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Differential effects of ocean acidification on growth and photosynthesis among phylotypes of *Symbiodinium* (Dinophyceae)

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Abstract

We investigated the effect of elevated partial pressure of CO_2 (pCO_2) on the photosynthesis and growth of four phylotypes (ITS2 types A1, A13, A2, and B1) from the genus *Symbiodinium*, a diverse dinoflagellate group that is important, both free-living and in symbiosis, for the viability of cnidarians and is thus a potentially important model dinoflagellate group. The response of *Symbiodinium* to an elevated pCO_2 was phylotype-specific. Phylotypes A1 and B1 were largely unaffected by a doubling in pCO_2; in contrast, the growth rate of A13 and the photosynthetic capacity of A2 both increased by ~ 60%. In no case was there an effect of ocean acidification (OA) upon respiration (dark- or light-dependent) for any of the phylotypes examined. Our observations suggest that OA might preferentially select among free-living populations of *Symbiodinium,* with implications for future symbioses that rely on algal acquisition from the environment (i.e., horizontal transmission). Furthermore, the carbon environment within the host could differentially affect the physiology of different *Symbiodinium* phylotypes. The range of responses we observed also highlights that the choice of species is an important consideration in OA research and that further investigation across phylogenetic diversity, for both the direction of effect and the underlying mechanism(s) involved, is warranted.

Ocean acidification (OA) describes the decline in pH of the ocean as a consequence of increased absorption of anthropogenically released CO_2 from the atmosphere (Caldeira and Wickett 2003). Key to OA is a complex change in seawater carbon chemistry. In particular, increases in bicarbonate ion (HCO_3^-) concentration and decreases in carbonate ion (CO_3^{2-}) concentration will occur as the partial pressure of CO_2 (pCO_2) increases. Algal photosynthesis is directly dependent on the concentration of CO_2 at the site of carbon fixation and, as such, it has been suggested that OA could have significant implications for marine primary productivity (Rost et al. 2008). For example, the productivity of certain species of marine phytoplankton has been predicted to increase by up to 40% if atmospheric pCO_2 were to double (Schippers et al. 2004).

To date, a wide range of physiological responses by marine phytoplankton to OA have been reported, notably increases of growth rate (Kim et al. 2006; Levitan et al. 2007), photosynthesis and carbon fixation rate (Hutchins et al. 2007; Levitan et al. 2007; Fu et al. 2008), cellular carbon and nitrogen content (Leonardos and Geider 2005; Fu et al. 2007; Levitan et al. 2007), and production of dissolved organic carbon (DOC; Engel et al. 2005), as well as changes to pigment composition (Fu et al. 2007; Levitan et al. 2007). Not surprisingly, these studies have focused primarily on species from taxonomic groups with fundamental biogeochemical roles, (i.e., highly productive diatoms and coccolithophores, as well as nitrogen-fixing cyanobacteria). These groups appear to be physiologically distinct, especially with regard to their mode of inorganic carbon acquisition (Tortell 2000), perhaps explaining the wide variation in their sensitivity to OA. However, taxonomic differences in physiology cannot fully explain this variation because studies that examine different species (or indeed different genotypes of the same species) within these taxonomic groups also appear to yield contrasting responses to OA (Fu et al. 2007). Phytoplankton genotypes of the same genus, and even of the same species, are well known to be physiologically distinct (Robison and Warner 2006; Suggett et al. 2007), and genotype-specific responses to OA are, thus, to be expected (Tortell et al. 2002). This raises the important question as to whether OA will preferentially select not only between the different phytoplankton groups but also within closely related phytoplankton genotypes. Although both scenarios carry inherent implications toward shifting ecosystem function, it is currently impossible to answer from the current array of data sets because methodological differences between studies further confound interpretation of alternative OA responses (reviewed in Rost et al. 2008; Hurd et al. 2009); as yet, only a single study has examined the effect of OA on the functionality of closely related genotypes (Hutchins et al. 2007).

Dinoflagellates are a diverse taxon and represent a major component of phytoplankton communities in all oceans (Taylor et al. 2008); yet, studies that directly examine the effects of OA on dinoflagellates are surprisingly limited (Fu et al. 2008). As a group, dinoflagellates are unique in that they are the only eukaryotic algal taxon currently known to possess form II Ribulose bisphosphate carboxylase oxygenase (Rubisco; Whitney et al. 1995). This form of Rubisco, typically associated with anaerobic proteobacteria, has a particularly low specificity for CO_2—much lower than that of form I Rubisco found in the other key phytoplankton taxa (reviewed in Tortell 2000). As such, the Rubisco of dinoflagellates would be considerably under-

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saturated with respect to CO_2 under present day pCO_2 levels without the operation of an efficient carbon concentrating mechanism (CCM; Leggat et al. 1999; Tortell 2000), which incurs both energetic and metabolic costs. However, further rises in CO_2 will increase the capacity for carbon acquisition by passive diffusion of CO_2; as such, OA should reduce the need for CCM activity, thereby alleviating some of the resource costs associated with carbon acquisition (Beardall and Giordano 2002).

Dinoflagellates play extremely important ecological roles in aquatic environments. For example, the ability of some species to form toxic red tides significantly affects coastal ecology and economics (Anderson et al. 2002). The dinoflagellate genus *Symbiodinium* is widely known for its symbiotic relationship with a number of marine invertebrates. In particular, its symbiosis with reef-building corals is essential for the persistence, resilience, and distribution of coral reef ecosystems (Iglesias-Prieto et al. 2004; Berkelmans and van Oppen 2006). *Symbiodinium* is not an exclusively symbiotic genus and can also exist as free-living cells, although little is known about this aspect of their ecology (Manning and Gates 2008). The *Symbiodinium* genus is taxonomically diverse, with hundreds of phylotypes, or "species," thought to exist across the world's oceans (Coffroth and Santos 2005). These phylotypes of *Symbiodinium* are able to occupy different environmental niches, most likely as a result of their substantial phylotype-specific range of physiological responses to environmental change (Hennige et al. 2009) and stress (Robison and Warner 2006; Suggett et al. 2008; Ragni et al. 2010), as well as the generalist vs. specialist modes of specificity with their cnidarian hosts (Finney et al. 2010; LaJeunesse et al. 2010). *Symbiodinium* is, thus, an important model organism to examine genotypic-specific responses to environmental change; however, their potential sensitivity to OA is currently unknown. Examining the response of different *Symbiodinium* phylotypes to OA could not only provide insight as to how dinoflagellates as a group might respond to OA but ultimately hold implications for specific associations with reef-building corals.

