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REPORT

Juvenile corals can acquire more carbon from high-performance algal symbionts

N. E. Cantin · M. J. H. van Oppen · B. L. Willis · J. C. Mieog · A. P. Negri

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Abstract Algal endosymbionts of the genus *Symbiodinium* play a key role in the nutrition of reef building corals and strongly affect the thermal tolerance and growth rate of the animal host. This study reports that ^{14}C photosynthate incorporation into juvenile coral tissues was doubled in *Acropora millepora* harbouring *Symbiodinium* C1 compared with juveniles from common parentage harbouring *Symbiodinium* D in a laboratory experiment. Rapid light curves performed on the same corals revealed that the relative electron transport rate of photosystem II ($rETR_{MAX}$) was 87% greater in *Symbiodinium* C1 than in *Symbiodinium* D *in hospite*. The greater relative electron transport through photosystem II of *Symbiodinium* C1 is positively correlated with increased carbon delivery to the host under the applied experimental conditions ($r^2 = 0.91$). This may translate into a competitive advantage for juveniles harbouring *Symbiodinium* C1 under certain field conditions, since rapid early growth typically limits mortality. Both symbiont types exhibited severe reductions in ^{14}C incorporation during a 10-h exposure to the electron transport blocking herbicide diuron (DCMU), confirming the link between electron transport through PSII and photosynthate incorporation within the host tissue. These findings advance the current understanding of symbiotic relationships between corals and their symbionts, providing evidence that enhanced growth rates of juvenile corals may result from greater translocation of photosynthates from *Symbiodinium* C1.

Keywords *Symbiodinium* · Coral · Symbiosis · Pigment · DCMU · Diuron

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Introduction

Zooxanthellae (symbiotic dinoflagellates of the genus *Symbiodinium*) are critical to the survival of reef-building corals, providing a major source of energy from photosynthesis for cell maintenance, growth and reproduction of their coral hosts (Crossland et al. 1980; Muscatine et al. 1984). Molecular techniques using nuclear 28S, ITS1, ITS2, and organellar 23S and cox1 markers have uncovered a large diversity describing eight major groupings or clades within the genus *Symbiodinium* (van Oppen et al. 2001; LaJeunesse 2002; Santos et al. 2002; Baker 2003; Pochon et al. 2004; Rowan 2004; Takabayashi et al. 2004; Coffroth and Santos 2005; Apprill and Gates 2007). The term 'clade' refers to one of the eight major groupings of *Symbiodinium* named A-H. Sub-cladal groups are referred to as 'types' within this paper. Differences in photobiology are known to exist between *in hospite Symbiodinium* types, especially under conditions of thermal or irradiance stress (Rowan 2004; Goulet et al. 2005; Berkelmans and van Oppen 2006). As ocean warming associated with climate

change is predicted to cause an increase in the frequency and intensity of coral bleaching events, assessing the performance of distinct coral-zooxanthella associations under conditions of photosystem stress will enhance the understanding of the future health of coral reefs (Little et al. 2004; Abrego et al. 2008).

Physiological traits of the coral host are at least partly shaped by the dominant symbiont type present within its tissues. For instance, the genetic type of symbiont within juveniles of *Acropora millepora* and *Acropora tenuis* has been linked to a 2- to 3-fold increase in growth rate within the first 6 months of development on the reef (Little et al. 2004). Furthermore, adult *A. millepora* corals on the Great Barrier Reef (GBR) have been shown to acquire a 1–1.5°C increase in thermal tolerance by shuffling the dominant symbiont type present within coral tissues (Berkelmans and van Oppen 2006). Likewise, in Guam, colonies of *Pocillopora* spp. associating with *Symbiodinium* D exhibited greater tolerance to thermal stress compared to corals associating with *Symbiodinium* C (Rowan 2004). Capacity for photoacclimation and tolerance to high irradiance stress has also been linked to the genetic type of *Symbiodinium* spp., both in culture and within multiple coral host species (Robison and Warner 2006; Warner et al. 2006). Recent studies have shown that symbiont type can affect the incorporation of algal-derived photosynthetic carbon (^{14}C) into host tissues of an anemone (Loram et al. 2007) and the total amount of photosynthetically fixed carbon released from the symbiont (Stat et al. 2008), further supporting the notion that symbiont type can affect growth and resilience of invertebrate hosts to stress. Anemones hosting *Symbiodinium* clade A obtained a greater proportion of photosynthetically fixed carbon into animal lipids and amino acid pools than those hosting clade B. Total fixation increased at high temperatures for clade A anemones and was depressed for clade B anemones, demonstrating that these two distinct *Symbiodinium* clades are not functionally equivalent (Loram et al. 2007). The ability of some corals to associate with a diverse range of symbiont types (van Oppen et al. 2001; Baker 2003; Rowan 2004) may provide ecological advantages to the host colony, enabling it to colonize a variety of reef habitats and survive a changing global climate.

Acropora millepora is typical of most broadcast spawning corals, acquiring symbionts from the environment just prior to or following larval metamorphosis. It associates with *Symbiodinium* D on reefs surrounding Magnetic Island, a nearshore island in the central GBR. However, it commonly harbours *Symbiodinium* C1 and C2 and occasionally with C3 on inner-, mid- and outer-shelf reefs (van Oppen et al. 2001, 2005). On the GBR, *Symbiodinium* D has a predominantly inshore distribution and hence experiences high temperatures, turbidity and pollution events relatively frequently. Its rarity on mid- or outer-shelf reefs is possibly due to higher light intensities common at these sites (van Oppen et al. 2005).

