of average PS vs. irradiance within selected areas of interest were extracted from images using the system software.

Most cyanobacteria have phycobilisomes as the major light-harvesting complex associated with PSII, showing a low optical cross section for blue light and therefore a low fluorescence yield upon excitation with blue light^{S9}. *Acaryochloris marina* has low amounts of phycobiliproteins (but no phycobilisomes)^{S2} but Chl *d* is the major light-harvesting pigment for both photosystems in *A. marina*^{S2} with blue light (440-480 nm) showing ~50-80% efficiency in photosynthetic action spectra as compared to the maximal activity induced by 700-720 nm light^{S10} in relatively dilute suspensions.

Due to the pigment flattening effect the equivalence of blue and far-red light should be even higher in the highly scattering biofilm. This enabled us to monitor the photosynthesis of the *Acaryochloris*-like cells growing below the ascidians using blue excitation. *Prochloron* sp. has no phycobiliproteins and major light-harvesting is due to Chl *a* and Chl *b*. Therefore blue excitation is also well suited for variable fluorescence analysis of *Prochloron*^{S11} and fluorescence imaging of the *Prochloron* layer inside the ascidians.

Acknowledgements.

This study was supported by the *Danish Natural Science Research Council* (MK) and the *Australian Research Council* (PJR, AWD).

References.

- S1. Kott, P. Algal-bearing didemnid ascidians in the Indo-West-Pacific *Mem. Qd. Mus.* **20**, 1-47 (1980).
- S2. Chen, M., Quinell, R. G. & Larkum, A. W. D. Chlorophyll *d* as the major photopigment in *Acaryochloris marina*. *J. Porphyrins Phthalocyanines* **6**, 763-773 (2002).
- S3. Chen, M., Hiller, R. G., Howe, C. J. & Larkum, A. W. D. Unique Origin and Lateral Transfer of Prokaryotic Chlorophyll-*b* and Chlorophyll-*d* Light-harvesting Systems. *Molec. Biol. Evol.* **22**, 12-20 (2005).
- S4. Kühl, M. & Fenchel, T. Bio-optical characteristics and the vertical distribution of photosynthetic pigments and photosynthesis in an artificial cyanobacterial mat. *Microb. Ecol.* **40**, 94-103 (2000).
- S5. Kühl, M. & Jørgensen, B.B. Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector. *Limnol. Oceanogr.* **37**, 1813-1823 (1992).
- S6. Schreiber, U., Walz, H. & Kolbowski, J. Propagation of spatial variations of chlorophyll fluorescence parameters in dandelion leaves induced by laser spot heating. PAM News 1 (2003), <http://www.pam-news.com/ar/03-01/PAM-News-03-01.html>.
- S7. Ralph, P. J., Schreiber, U., Gademann, R., Kühl, M. & Larkum, A.W.D. Coral photobiology studied with a new imaging PAM fluorometer. *J. Phycol.* (IN PRESS)
- S8. Schreiber, U. Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. In *Chlorophyll fluorescence: a signature of photosynthesis* (Eds. Papageorgiou, G. C. & Govindjee) 279-319 (Kluwer, Dordrecht, 2004).
- S9. Campbell, D., Hurry, V., Clarke, A. K., Gustafsson, P. & Öquist, G. Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. *Microbiol. Mol. Biol. Rev.* **62**, 667-683 (1998).
- S10. Boichenko, V. A., Klimov, V. V., Miyashita, H. & Miyachi, S. Functional characteristics of chlorophyll *d*-predominating photosynthetic apparatus in intact cells of *Acaryochloris marina*. *Photos. Res.* **65**, 269-277 (2000).
- S11. Schreiber, U., Gademann, R., Ralph, P. J. & Larkum, A. W. D. Assessment of photosynthetic performance of *Prochloron* in *Lissoclinum patella* in hospite by chlorophyll fluorescence measurements. *Plant Cell Physiol.* **38**, 945-951 (1997).