Here, we examined the response of four different phylotypes of *Symbiodinium* to OA by experimentally examining their physiological responses to elevated pCO_2 levels representative of the expected rise for this century (Solomon et al. 2007). Given the low specificity of form II Rubisco to *Symbiodinium* and the requirement of a CCM to support photosynthesis under present day pCO_2, we hypothesized that the increase in CO_2 availability associated with OA would affect changes in resource allocation away from carbon acquisition and into other important metabolic processes, such as growth and cell synthesis.

Methods

Phylotype selection—We focused on four phylotypes of *Symbiodinium* that have previously been characterized for their photobiological (Robison and Warner 2006; Hennige et al. 2009) and stress tolerance (Robison and Warner 2006; Suggett et al. 2008; Ragni et al. 2010) characteristics: three phylotypes from clade A (A1, A13, and A2) and one phylotype from clade B (B1). These phylotypes have previously been isolated from reef-building corals, as well as other host types, including jellyfish, soft corals, bivalves, gorgonian sea fans, and sea anemones (Freudenthal 1962; LaJeunesse 2001; Rogers and Davis 2006; Santos and LaJeunesse 2009), and identified in hospite across a range of geographic locations—including the Caribbean; western, eastern, and central Pacific; and the Red sea—apart from phylotype A13 (formally designated as A1.1 in Robison and Warner [2006] and LaJeunesse [2001], but now reclassified as A13 in LaJeunesse et al. 2009) which, to date, has only been isolated in the Caribbean (LaJeunesse 2001). Few studies have been published regarding the diversity of free-living populations of *Symbiodinium* (Coffroth et al. 2006; Manning and Gates 2008; Pochon et al. 2010); however, of the phylotypes used in this study, both A1 and B1 have previously been cultured from environmental water samples taken from Caribbean reefs (Coffroth et al. 2006). The genetic signature of B1 has also been identified in water samples from both the Caribbean and the eastern Pacific (Manning and Gates 2008). There are a number of difficulties with identifying and estimating the diversity of free-living populations of *Symbiodinium*—namely, the low occurrence within a single water sample and the sampling effort that would be required to confidently state that a fully representative community has been characterized—so it is perhaps unsurprising that not all *Symbiodinium* isolates have been found to be free-living within the water column. However, the fact remains that the isolates used in this study have been grown and maintained in culture for at least 5 yr, which demonstrates that all four phylotypes are capable of surviving and reproducing ex hospite and are thus not obligate symbionts.

Experimental design—Continuous cultures (3-liter volume) of the selected four phylotypes of *Symbiodinium* were grown at 26°C under a photon flux density (PFD) of $\sim$ 350 μmol photons m^{-2} s^{-1} (measured with a 4π Biospherical Instruments, Quantum Scalar Laboratory) on a 14 : 10 h light : dark cycle. Phylotypes were grown in nutrient-replete ASP-8A artificial seawater (Provasoli et al. 1957), modified to exclude the tris(hydroxymethyl)aminomethane buffer that would confound adjustments of the inorganic carbon chemistry by pH modification. Dried sodium bicarbonate was added to the media to a final total carbon alkalinity (TA_C) of $\sim$ 2300 μEq kg^{-1} seawater (SW). Fresh medium was continuously supplied to each culture vessel from dedicated 20-liter reservoirs, through 0.2-μm pore-sized filters (Polycap 36AS, Whatman Filters), at a rate controlled to match the individual growth rates of each phylotype. Cultures were gently stirred continuously to minimize the settling of algal cells. The cultures were first grown at a pCO_2 representative of present day levels (40 Pa, equivalent to $\sim$ 390 ppmv). The pCO_2 within all cultures was then raised by $\sim$ 10.1 Pa increments every 96 h, until it was approximately at a level predicted for the year 2100 (81 Pa, equivalent to $\sim$ 800 ppmv; Solomon et al. 2007). The pCO_2 was continuously adjusted to the desired levels following the approach described below. The cultures were acclimated into steady-state growth over $\sim$ 2 weeks ($>$ 10 generations), at

both present day (control) and elevated (treatment) pCO_2 conditions (Table 1), before sampling.

pH-stat system—The treatment pCO_2 levels used in this experiment were maintained by manipulating the pH (total hydrogen scale) of the cultures. The pH in each of the cultures was recorded at 30-s intervals with a Ross Ultra pH electrode (model 8103BNUWP, Thermo Fisher Scientific), monitored by computer software (KSgrowstat, developed by K. Oxborough, Chelsea Technologies Inc.) that controlled the aeration of the cultures. The system was set to enrich the aeration into a culture with a short pulse of CO_2 (99.8% concentration) when the software detected a pH (> 0.01 pH units) above the target pH, which was set according to calculations based on the desired pCO_2 and TA_C (2300 μEq kg^{-1} SW) using CO2SYS (Pierrot et al. 2006). If the pH fell below the target pH, the airflow would be redirected through soda lime to remove CO_2 before entering the culture. Under the 40 Pa pCO_2 treatment, the pH was maintained at 8.05, and under the 81 Pa pCO_2 treatment, the pH was maintained at 7.79.

Measurements of total CO_2 (TCO_2, μmol CO_2 kg^{-1} SW) were conducted weekly throughout the course of the experiment to characterize the variability of carbonate chemistry within the treatments. Measurements of TCO_2 were performed on the filtrate of culture samples passed through glass fiber filters (0.7 μm pore size, Fisher Scientific). The inorganic carbon content (TCO_2) of the filtrate was measured on a carbon analyzer (Shimadzu Total Organic Carbon Analyzer). The carbon chemistry of the culture media at the time of sampling could then be fully characterized by entering the values of TCO_2 and pH into the CO2SYS software. The true average pCO_2 values for the 40 and 81 Pa treatments are summarized in Table 1. The discrepancies between the target and actual pCO_2 attained were largely driven by small variability in the TA_C of the media fed into the culture vessels. This discrepancy was largest for the high pCO_2 treatment as a result of a calibration error (underestimation in the carbon standards used for the calibration) in the TCO_2 data which, when corrected for, increased the pCO_2 even further.