This study compared the effect of hosting *Symbiodinium* C1 and D on the translocation of carbon-based energy (measured as tissue ^{14}C incorporation) to the coral host. The relative electron transport rates of photosystem II ($rETR_{MAX}$) of these symbiont types were then measured *in hospite* as a secondary measure of photosynthetic performance. Photosynthetic performance of the *Symbiodinium* types were also compared following exposure to the photosystem II (PSII) blocker DCMU (diuron), which is also an environmentally relevant contaminant (Jones et al. 2003).

Materials and methods

The experiment was conducted under controlled light and temperature conditions using 9-month-old *A. millepora* juveniles. In this set-up, juveniles experimentally infected with *Symbiodinium* C1 or D originated from crosses involving the same parent corals, thereby minimizing potential host genetic differences that may influence the physiology of the holobiont (host–symbiont partnership). The experimental design therefore controlled for both environmental and host influences on symbiont physiology (Coles and Brown 2003; Rowan 2004).

Juvenile inoculation and treatments

Gametes were collected following spawning from eight colonies of *A. millepora* and mixed for fertilization. Larvae were raised in filtered seawater (1 μm). On day 4 following spawning, when larvae were first observed to exhibit settlement behaviour, preconditioned (for 16 weeks on the reef at Magnetic Island), autoclaved (to kill any *Symbiodinium* spp. present on the tiles) terracotta tiles were placed on the bottom of two 500 l tanks as settlement surfaces. *Symbiodinium* type C1 (GenBank Accession No. AF380555) and D (GenBank Accession No. EU024793) based on ITS1 were selected for experimental infection of juveniles because both associate with *A. millepora* on inshore reefs of the GBR (van Oppen et al. 2005). *Symbiodinium* C1 and D were obtained from adult colonies of *A. tenuis* and *A. millepora* at Magnetic Island, respectively, by airbrushing the coral tissue and isolating the *Symbiodinium* cells from the coral-algal slurry through centrifugation (5 min at 350 × *g*). These isolated symbionts were offered to larvae and newly settled juveniles at 10^8 cells/tank after 3 and 5 days following spawning. Infection of the coral juveniles was confirmed by microscopic observation of squash

preparations indicating the presence of symbionts within the tentacles of each juvenile. *Symbiodinium* genotypes were confirmed following infection prior to field deployment by single-stranded conformation polymorphism (SSCP, following methods described in van Oppen et al. 2001) of the ITS1 regions and potential presence of unexpected background strains was tested using quantitative PCR at the end of the experiment (see below). The juvenile corals were allowed to develop for a further 2 weeks in the laboratory, after which the tiles were attached vertically to racks on a fringing reef (Nelly Bay, Magnetic Island) in a zone where *A. millepora* is common, and were randomly arranged to minimize any effects of partial shading during this grow-out period. The juveniles were collected 9 months later and acclimated horizontally under identical natural illumination (75% shading, approximately 200 μmol photons m^{-2} s^{-1}) in an outdoor flow-through aquarium for 2 days prior to experimental testing.

The juvenile corals were exposed to the herbicide DCMU (diuron or 3(3,4-dichlorophenyl)-1,1-dimethylurea, photosystem II inhibitor), an environmentally relevant contaminant that acts by inhibiting photosynthesis by blocking electron transport and causes photoinactivation of photosystem II (PSII) (Jones et al. 2003). Unlike high temperature and irradiation stresses, DCMU exposure influences the symbiont's performance without directly affecting the coral host (Schreiber et al. 1997; Negri et al. 2005; Cantin et al. 2007), therefore enabling the effects of photoinhibition to be distinguished between symbiont types. Nine-month-old juveniles were randomly distributed within three glass tanks (4 l filtered sea water, 0.25 μm) per DCMU treatment [0 (control), 1 and 10 μg l^{-1}; $n = 9$ juveniles per DCMU treatment and symbiont type; 10 h DCMU exposure]. Tanks were placed under metal halide lamps exposing the corals to a constant illumination of 180–200 μmol photons m^{-2} s^{-1}.

Pulse amplitude modulation (PAM) fluorometry

All fluorescence measurements were taken with a Diving-PAM (Walz, Germany) by holding a 2-mm fibre-optic probe at a consistent distance of 2 mm directly above each juvenile coral using the manufacturer-supplied leaf clip. Rapid light curves (RLCs) can be used to assess the current photosynthetic capacity of PSII as a function of irradiance under increasing levels of light intensity (PAR) (Schreiber et al. 1997; Ralph and Gademann 2005). RLCs are constructed by plotting the effective quantum yield as measured with a PAM fluorometer against PAR. Cardinal points calculated from a RLC, such as the maximum relative electron transport rate of PSII ($rETR_{MAX}$), reflect the present state of photosynthesis (of PSII) and are strongly dependent on the immediate light pre-history of the sample (Schreiber 2004). These measurements differ from traditional photosynthesis-irradiance (*P-E*) curves derived from gas exchange measurements, which effectively describe how the entire photosynthetic apparatus acclimates to different light intensities and are less dependent on light pre-history. RLC measurements calculated within this study were used comparatively among the different symbiont and DCMU treatments to reflect the current photosynthetic performance under the experimental light conditions.