Cell volume and growth rate—Daily cell counts were obtained microscopically with a Neubauer hemocytometer (Fisher Scientific) to monitor the growth rates and health of the cultures. Additional measurements of cell number and volume were performed on a particle counter and size analyzer (Z2, Beckman Coulter) to yield the volume per cell (μm^3 $cell^{-1}$). Because of the nature of continuous culturing, changes in cell number were further corrected for the dilution rate and sampling volumes to provide a true measure of growth rate (Eq. 1),

$$\mu = \frac{\ln\left(\frac{C_{t+\Delta t} \times \mathrm{Vol}_{t+\Delta t}}{C_t \times \mathrm{Vol}_t}\right) + \left(\frac{\mathrm{Vol}_{\mathrm{waste}}}{\mathrm{Vol}_{\mathrm{vessel}}}\right)}{\Delta t}, \quad (1)$$

where t = time (days), Δt = time interval between sampling, C = concentration of cells (cells mL^{-1}), Vol = volume of medium (mL) in the culture vessel at time of sampling, $\mathrm{Vol}_{\mathrm{waste}}$ = volume of waste medium or medium outflow (mL) generated by the continuous dilution of the culture during the time period between sampling, and $\mathrm{Vol}_{\mathrm{vessel}}$ = maximum volume of medium in the vessel (mL). The first term,

$$\ln\left(\frac{C_{t+\Delta t} \times \mathrm{Vol}_{t+\Delta t}}{C_t \times \mathrm{Vol}_t}\right),$$

describes the change in cell number for the total population during the sampling interval, and the second term,

$$\left(\frac{\mathrm{Vol}_{\mathrm{waste}}}{\mathrm{Vol}_{\mathrm{vessel}}}\right),$$

describes the dilution rate. The specific growth rate (μ, d^{-1}) was calculated as a change in cell concentration per day (d^{-1}). After the daily cell count, the dilution rate for each culture vessel was adjusted to maintain a constant cell density within each culture. So as to maintain an approximately similar optical density within the cultures of each phylotype, the culture vessels of A1 and A13 were maintained at a mean cell density of $\sim$ 100,000 cells mL^{-1}, A2 at $\sim$ 60,000 cells mL^{-1}, and B1 at $\sim$ 140,000 cells mL^{-1}. These cell densities for each phylotype did not differ significantly between the two pCO_2 treatments (data not shown).

Chlorophyll a—Aliquots of $\sim$ 50–100 mL were taken from each culture and filtered through glass fiber filters. Each filter was snap-frozen in liquid nitrogen and stored at -80°C for analysis at a later date. To extract the chlorophyll (Chl), each filter was ground in 5 mL of 100% methanol and kept in darkness at -20°C overnight. The samples were then centrifuged at 4500 revolutions per minute (rpm) for 5 minutes. The absorption of the supernatants between 600 and 760 nm were read with a spectrophotometer (U-3000, Hitachi High Technologies). Chl *a* concentration was quantified with the use of the equations of Ritchie (2006) and normalized to cell number.

Particulate organic carbon—Aliquots (100 mL) of culture were filtered onto precombusted glass fiber filters and stored at -80°C for subsequent analysis for cellular particulate organic carbon (POC) content. Inorganic carbonates were removed from the filters by acidification with $\sim$ 15% hydrochloric acid. Each filter was dried at 60°C for 48 h, packaged in precombusted aluminum foil (Hilton et al. 1986), and analyzed on a Thermo Finnegan flash EA1112 elemental analyzer with acetanilide as the calibration standard.

Oxygen production—The photosynthetic light response of each culture was characterized with the oxygen-18 isotope ($^{18}O_2$) dilution technique (Kana 1990) and measured on a Membrane-Inlet Mass Spectrometer (MIMS; model QMG 422 Pfeiffer Vacuum) as described previously (Suggett et al. 2008). Briefly, samples were first concentrated by gravity filtration onto membrane filters (3 μm pore size, Nuclepore Track-Etch Membrane) followed by resuspension into a smaller volume of media. Nitrogen gas was bubbled through 200 mL of concentrated sample,

contained within a sealed syringe, to decrease the concentration of dissolved $^{16}O_2$ within the sample. Any remaining headspace within the syringe was then removed, and 1.5 mL of $^{18}O_2$ gas was injected into the sample. The sample was then gently stirred in a low-light environment for 20 min to allow even diffusion of $^{18}O_2$. Following this mixing period, the headspace gas was once again removed from the syringe and the sample was rapidly decanted into 6-mL glass cuvettes. Each cuvette was completely filled with sample before being sealed with glass stoppers, thereby preventing gas exchange with the atmosphere.

Ten cuvettes were immediately placed within a custom-built linear incubator illuminated via a block of blue light–emitting diodes (LEDs, peak emission ~ 470 nm) to expose the cuvettes to a gradient of photon flux densities (PFDs), from ~ 4 to 650 μmol photons m^{-2} s^{-1}. An additional cuvette was incubated in complete darkness, and three other cuvettes were immediately sampled by the MIMS to provide a measurement of the initial concentrations of $^{16}O_2$ and $^{18}O_2$ within the sample. After 25 min, the cuvettes were sequentially analyzed by the MIMS in continuous succession, from the highest to lowest PFD exposure. The concentrations of $^{16}O_2$ and $^{18}O_2$, within each cuvette, were measured and recorded. The membrane inlet was maintained at the growth temperature of the cultures (26°C) with the use of a water bath, allowing samples to be analyzed rapidly at a constant temperature. Regular standards ($n = 3$) of air-equilibrated deionized water, at a known temperature and pressure, were also analyzed. A zero standard for $^{16}O_2$ was prepared from a filtered sample treated with sodium dithionite. The rates of gross O_2 evolution and consumption (and hence also net O_2 production) for each sample along the light gradient was then determined by equations described previously (Kana 1990) and normalized to cell concentration, cell volume, or Chl *a* concentration.

Photosynthetically available radiation (PAR) measured from the blue LEDs used to generate the MIMS light–response curves was adjusted to match the spectral quality of white fluorescent tubes used for originally growing each culture. First, an effective rate of light absorption ($\bar{a}$) was determined for each *Symbiodinium* phylotype and treatment as

$$\bar{a} = \frac{\sum_{380}^{750}[E(\lambda) \times a(\lambda)]}{\sum_{380}^{750} E(\lambda)}, \quad (2)$$

where $a(\lambda)$ is the absorption spectrum of the alga and $E(\lambda)$ is the emission spectra of the light source. Values of $E(\lambda)$ and $a(\lambda)$ were measured between 40 and 750 nm with a spectroradiometer (SR-9910a, Macam) and a spectrophotometer (U-3000, Hitachi High Technologies), respectively, following Suggett et al. (2008). Values for $\bar{a}$ were determined on the basis of the spectral output for the blue LEDs ($\bar{a}^{LED}$) and for the white fluorescent tubes ($\bar{a}^{growth}$) and thus used to adjust the absolute PFD ($\overline{PFD}$, μmol photons m^{-2} s^{-1}) as

$$\overline{PFD} = PFD\left(\frac{\bar{a}^{LED}}{\bar{a}^{growth}}\right). \quad (3)$$

The light-limited photosynthetic efficiency (initial slope, α)

Table 1. Changes to the inorganic carbon chemistry of oceans in tropical regions expected for the turn of the century, and the actual inorganic carbon chemistry attained during the experiment for each culture and treatment. Scenario values are calculated assuming a total carbon alkalinity of 2300 μEq kg^{-1} SW, a salinity of 35, and an average sea surface temperature of 26°C. Predicted changes in pCO_2 are taken from the IS29a "business as usual" scenario (Solomon et al. 2007). The true values of TA_C within the cultures were calculated from direct measurements of DIC and pH in discrete samples taken from each of the cultures ($n = 15$). The mean values (± SE) of inorganic carbon chemistry were then calculated using the true TA_C and the pH maintained (to within ± 0.01 pH units) under each treatment by the pH-stat system (i.e., pH 8.05 for 40 Pa pCO_2 and pH 7.79 for 81 Pa pCO_2; *see* Methods). Values in parentheses are the standard error of the mean. All calculations of inorganic carbon chemistry were performed with the CO2SYS program developed by Pierrot et al. (2006) with the constants taken from Millero et al. (2006). Ω_{calc} and Ω_{arag} are the saturation states for calcite and aragonite, respectively, and both have dimensionless units.

			Ambient pCO_2 treatment				Elevated pCO_2 treatment			
	Present day	2100	A1	A2	A13	B1	A1	A2	A13	B1
pCO_2 (Pa)	~40	~81	41.0(0.3)	42.4(0.3)	41.0(0.3)	40.3(0.3)	87.2(0.3)	88.1(0.6)	91.9(0.5)	85.4(0.4)
pH (total hydrogen scale)	8.05	7.79	8.05	8.05	8.05	8.05	7.79	7.79	7.79	7.79
TA_c (μEq kg^{-1} SW)	2300	2300	2357(17)	2430(19)	2355(15)	2318(15)	2460(10)	2486(17)	2588(13)	2411(12)
TCO_2 (μmol kg^{-1} SW)	1992	2127	2043(16)	2110(17)	2042(14)	2008(14)	2279(9)	2303(16)	2401(13)	2232(12)
$[CO_2]$ (μmol kg^{-1} SW)	10.9	22.2	11.2(0.1)	11.5(0.1)	11.2(0.1)	11.0(0.1)	23.8(0.1)	24.0(0.2)	25.0(0.1)	23.3(0.1)
$[HCO_3^-]$ (μmol kg^{-1} SW)	1763	1971	1808(14)	1867(15)	1807(12)	1778(12)	2112(8)	2134(15)	2225(12)	2069(11)
$[CO_3^{2-}]$ (μmol kg^{-1} SW)	218	134	224(2)	231(2)	223(2)	220(2)	144(1)	145(1)	151(1)	141(1)
Ω_{calc}	5.26	3.48	5.39(0.04)	5.57(0.05)	5.39(0.04)	5.30(0.04)	3.46(0.01)	3.50(0.02)	3.65(0.02)	3.39(0.02)
Ω_{arag}	3.23	2.14	3.56(0.03)	3.68(0.03)	3.56(0.02)	3.50(0.02)	2.29(0.01)	2.31(0.02)	2.41(0.01)	2.24(0.01)

and the light-saturated photosynthetic capacity (maximum rate of oxygen evolution, P^{max}) of gross photosynthesis were determined for each light–response curve, generated by the MIMS, by fitting a modified version of the model of Platt et al. (1980) with SigmaPlot 10.0 curve-fitting software,

$$P^B = P_S^B \times \left(1 - e^{\left(-\alpha^B \overline{PFD}\right)/P_S^B}\right) \times \left(e^{\left(-\beta^B \overline{PFD}\right)/P_S^B}\right), \quad (4)$$

where

$$P^{max} = P_S^B \times \left(\frac{\alpha}{\alpha+\beta}\right) \times \left(\frac{\beta}{\alpha+\beta}\right)^{\beta/\alpha}$$

describes the reduction in photosynthetic rate at high irradiance (photoinhibition, [fmol O_2 cell^{-1} h^{-1}] [μmol photons m^{-2} s^{-1}]$^{-1}$). P^{max} (fmol O_2 cell^{-1} h^{-1}) and α ([fmol O_2 cell^{-1} h^{-1}] [μmol photons m^{-2} s^{-1}]$^{-1}$) are normalized to cell number. The light saturation PFD (E_k) was calculated as P^{max}/α (μmol photons m^{-2} s^{-1}). Oxygen consumption in the dark (respiration) and maximum oxygen consumption in the light (fmol O_2 cell^{-1} h^{-1}) were calculated from PFDs corresponding to P^{max} (Suggett et al. 2008).