After 2 h of light exposure, the corals were placed in total darkness for a short time (2-min dark adaptation) to allow substantial re-oxidation of the primary electron acceptor (Q_A) (Schreiber 2004) and minimize the total time required for RLC measurements. RLC measurements were performed in the dark on three juveniles for each symbiont type and DCMU treatment ($n = 3$), taking a total of 90 min. Each tank was immediately returned to the light regime following each RLC. The RLCs were measured using a pre-installed software routine, where the actinic measuring light was incremented over eight light steps (0, 44, 72, 116, 147, 222, 283, 428 and 653 μmol photons m^{-2} s^{-1}), each with a duration of 10 s. The relative electron transport rate (rETR) (Eq. 1) obtained from RLCs provides a reliable approximation of relative electron flow through PSII when absorbance between samples is identical (Genty et al. 1989). In the present experiments, this was assumed since all juveniles (i) had similar colony heights (2–4 mm), (ii) received the same irradiance, (iii) contained the same quantity of symbionts (see below) and pigment concentrations in control treatments (see Results section) and (iv) were tested using the same host coral species; therefore, the corallite shapes were identical, thus reducing the influence of light scattering based on coral skeleton morphologies. Correction factors for absorbance were not used in calculations of rETR.

$$\text{Relative electron transportate of PSII (rETR)} = \Delta F/F_{m'} \times \text{PAR} \quad (1)$$

Minimum fluorescence (F in illuminated samples or F_o in dark-adapted samples) was determined by applying a weak pulse-modulated red measuring light (0.15 μmol photons m^{-2} s^{-1}). The maximum fluorescence ($F_{m'}$ in illuminated samples or F_m in dark-adapted samples) was then measured following the application of a saturating pulse of actinic light (>3,000 μmol photons m^{-2} s^{-1}). The maximum quantum yield (F_v/F_m, Eq. 2) is the proportion of light absorbed by chlorophyll (in PSII) used for photochemistry in the dark-adapted organisms, when all reaction centres are open (Genty et al. 1989). Maximum quantum yield (F_v/F_m) values were obtained from dark-adapted symbionts ($n = 3$ juvenile corals) following each RLC and an additional 10-min dark adaptation.

$$\text{Maximum quantum yield of PSII } (F_v/F_m) = (F_m - F_o)/F_m \quad (2)$$

Radio-labelled ^{14}C incorporation

Following the fluorescence measurements, the volume of filtered seawater was reduced to 1 l and 1 ml of $NaH^{14}CO_3$ (specific activity 74 MBq ml^{-1}, Amersham Biosciences, USA) was added to each tank. The water level was subsequently raised to 2 l to ensure equal distribution of radiolabel throughout the tank. ^{14}C incubation was carried out for 6 h under constant illumination (180–200 µmol photons m^{-2} s^{-1}). At the end of the incubation, each tile was removed from the experimental light exposure and rinsed twice with fresh filtered sea water for 5 min to remove unincorporated ^{14}C from the surface of coral tissues. Each juvenile ($n = 9$) was removed from the terracotta tile with a scalpel, snap frozen in liquid nitrogen and stored at −80°C until analysis. The time frame used to rinse each juvenile coral at lower light intensity (approx 15–30 µmol photons m^{-2} s^{-1} for 10–15 min) prior to freezing may have influenced the xanthophyll pigment ratios [conversion of diatoxanthin back to diadinoxanthin in the absence of light stress (Brown et al. 1999)]; however, this was not likely to influence the total xanthophyll pool. Tissue from each juvenile *A. millepora* ($n = 9$) was removed by airbrushing and the host tissue was separated from the symbionts by centrifugation (490 × *g*). Host tissue samples (100 µl) were acidified with 0.1 M HCl (100 µl) prior to scintillation counting to remove unincorporated ^{14}C. Host tissue samples were counted on a 1450 Microbeta Plus scintillation counter (Perkin Elmer) for 2 min to determine disintegrations per minute (dpm) and the amount of ^{14}C incorporation. *Symbiodinium* cells were resuspended in 10% formalin and cell densities were determined with a haemocytometer. Mean cell densities were not different between symbiont types: *Symbiodinium* C1 = 5.5 ± 0.4 (SE) × 10^5 cells cm^{-2} and Symbiodinium D = 4.6 ± 0.8 × 10^5 cells cm^{-2}, $P = 0.20$]. Digital images of the juveniles were taken from a standardized height on a tripod and the perimeter of each juvenile coral was then traced using image analysis software (Optimas, Media Cybernetics, Silver Spring, MD, USA) and surface area of each juvenile coral was then calculated using Optimas (Negri et al. 2005). The radiolabelled photosynthate incorporation was expressed as radioactivity per unit area of juvenile per dinoflagellate cell (dpm $zoox^{-1}$ cm^{-2}) from the calculated surface area measurements to standardize for the variations in the size of individual juveniles and the *Symbiodinium* densities they host.

Extraction, amplification and sequencing of *Symbiodinium* chloroplast 23S ribosomal DNA (cp23S-rDNA)

In order to develop a clade-specific quantitative real-time PCR assay based on chloroplast DNA (cpDNA), small branches were collected from colonies of *A. tenuis* harbouring *Symbiodinium* C1 (from Magnetic Island, Great Barrier Reef (GBR)) and *A. millepora* harbouring *Symbiodinium* D (collected from Magnetic Island), *Symbiodinium* C2 (from Davies Reef) or *Symbiodinium* C2 or D (from Keppel Islands, GBR) and fixed in absolute ethanol. DNA was extracted following a previously published DNA isolation method (Wilson et al. 2002). An approximately 0.7 kb region of *Symbiodinium* cp23S-rDNA was PCR amplified using the primer pair 23S1 (5′-GGCTGTAACTATAACG GTCC-3′) and 23S2 (5′-CCATCGTATTGAAC CCAGC-3′) (Santos et al. 2002). PCRs were performed in 25 µl volumes containing 2 mM MgCl, 200 µM dNTPs, 1 × DNA Polymerase PCR buffer, 0.02 U *Taq* polymerase (Fisher Biotec, Australia), 40 pmol of each primer and 50–70 ng of template DNA. Reactions were carried out in a Corbett Research PC-960G Gradient Thermal Cycler (Corbett Research, NSW Australia) under the following conditions: initial denaturing period of 1 min at 95°C, 35 cycles consisting of 95°C for 45 s, 55°C for 45 s and 72°C for 1 min, followed by a final extension period at 72°C for 7 min. PCR products were precipitated by adding 100% ethanol, 0.3 M ammonium acetate and then centrifuged at 1,530 × *g* in a bench top centrifuge at 4°C for 20 min. The products were cloned using the TOPO TA Cloning kit (Invitrogen, Victoria Australia) according to the manufacturer's directions. Colony PCR was conducted using the universal M13 plasmid primers M13F (5′-GTAAAACGACGGCCAG-3′) and M13R (5′-CAGGA AACAGCTATGAC-3′). The products were then purified and sequenced at the Macrogen Sequencing Service (Macrogen Inc., South Korea).