Statistical analysis—The dataset for each phylotype was analyzed separately with an independent samples *t*-test to compare the effect of pCO_2 (40 Pa vs. 81 Pa) on growth, cell volume, Chl *a* and POC concentration, and photosynthesis. Additional one-way analysis of variance (ANOVA) statistical tests were used to compare the four phylotypes when grown under ambient conditions (40 Pa pCO_2). Post hoc Tukey's analysis was performed when samples sizes were equal, and Gabriel's pairwise test when samples sizes were slightly different, to identify subsets within the group of phylotypes. The threshold of statistical significance was set at $p < 0.05$. The statistical tests were performed with SPSS statistical analysis software (version 16.0).

Results

Growth rate, cell volume, Chl a*, and POC*—Under steady-state growth at 40 Pa pCO_2, the growth rates (μ, d^{-1}) were similar for all four phylotypes ($\sim$ 0.37–0.39 d^{-1}; Fig. 1a). Of all the phylotypes, only A13 exhibited a significant response to elevated CO_2 (81 Pa pCO_2), with an increase in growth rate of $\sim$ 60% from that at 40 Pa pCO_2 (*t*-test, $t = -4.753$, 43 df, $p = 0.000$). Cellular volumes and Chl *a* concentrations both varied significantly between the phylotypes when grown under steady state at 40 Pa pCO_2 (cell volume data: ANOVA, $F_3 = 177$, $p = 0.000$; Chl *a* concentration data: ANOVA, $F_3 = 61.3$, $p = 0.000$). The smallest and largest cellular volumes (< 240 m^3 and > 740 m^3) and Chl *a* concentrations (< 400 and > 2200 fg Chl *a* cell^{-1}) were observed for phylotypes B1 and A2, respectively (Table 2); however, neither parameter was affected by the increase in pCO_2 (and hence OA) for any phylotype. Cellular POC concentrations (pg POC cell^{-1}) also varied between phylotypes at 40 Pa pCO_2 (Table 2; ANOVA, $F_3 = 67.3$, $p = 0.000$); these concentrations increased with pCO_2 by $\sim$ 20–40% for all phylotypes, except A13 (Table 2); however, these increases were not statistically significant.

Values of POC and Chl *a* per cell were subsequently used to estimate the C : Chl *a* for all phylotypes. Under steady-state growth at 40 Pa pCO_2, C : Chl *a* was highest for phylotype B1 ($\sim$ 120 g C [g Chl *a*]$^{-1}$) and lowest for A1 and A2 ($\sim$ 75 g C [g Chl *a*]$^{-1}$). Interestingly, the C : Chl *a* of A1, A2, and B1 significantly increased under elevated pCO_2 by $\sim$ 30–50%, despite the lack of a significant pCO_2 effect on POC (cell^{-1}) and Chl *a* (cell^{-1}) (Fig. 1b). In this case, the differences in C : Chl *a* between the treatments for each of these three phylotypes could be a propagation of the small variability inherent in the POC (cell^{-1}) and Chl *a* (cell^{-1}) data.

Primary productivity and respiration—Under steady-state growth at 40 Pa pCO_2, values of cell-normalized maximum gross O_2 production (P^{max}) and light-limited photosynthetic efficiency (initial slope, α) were highest for A2 and lowest B1 (Fig. 2a,b). Under the same conditions, the light saturation parameter (E_k) was highest for B1 and lowest in A2 (Fig. 2c). The P^{max}, α, and E_k of A1 and A13 at 40 Pa pCO_2 were statistically the same. Values of cell-normalized P^{max} and α of gross light-dependent O_2 production for A1, B1 and A13 were unaffected by the increase of pCO_2 to 81 Pa pCO_2 (Fig. 2a,b); however, values for both P^{max} and α of A2 increased by $\sim$ 60% (such that E_k remained unchanged; Fig. 2c) from that at 40 Pa pCO_2 (P^{max} data *t*-test, $t = -3.762$, 4 df, $p = 0.020$). It should be noted that the increase of α for A2 was not statistically significant (α data *t*-test, $t = -2.486$, 4 df, $p = 0.068$) as a result of the high variability observed between replicates for cells grown at 81 Pa pCO_2. When normalized to Chl *a*, the increase in P^{max} and α with increased pCO_2 for A2 were not as great and no longer statistically significant (data not shown). Small variability in Chl *a* concentration on the days of sampling might, therefore, be partially responsible for changes in the cell-normalized α in response to OA.

Cell-normalized rates of oxygen consumption in the dark (i.e., mitochondrial respiration) under 40 Pa pCO_2 differed greatly between phylotypes and was greatest for A2 and lowest for B1 (ANOVA, $F_3 = 12.91$, $p = 0.002$). This trend in cell-normalized respiration is probably related to phylotype-specific cell size; when normalized to the cell volume, the rates of respiration (amol O_2 μm^{-3} cell h^{-1}, data not shown) were similar for all phylotypes. Cell-normalized respiration was unaffected by the increased pCO_2 to 81 Pa, and the trend between phylotypes remained the same as at 40 Pa pCO_2 (Table 3). Light-dependent rates of O_2 consumption exhibited a similar pattern to dark respiration (i.e., were highest for phylotype A2 under ambient pCO_2 [ANOVA, $F_3 = 5.320$, $p = 0.026$] and were unaffected by increased pCO_2). In general, the maximum rate of light-enhanced O_2 consumption was $\sim$ 35% (B1), $\sim$ 45% (A1, A13), and $\sim$ 55% (A2) of the gross P^{max} under 40 Pa pCO_2; these percentages did not change for growth under 81 Pa pCO_2, except for A2, in which it decreased to $\sim$ 41% as a consequence of the significant increase in gross P^{max} for this phylotype. Given these various responses of

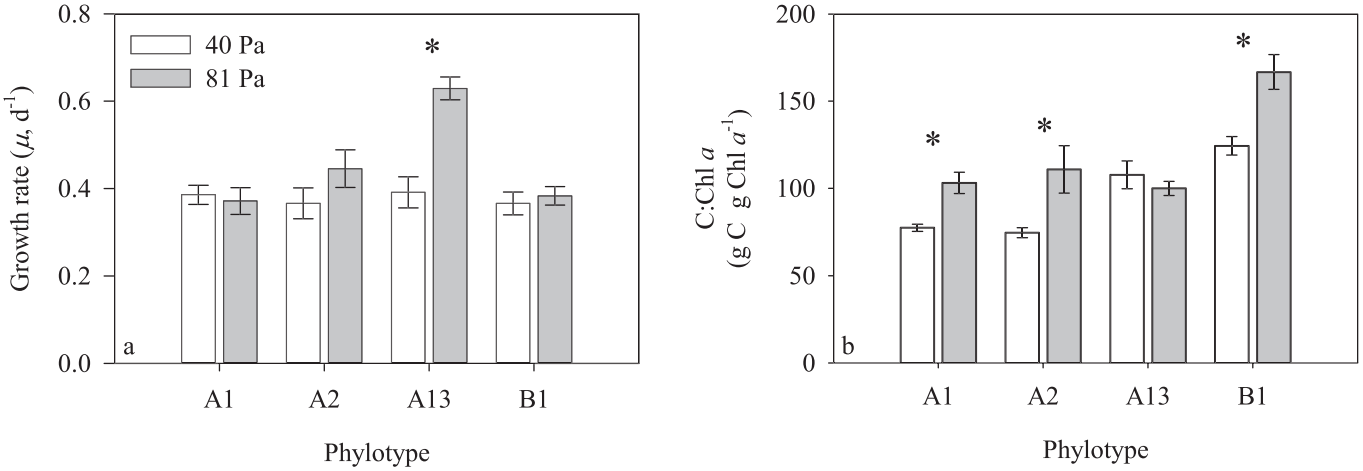

Fig. 1. Mean (± SE) cell-specific (a) growth rate (d^{-1}, $n = 15$) and (b) C : Chl *a* (g C [g Chl *a*]$^{-1}$, $n = 3$) for the four *Symbiodinium* phylotypes. Data shown are from continuous cultures acclimated to both 40 and 81 Pa pCO_2 (Table 1). The use of an asterisk above the data indicates statistical differences between the two treatments for each phylotype.

both maximum gross O_2 production and light-driven O_2 consumption to OA, only the net productivity of A2 was estimated to have increased under elevated pCO_2 (data not shown). Finally, the ratio of maximum light-dependent O_2 consumption to dark respiration (dimensionless), which indicates by how much alternative oxygen consumption pathways other than mitochondrial respiration (e.g., Mehler reaction and mitochondrial alternative oxidase respiration) are increased in the light, was greatest in B1 (~ 3.8) and lowest in A2 (~ 2.3) under both pCO_2 regimes and was not affected by increased pCO_2.

Discussion

The response of *Symbiodinium* phylotypes examined here to simulated conditions of seawater carbon chemistry under present day and turn of the century conditions (i.e., 40 Pa vs. 81 Pa pCO_2; Solomon et al. 2007) was phylotype specific, whereby elevated pCO_2 induced (1) no response at all (phylotypes A1 and B1); (2) increased photosynthetic capacity, without an apparent increase in growth (phylotype A2); or (3) increased growth, without an apparent increase in photosynthetic capacity (phylotype A13).

Our data for phylotypes A1 and B1 are consistent with a general lack of response by marine phytoplankton to elevated pCO_2 levels previously observed (e.g., *Asterionella glacialis*, *Thalassiosira punctigera*, *Coscinodiscus wailesii*, *Phaeodactylum tricornutum*, and *Prochlorococcus* sp.; Burkhardt et al. 1999; Fu et al. 2007). Most likely, these species are solely dependent on HCO_3^- uptake to support photosynthesis, lacking the sensitivity to changes in the environmental concentration of CO_2 and the failure to regulate the CCM according to variability in the environmental $HCO_3^- : CO_2$ ratio (Fig. 3d; Burkhardt et al. 1999; Fu et al. 2007). Consequently, such species are unaffected by OA and unable to benefit from changes in the inorganic carbon pool. In contrast, the positive responses of growth and productivity for A13 and A2, respectively, have implications toward the interspecific adaptability of *Symbiodinium* spp. to carbon use under steady-state growth.

Symbiodinium *photosynthesis and ocean acidification*—The photosynthetic performance of several marine phyto-

Table 2. Mean values (± SE) for cell volume ($n = 3$, μm^3 cell^{-1}), Chl *a* concentration ($n = 7$, fg Chl *a* cell^{-1}), and the ratio of POC to Chl *a* ($n = 3$, g C [g Chl *a*]$^{-1}$). Values are for the four different *Symbiodinium* phylotypes, grown under ambient (40 Pa) and elevated (81 Pa) pCO_2. Values in parentheses are the standard error of the mean. Statistical analysis (*t*-test) of the means of each variable, at ambient and elevated pCO_2, for each phylotype indicated no significant effect of the treatment.

Algal phylotype	pCO_2 (Pa)	Cell volume (μm^3 cell^{-1})	Chl *a* (fg Chl *a* cell^{-1})	POC (pg C cell^{-1})
A1	40	377(5)	1142(58)	91(8)
	81	390(20)	1096(39)	109(3)
A2	40	921(63)	2783(254)	235(17)
	81	744(59)	2712(240)	286(41)
A13	40	433(56)	915(23)	100(4)
	81	351(20)	1016(57)	98(7)
B1	40	234(4)	412(15)	53(5)
	81	233(6)	417(7)	71(4)