Symbiodinium *cp23S-rDNA quantitative real-time PCR assay*

In order to determine the relative abundance of symbiont types within *A. millepora* juveniles used in this ^{14}C study, local GBR cp23S-rDNA *Symbiodinium* sequences (one C1 from Magnetic Island, GenBank Accession No. EF140804; two C2 from Davies Reef, GenBank Accession No. EF140806 and Keppel Islands, GenBank Accession No. EF140805; and one D from Magnetic Island, GenBank Accession No. EF140808) were added to an existing alignment (Santos et al. 2002) to design *Symbiodinium* clade C and D specific primers for the quantitative real-time PCR (qPCR) assay. Relative symbiont abundance within the

A. millepora juveniles was estimated from qPCR of the cp23S-rDNA region. The primer pairs 23S C FP2 (5′-GGGAT AAAACTTGGGTAACATTC-3′) and 23S C RP2 (5′-CCA ATTAAACAGTGGTCTTAGGAG-3′) were used to amplify *Symbiodinium* C and 23S D FP1 (5′-AACCCCC GATTGGCCTAG-3′) and 23S D RP1 (5′-CTTGATTG GGCCATTAAGCA-3′) were used to amplify *Symbiodinium* D. Clade specificity of each primer pair was confirmed by the absence of D amplification with the C primers and vice versa in qPCR assays with cloned *Symbiodinium* C1 and D 23S DNA. Each DNA sample was analyzed in duplicate with a *Symbiodinium* C specific reaction, a *Symbiodinium* D specific reaction and a quantified standard with DNA isolated from 100,000 cells of each *Symbiodinium* type, along with no-template controls. qPCR reactions were performed in 20 µl volumes containing 10.0 µl of Platinum SYBR Green qPCR Supermix UDG (Invitrogen, Victoria Australia), 1.0 µl of 4 µM C/D specific FP, 1.0 µl of 4 µM C/D specific RP, 6.0 µl of MilliQ water and 2.0 µl of DNA template. Reactions were carried out in the Rotor-Gene RG-3000A thermal cycler (Corbett Research, NSW Australia) under the following conditions: an initial denaturing period of 2 min at 50°C and 2 min at 95°C, 40 cycles of 15 s at 95°C and 30 s at 60°C. In order to check for the formation of primer dimers and to verify *Symbiodinium* type primer specificity, a melt curve was generated at the end of the run and the melting point (T_m) for each symbiont specific reaction was determined by starting at 60°C for 30 s and raising the temperature by 1°C every 5 s until 95°C was reached. Fluorescent data collection took place after each 60°C step during cycling and after each temperature step of the melt curve. Serial dilutions over a range of 5 logs were run to ensure both the *Symbiodinium* C and D reactions had similar efficiencies (*Symbiodinium* C reaction efficiency = 0.92 and *Symbiodinium* D reaction efficiency = 0.95). Relative abundances (as a percentage of total copies per reaction) were determined from the calculated concentration (copies per reaction) within Rotor-Gene Analysis Software v. 6.0 (Corbett Research, NSW Australia) for each *Symbiodinium* type per reaction compared to the 100,000 copy standard after importing the standard curve from the serial dilution series. Although copy numbers may vary over the symbionts growth curve (Koumandou and Howe 2007), it is assumed that growth *in hospite* was not likely to be synchronous and that the application of duplicate 100,000 cell extracts is likely to have averaged out any differences. Quantification using multi-copy genes may also be affected by potential differences in copy numbers between symbiont types. A nuclear rDNA qPCR assay had previously been compared with the cpDNA assay used here and the quantification results were very similar (Mieog et al. 2007).

Although the cp23S-rDNA assay used here is *Symbiodinium* clade and not type specific, it is highly unlikely the C-juveniles would harbour C-types other than C1. First, the juveniles were experimentally infected with C1 and it was verified using SSCP of the rDNA ITS1 region that a symbiosis had been established with C1 prior to deployment of the juveniles in the field. Second, juveniles from the same batch that were used in other experiments only harboured C1 and D after the grow-out period in the field as verified by SSCP analysis (J.C. Mieog et al., pers. comm.). Third, C-types other than C1 are extremely rare at Magnetic Island; of 52 cnidarian species (136 specimens) surveyed at Magnetic Island (D. Abrego et al., pers. comm.), the vast majority harboured C1 or D, two *Montipora* species harboured C• (which has so far only been found in *Porites* and *Montipora* species and is not taken up by *Acropora* spp. in infection experiments), and a single colony harboured a mix of C1 and C2-like. Other previous reports on zooxanthellae types at Magnetic Island (van Oppen et al. 2001; van Oppen 2004; Ulstrup and van Oppen 2003) have shown only C1, C• and D with a single exception of an *A. tenuis* colony where C2 was present in addition to C1.

Pigment analysis by HPLC

Chlorophylls and carotenoids were extracted sequentially by sonication (Cole Parmer Ultrasonic Processor, Extech Equipment, Victoria, Australia) in 100% acetone from *Symbiodinium* cells that were separated from the host tissue ($n = 8$ juvenile corals). High performance liquid chromatography (HPLC) was used to analyse the extracts on a Waters 600 HPLC, combined with a Waters PDA 996 photodiode-array detector, on a 3 µm, 50 × 4.6 mm Phenomonex C-18 Gemini 110Å column (Phenomonex, NSW, Australia). A two solvent gradient with a flow rate of 1 ml min^{-1} was used to separate the pigments with a run time of 18 min. Percentages of the solvents A and B, respectively, were as follows: 0 min: 75, 25%; 0 to 5 min linear gradient to: 0, 100%; 5 to 10 min hold at: 0, 100%; 10–11 min linear gradient to: 75, 25%; 11–18 min hold at 75, 25%. Solvent A was 70:30 v/v methanol:28 mM tetrabutyl ammonium acetate (TBAA, 1.0 M aq. Sigma-Aldrich, Australia) and solvent B was 50:50 v/v methanol:acetone. Chlorophyll *a*, c_2, peridinin and diadinoxanthin standards were obtained from the International Agency for ^{14}C Determination (DHI, Denmark). The peaks reported were identified by comparison of retention times and absorption spectra with standards and published data (Wright and Jeffrey 1997).

Data analysis

Two way, Model I ANOVA ($\alpha = 0.05$) was used to test the effect of *Symbiodinium* type and DCMU concentration on $rETR_{MAX}$, F_v/F_m, ^{14}C incorporation and total pigment

concentrations. Fishers LSD post hoc test ($P < 0.05$) was used to identify statistical differences between treatments. Data were tested for assumptions of normality and homogeneity of variances and transformations were not required. Rapid light curves were fitted according to the functions of Platt et al. (1980) as outlined by Ralph et al. (2002). All figures and curve-fitting to determine the characteristic parameters of the rapid light curves (Ralph et al. 2002) were created using Sigmaplot 2001 for Windows (v. 7.1, SPSS Inc.). Statistica v. 6.0 (StatSoft, Inc. Oklahoma, USA) was used for all statistical analyses. Rotor-Gene Analysis Software v. 6.0 (Corbett Research, NSW Australia) was used for all real-time qPCR analysis.

Results

Quantitative real-time PCR assays indicated that the juvenile *A. millepora* colonies were dominated by the *Symbiodinium* types that they were exposed to during experimental infections after 9 months of growth on the reef at Magnetic Island in GBR. Juveniles exposed to *Symbiodinium* C1 were estimated to contain 98–100% relative abundance C ($n = 27$), while juveniles exposed to *Symbiodinium* D contained 94–100% relative abundance D ($n = 27$).

Incorporation of radiolabelled photosynthate (^{14}C, energy) into the host tissue was 121% greater within juvenile *A. millepora* corals hosting *Symbiodinium* C1 than the colonies associated with *Symbiodinium* D in control treatments at 200 µmol photons m^{-2} s^{-1} (0 µg l^{-1}, Fig. 1a, Two-way ANOVA, symbiont type: $F_{1,48} = 6.69$, $P = 0.02$, DCMU: $F_{2,48} = 4.11$, $P = 0.01$, symbiont*DCMU interaction: $F_{2,48} = 0.84$, $P = 0.4$). Exposure to the electron transport inhibitor DCMU for 10 h reduced the incorporation of photosynthate into the tissues of corals hosting both *Symbiodinium* C1 and D (Fig. 1a). Corals hosting *Symbiodinium* C1 exhibited a 58% drop in photosynthate accumulation when exposed to 10 µg l^{-1} DCMU, while photosynthate level was reduced by 42% in corals hosting *Symbiodinium* D (Fig. 1a).

Comparison of rapid light curves (RLCs, Fig. 2a, b) indicated that *Symbiodinium* C1 had an 87% greater relative electron transport rate through PSII ($rETR_{MAX}$, Fig. 1b) than *Symbiodinium* D when associated with the same coral species in the absence of DCMU (Two-way ANOVA, symbiont type: $F_{1,12} = 6.85$, $P = 0.02$, DCMU: $F_{2,12} = 14.77$, $P < 0.001$, symbiont*DCMU interaction: $F_{2,12} = 2.07$, $P = 0.2$). A strong, positive correlation was observed between $rETR_{MAX}$ and incorporation of ^{14}C labelled photosynthates ($r^2 = 0.91$, $P = 0.01$, $n = 6$). Exposure of the juvenile colonies to DCMU significantly reduced the $rETR_{MAX}$ in both *Symbiodinium* C1 and D (Fig. 1b). At 10 µg l^{-1} DCMU, the $rETR_{MAX}$ was reduced by 86% in *Symbiodinium* C1 and by 71% in *Symbiodinium* D under the same experimental conditions (Fig. 1b). There was no difference between the maximum quantum yields (F_v/F_m) in dark-adapted corals for each symbiont type in the absence of DCMU (Fig. 3, Two-way ANOVA, symbiont type: $F_{1,102} = 0.05$, $P = 0.8$, DCMU: $F_{2,102} = 103.76$, $P < 0.001$, symbiont*DCMU interaction: $F_{2,102} = 0.61$, $P = 0.5$), indicating similar efficiencies of excitation energy capture by PSII for each symbiont (Genty et al. 1989). DCMU exposure caused similar reductions in F_v/F_m in both symbionts (Fig. 3), indicating equivalent photoinactivation of PSII in both symbiont types (Schreiber 2004).