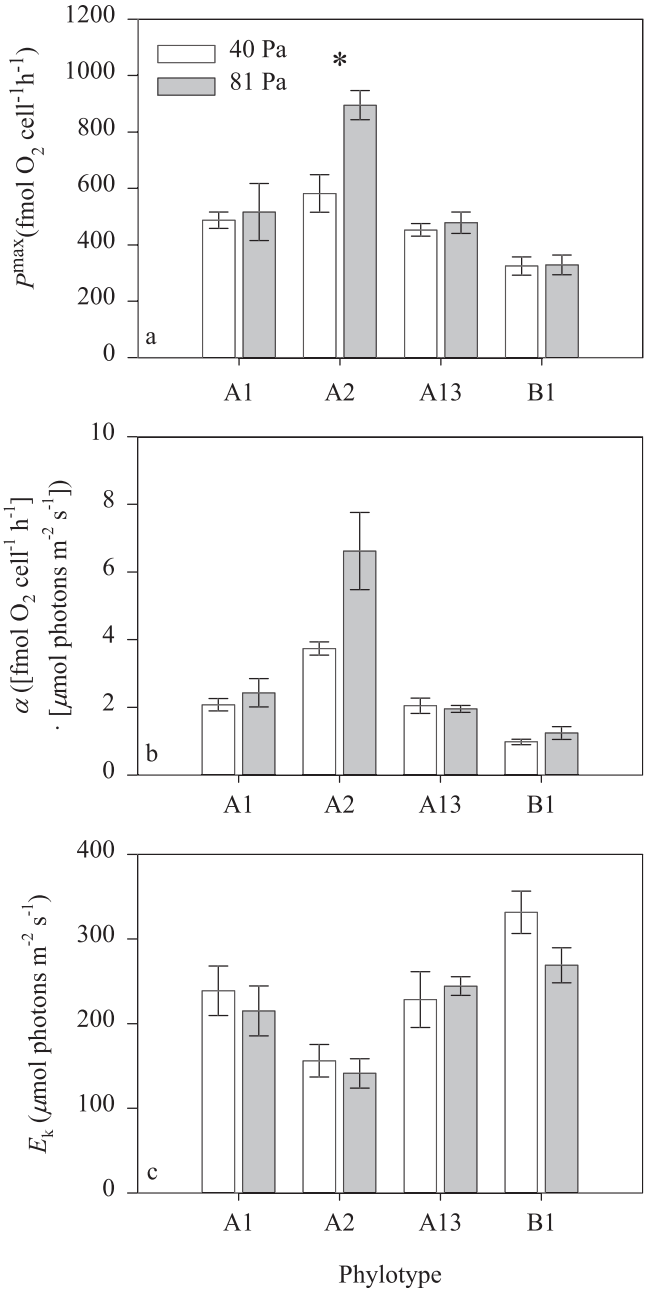

Fig. 2. Mean (± SE, $n = 3$) cell-normalized (a) P^{max} (photosynthetic capacity, fmol O_2 cell^{-1} h^{-1}), (b) α (initial slope or photosynthetic efficiency, [fmol O_2 cell^{-1} h^{-1}] [μmol photons m^{-2} s^{-1}]), and (c) E_k (light saturation PFD, μmol photons m^{-2} s^{-1}) for the four *Symbiodinium* phylotypes. Data shown are from continuous cultures acclimated to both 40 and 81 Pa pCO_2 (Table 1). The use of an asterisk above the data indicates statistical differences between the two treatments for each phylotype.

plankton species has been demonstrated to be carbon-limited under natural concentrations of dissolved inorganic carbon (DIC), inferred from an increase in carbon acquisition and rates of photosynthesis under elevated concentrations of aqueous CO_2 ($CO_{2(aq)}$; e.g., *Gephyrocapsa oceanic* [coccolithophore], several strains of *Emiliania huxleyi* [coccolithophore], *Heterosigma carterae* [raphidophyte], and *Stichococcus bacillaris* [chlorophyte]; Riebesell et al. 2000; Zondervan et al. 2002; Schippers et al. 2004). Similarly, a > 15% stimulation of primary productivity (measured as carbon-14 uptake) has also been observed for natural seawater samples taken from the Atlantic Ocean exposed to elevated $CO_{2(aq)}$ (Hein and Sand-Jensen 1997). Such inorganic carbon limitation is considered to be a consequence of a low affinity for bicarbonate because of the absence of, or possession of a highly inefficient, CCM (Hein and Sand-Jensen 1997; Zondervan et al. 2002; Schippers et al. 2004). Recent OA studies observing increases in the maximum rates of photosynthesis for *Trichodesmium* sp. and *Prorocentrum minimum* under elevated pCO_2 (Hutchins et al. 2007; Fu et al. 2008) further suggest that inorganic carbon limitation of photosynthesis results from a partial dependence on carbon acquisition by diffusive uptake from the variable pool of $CO_{2(aq)}$ (Rost et al. 2008). Our data for phylotype A2 (assuming a constant photosynthetic quotient [PQ] of net O_2 evolution: carbon uptake) is thus consistent with the response of microalgae that lack an efficient CCM.

Table 3. Mean values (± SE; $n = 3$) of oxygen consumption in the dark (respiration, fmol O_2 cell^{-1} h^{-1}) and maximum oxygen consumption in the light (fmol O_2 cell^{-1} h^{-1}). Values are for the four different *Symbiodinium* phylotypes, grown under ambient (40 Pa) and elevated (81 Pa) pCO_2. Values in parentheses are the standard error of the mean. Statistical analysis (*t*-test) of the means of each variable, at ambient and elevated pCO_2, for each phylotype indicated no significant effect of the treatment.

Algal phylotype	pCO2 (Pa)	O2 consumption (fmol O2 cell^{-1} h^{-1})	
		Dark	Light
A1	40	83(15)	232(46)
	81	82(11)	205(40)
A2	40	142(11)	329(44)
	81	169(30)	363(31)
A13	40	60(19)	209(44)
	81	69(5)	206(10)
B1	40	29(4)	110(11)
	81	32(5)	104(16)

Symbiodinium spp. are known to possess a CCM to increase the concentration of CO_2 available to Rubisco and suppress the competing oxygenation pathway (Leggat et al. 1999). CCMs generally differ in their design and efficiency between algal species (Tortell 2000; Giordano et al. 2005); thus, it is conceivable that some *Symbiodinium* phylotypes possess less efficient CCMs (relying to a greater extent on the passive, diffusive uptake of $CO_{2(aq)}$) than others. Our results would suggest that the operation of an inefficient CCM requires A2 to be partially dependent upon the diffusive uptake of $CO_{2(aq)}$ from the external medium (Fig. 3a), a mechanism of carbon acquisition enhanced by the increased availability of $CO_{2(aq)}$ under OA conditions (Schippers et al. 2004; Rost et al. 2008). Although the increase of P^{max} for A2 could also conceivably be explained by a simultaneous increase in the amount of Rubisco present in its chloroplast (which would increase its ability