The pigments, chlorophyll *a*, c_2 and peridinin, which constitute the major light harvesting complex (LHC) within

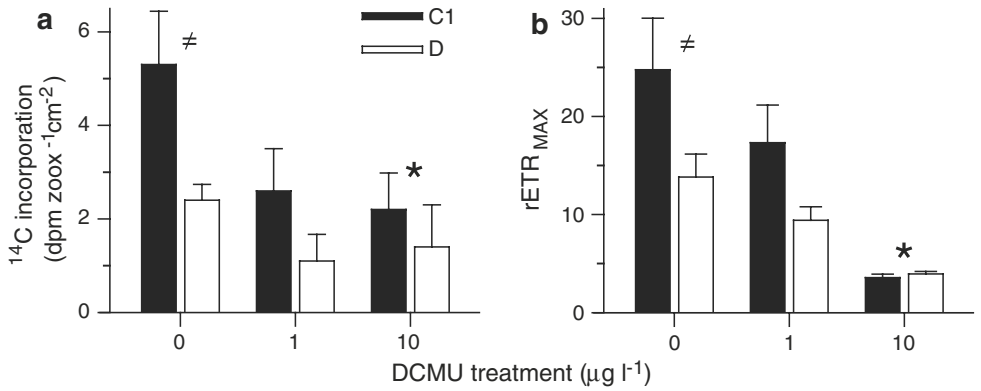

Fig. 1 Photosynthate incorporation into juvenile corals and photosynthetic capacity of symbionts exposed to 3 DCMU (diuron) treatments (0, 1 and 10 µg l^{-1}). **a** Photosynthate incorporation into juvenile *A. millepora* colonies hosting *Symbiodinium* C1 and *Symbiodinium* D as measured by uptake of ^{14}C into host tissue $n = 9$ juveniles, mean ± SE. **b** Photosynthetic capacity of PSII in *Symbiodinium* C1 and D hosted by *Acropora millepora* juveniles as measured by relative electron transport ($rETR_{MAX}$) derived from rapid light curve. $n = 3$ juveniles (mean ± SE). Asterisks (*) indicate significant differences ($P < 0.05$) between DCMU treatments compared to the control (0 µg l^{-1}) and inequalities (≠) indicate significant differences between *Symbiodinium* C1 and D

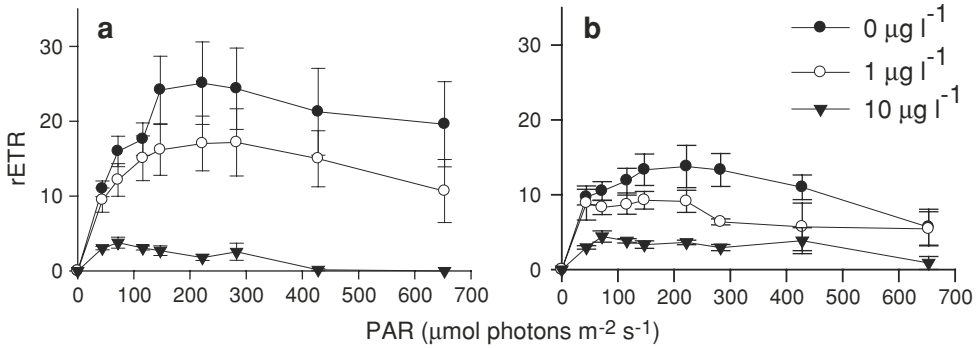

Fig. 2 Rapid light curves from juvenile colonies of *Acropora millepora*. Relative electron transport rate (rETR) as a function of photosynthetically active radiation (PAR, µmol photons m^{-2} s^{-1}) derived from colonies hosting **a** *Symbiodinium* C1 and **b** *Symbiodinium* D exposed to 3 DCMU (diuron) treatments (0, 1 and 10 µg l^{-1}). $n = 3$ juveniles (mean ± SE)

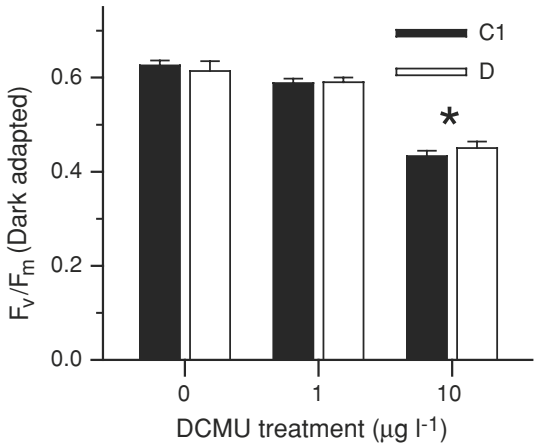

Fig. 3 Quantum yields of *Symbiodinium* C1 and D. Maximum quantum yields (F_v/F_m, mean ± SE, $n = 3$ juveniles) for dark-adapted *Acropora millepora* juveniles hosting *Symbiodinium* C1 and *Symbiodinium* D. Asterisks (*) indicate significant differences ($P < 0.05$) between DCMU treatments compared to the control (0 µg l^{-1}) and inequalities (≠) indicate differences between *Symbiodinium* C1 and D

dinoflagellates, were detected with a molar ratio of 1:0.3:0.5 within the control juveniles. Diadinoxanthin was the major xanthophyll carotenoid within coral symbionts, along with low concentrations of diatoxanthin. No differences in light harvesting and xanthophyll pigments were evident between *Symbiodinium* types in the absence of DCMU (Fig. 4a and b) (Two-way ANOVA LHC symbiont type: $F_{1,42} = 0.25$, $P = 0.6$, DCMU: $F_{2,42} = 3.14$ $P = 0.05$, symbiont*DCMU interaction: $F_{2,42} = 2.54$, $P = 0.09$, Fishers LSD, $P = 0.04$. Two-way ANOVA xanthophylls, symbiont type: $F_{1,42} = 0.52$, $P = 0.5$, DCMU: $F_{2,42} = 2.76$, $P = 0.07$, symbiont*DCMU interaction: $F_{2,42} = 2.91$ $P = 0.07$, Fishers LSD, $P = 0.02$). Severe inhibition of PSII electron transport (10 µg l^{-1} DCMU treatment) for 10 h resulted in significant increases in both the total light harvesting and total xanthophyll pigments (by 108 and 114% respectively) in *Symbiodinium* C1, whereas pigment concentrations did not change in type D symbionts (Fig. 4). While the total light harvesting and xanthophyll pools increased for *Symbiodinium* C1, no changes in pigment ratios were observed for both symbiont types at any of the DCMU concentrations.

Discussion

Distinct patterns in rates of photosynthate (^{14}C) incorporation and RLCs revealed physiological differences between *Symbiodinium* C1 and D when associated with the same host species. *Symbiodinium* C1 exhibited a 121% greater capacity for translocation of photosynthate to *A. millepora* juveniles along with 87% greater relative electron transport through photosystem II under identical environmental conditions. *A. tenuis* and *A. millepora* juveniles in a previous study exhibited 2 to 3 times faster growth rates when associated with *Symbiodinium* C1 compared to those associated with *Symbiodinium* D (Little et al. 2004) at the same field site where juveniles were reared in the present study. The higher growth rate previously found within C1 juveniles (Little et al. 2004) may result from greater translocation of photosynthate by colonies hosting *Symbiodinium* C1, as demonstrated here. The differences in carbon-based energy transfer between symbiont types may provide a competitive advantage to corals associating with *Symbiodinium* C1, particularly during their early life histories, when greater energy investment into rapid tissue and skeletal growth can prevent overgrowth of juveniles by competitors and mortality from grazers (Hughes and Jackson 1985).

The large differences in photosynthate incorporation into host tissue and photosynthetic performance observed between symbiont types in coral hosts of the same parentage highlights the functional influence of symbionts on the nutritional physiology of corals. *Symbiodinium* clade has also been shown to affect photosynthate transfer within the sea anemone, *Condylactis gigantea* (Loram et al. 2007). Photosynthetically fixed ^{14}C incorporation into lipids and low molecular weight amino acids within the host tissue of anemones was significantly greater for those hosting clade A symbionts than B symbionts (Loram et al. 2007). Freshly isolated *Symbiodinium* C was also recently

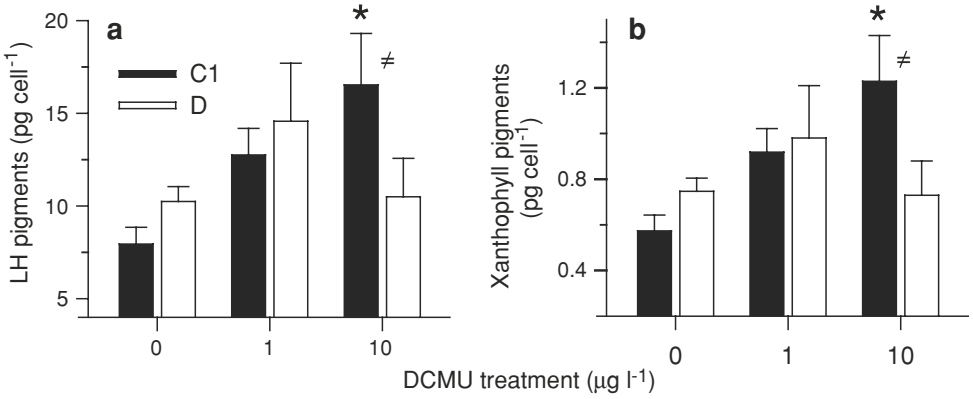

Fig. 4 Pigment concentrations of *Symbiodinium* C1 and D. Pigments of *Symbiodinium* C1 and D within juveniles of *Acropora millepora* exposed to three different DCMU (diuron) treatments (0, 1 and 10 µg l^{-1}). **a** Concentrations of total light harvesting pigments (LH), comprising chlorophyll a and c_2 and peridinin (pg $cell^{-1}$). **b** Concentrations of xanthophyll pigments, diadinoxanthin and diatoxanthin (pg $cell^{-1}$). $n = 8$ juveniles (mean ± SE). Asterisks (*) indicate significant differences ($P < 0.05$) between DCMU treatment compared to the control (0 µg l^{-1}) and inequalities (≠) indicate differences between *Symbiodinium* C1 and D

shown to fix higher amounts of carbon in the presence of synthetic host factor compared to *Symbiodinium* A (Stat et al. 2008). The efficiency of photosynthate transfer to the host and specific molecular allocation of fixed carbon have not been compared between symbiont types in corals, but are likely to contribute to the large (121%) differences in total photosynthate incorporation observed in the present study. Differences in the performance of *Symbiodinium* types are likely to be widespread in anthozoan endosymbioses and an important feature in the nutritional economy of reef corals.

Although both ^{14}C uptake and $rETR_{MAX}$ were significantly higher for *Symbiodinium* C1 corals, the actual relationship between carbon fixation and symbiont performance is not likely to be simple due to the occurrence of both assimilatory and non-assimilatory electron flow (Jones et al. 1998). Hoogenboom et al. (2006) demonstrated that the saturation of O_2 evolution in corals can occur at lower PAR than rETR saturation, indicating non-assimilatory electron flow through PSII. Further, ^{14}C uptake in the present study was measured in the host tissue and not in the symbiont and translocation to the host tissue rather than total fixation of ^{14}C may differ between the symbiont types. Additional respirometry and ^{14}C fixation (and translocation) experiments over a wider range of PAR, in conjunction with detailed quenching analysis, are required to fully appreciate the complex relationship between the photosynthetic performance of different symbiont types and energetic benefit to the coral host.

The reduction of $rETR_{MAX}$ and ^{14}C in both symbiont types by 10 µg l^{-1} DCMU to indistinguishable levels suggests that, under these conditions of severe electron transport inhibition and at this level of PAR, C1 colonies would receive the same ^{14}C (energy) allocation from their symbionts as D-colonies. However, *Symbiodinium* C1 suffered a greater proportional drop since, in the absence of DCMU, its $rETR_{MAX}$ was 85% greater than that observed for *Symbiodinium* D (Fig. 1b). The relative reduction in ^{14}C incorporation into host tissues in the presence of DCMU was also greater for C1 colonies (Fig. 1a). Therefore, C1 colonies may lose their potential for more rapid growth and any competitive advantage over D-colonies at the juvenile stage under stressful conditions that limit electron transport. While future experiments under a full range of irradiance conditions are required to confirm differences in physiology between *Symbiodinium* C1 and D, the present results reveal how PSII herbicide exposures might affect corals differently, depending on the symbiont types they harbour.

The identical maximum quantum yields (F_v/F_m) in dark-adapted samples for each symbiont type in the absence of DCMU (Fig. 3) indicates similar efficiencies of excitation energy capture by PSII for each symbiont type (Genty et al. 1989). This result is consistent with other reports, which show similar F_v/F_m for *Symbiodinium* within different coral species at the same depth when measured in the absence of stress (Rowan 2004; Berkelmans and van Oppen 2006; Robison and Warner 2006; Warner et al. 2006). Reductions in F_v/F_m at 10 µg l^{-1} DCMU were similar for both symbiont types, indicating equivalent level PSII photoinactivation (Genty et al. 1989). It is plausible that longer exposure to DCMU and exposures at higher irradiances might reveal differences in photoinactivation to PSII between symbiont types, analogous to that observed during longer exposure experiments to thermal stress. For example, far greater reductions in F_v/F_m were observed in C2 symbionts in adult *A. millepora* exposed to elevated seawater temperatures than for the more thermally tolerant D symbionts in the same species, indicating greater PSII photoinactivation of C2 symbionts (Berkelmans and van Oppen 2006). *Pocillopora* spp. hosting *Symbiodinium* C exhibited identical F_v/F_m

values and higher levels of photosynthesis (measured as oxygen flux) at 28°C than *Symbiodinium* D, but suffered a greater decline in photosynthetic performance and more photoinactivation (damage) to PSII at elevated temperatures (Rowan 2004).

No differences in light harvesting and xanthophyll pigments were evident between *Symbiodinium* types in the absence of DCMU (Fig. 4). Thus, the greater incorporation of ^{14}C labelled photosynthates and the observed higher $rETR_{MAX}$ of *Symbiodinium* C1 in control treatments did not result from higher concentrations of light harvesting pigments. In order to fully explain the functional mechanism leading to the differences observed between the two symbiont types, *Symbiodinium* C1 and D, future studies need to be conducted to determine which photosynthetic processes limit the photosynthetic performance of *Symbiodinium* D. Severe inhibition of PSII electron transport (10 μg l^{-1} DCMU treatment) for 10 h resulted in significant increases in both the light harvesting and xanthophyll pigments (by 108 and 114%, respectively) in *Symbiodinium* C1, whereas pigment concentrations did not change in type D symbionts (Fig. 4). Under these exposures, a large proportion of the PSII reaction centres are inactive due to DCMU inhibiting reduction of the plastoquinone pool, therefore mimicking the physiological transition to low light (Escoubas et al. 1995). It is likely that rapid pigment biosynthesis was stimulated in *Symbiodinium* C1 in response to the reduced electron transport and the effects of DCMU on the redox state of the plastoquinone pool. This type of rapid pigment biosynthesis was reported for high light acclimated green alga *Dunaliella salina* following a 12-h transition to low illumination (Masuda et al. 2002) and following 12-h exposure of *Dunaliella tertiolecta* to DCMU (Escoubas et al. 1995). It is unclear why the light harvesting pigments of *Symbiodinium* D did not increase following photoinactivation by DCMU. Further experiments that subject *Symbiodinium* spp. to DCMU under both low and high irradiances are needed to confirm this photoacclimatory response mechanism in corals.

This study identifies the potential energetic consequences to the coral host of association with genetically distinct types of the algal endosymbiont, *Symbiodinium*, that differ intrinsically in their photophysiology. It demonstrated that photosynthetic performance, as measured by photosynthate incorporation (carbon-based energy) and PSII relative electron transport, was significantly greater within *Symbiodinium* C1 compared to *Symbiodinium* D, which might explain the influence that symbiont type has previously been shown to have on juvenile coral growth (Little et al. 2004). The results suggest that a physiological trade-off between stress (thermal, irradiance or contamination) tolerance and photosynthetic performance underlies the growth advantage gained by corals when associated with *Symbiodinium* C1 in early life history (Little et al. 2004). As the community structure of coral reefs shift in response to global climate change and water quality impacts (Hughes et al. 2003), opportunistic corals harbouring symbionts that enable maximum rates of growth may similarly gain a competitive advantage. A shift in an *A. millepora* population from dominance by *Symbiodinium* C to D, a symbiont commonly associated with high-temperature, was identified in the southern GBR following a severe bleaching events (Jones et al. 2008). Further, a similar shift was observed in *Pocillopora* spp. from Pacific Panama, and was implied for a range of coral species in the Persian (Arabian) Gulf and Kenya following episodes of severe, high temperature bleaching, a response which may increase the resistance of coral reefs to future bleaching (Baker et al. 2004). While such shifts may increase the survival of corals under warming conditions (Berkelmans and van Oppen 2006), non-elevated sea temperatures between bleaching events may favour corals that harbour more photosynthetically active *Symbiodinium* types such as C1 (ITS1, used in this study) and enhance the growth rate of coral colonies that maintain stable symbiotic relationships observed following bleaching events (Thornhill et al. 2006). These findings reveal underlying photophysiological differences between genetically distinct algal endosymbionts that advance the understanding of the dynamic relationship between the coral host and its symbiotic partner.

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