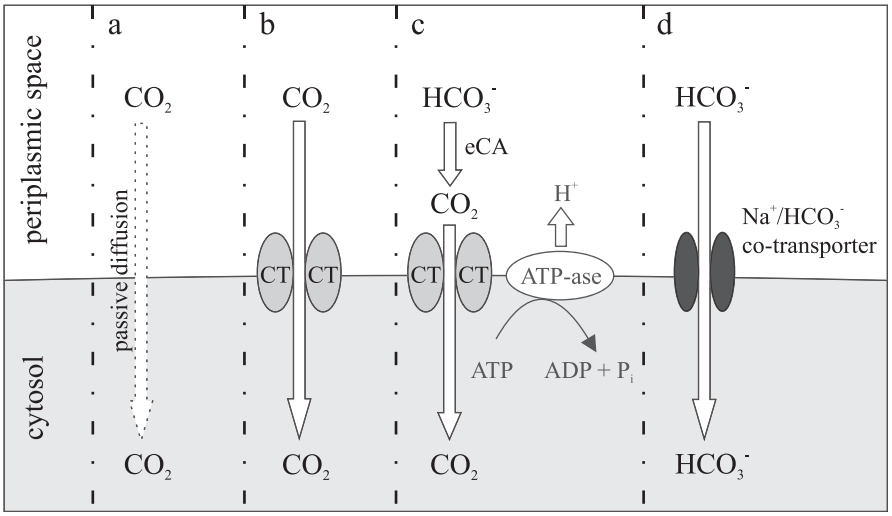

Fig. 3. Schematic diagram of carbon acquisition pathways potentially used by *Symbiodinium* spp. to transport inorganic carbon from the external environment (periplasmic space) into the cytosol of the cell: (a) passive diffusion of CO_2. (b) Active uptake via a CO_2 transporter (CT). (c) Indirect active uptake of HCO_3^- via the catalytic conversion of HCO_3^- to CO_2 by external carbonic anhydrase (eCA). (This pathway can also be facilitated by ATP-ase, which acidifies the immediate area within the periplasmic space). (d) Direct active uptake of HCO_3^- via a Na^+/HCO_3^- co-transporter.

to fix carbon), such a mechanism under OA conditions has not been previously been studied or reported in the literature.

In addition to the elevated pCO_2-induced increase of P^{max}, phylotype A2 also exhibited a proportionate (but not statistically significant) increase of α such that E_k remained constant. Such an acclimatory response of the light-harvesting apparatus following an increase in P^{max}, so as to maintain E_k, is perhaps unsurprising given that E_k is already optimized toward the growth irradiance (which was not changed). Typically, under a variable light environment, photoacclimation optimizes light harvesting to balance dynamically the rate of adenosine triphosphate (ATP) and reductant production to the rate of their consumption in carbon fixation (Falkowksi and Chen 2003). Therefore, it might be expected that alterations to P^{max} require concomitant modification of light harvesting to maintain balanced ATP and reductant production and consumption. Several strategies exist for optimizing light harvesting, including adjustment of cellular pigment content and arrangement, which ultimately affects the effective absorption cross section (a^*) and the quantum yield (i.e., the efficiency of photochemical production of oxygen per photon of light absorbed [Φ_{max}]; Falkowksi and Chen 2003). For A2, the cellular Chl *a* content did not increase under elevated pCO_2; however, calculation of both the Chl *a*–specific a^* (m^2 [mg Chl *a*]$^{-1}$, as in Suggett et al. 2007) and Φ_{max} (mol O_2 [mol photon]$^{-1}$, $\alpha^{Chl\ a}:a^*$; data not shown) demonstrates that Φ_{max} appears to increase by $\sim$ 50% under elevated pCO_2. Although this was not statistically significant ($p = 0.359$ for 40 vs. 81 Pa pCO_2) the change to Φ_{max} might be sufficient to account for the change in the cell-normalized α observed.

Although an increase in oxygen-based measurements of cell-normalized P^{max} and α was observed for A2 in response to elevated pCO_2, no obvious changes in any downstream fates of elevated productivity (e.g., POC and growth) occurred. In fact, both the growth rate and cellular POC of A2 did increase by $\sim$ 22% (but not significantly) at the elevated pCO_2. Other OA studies of dinoflagellates (*P. minimum*) also show that changes in carbon acquisition are not necessarily coupled with changes in growth rates under OA conditions (Fu et al. 2008) and that other sinks for fixed carbon might increase in response to OA instead, such as the release of dissolved organic carbon (DOC) and production of transparent exopolymer particles (TEP), as seen in other marine phytoplankton (Engel et al. 2004, 2005). However, increased DOC excretion and TEP production are typically associated with conditions in which carbon fixation exceeds the intake of the other essential nutrients (e.g., nitrogen-based compounds) required for growth (Dubinsky and Berman-Frank 2001; Engel et al. 2004). Thus, both elevated DOC release and TEP production, as a consequence of nutrient limitation, might be unlikely for A2 given that all cultures were nutrient replete. In the present study, DOC was not measured, so we cannot directly discount increased DOC production as a sink for the increased photosynthetic capacity observed in A2 under elevated pCO_2. However, it might be possible that propagation of the (nonsignificant) increases for both POC and growth of A2 are in fact sufficient to account for the increase of P^{max}. Again, this explanation assumes that PQ remains constant.

Symbiodinium *growth and ocean acidification*—Elevated growth of several marine phytoplankton species has been

observed at higher $CO_{2(aq)}$ (e.g., *Skeletonema costatum, Heterosigma akashiwo*, *H. caterae*, *Trichodesmium* spp., *Stichococcus bacillaris*, and *Emiliania huxleyi*; Schippers et al. 2004; Kim et al. 2006; Hutchins et al. 2007; Levitan et al. 2007; Fu et al. 2008). In many cases, the increase in growth in response to elevated pCO_2 is accompanied by a concomitant increase in the amount of fixed carbon (Schippers et al. 2004; Hutchins et al. 2007; Fu et al. 2008). However, OA-driven increases in growth rates can also occur in the absence of any apparent increase in carbon fixation. For example, *Trichodesmium* IMS101 exhibited an increase in growth rate under elevated pCO_2 (91.2 Pa), despite appearing not to be carbon limited at 35.5 Pa (Levitan et al. 2007). It should be noted, however, that other OA research conducted on the same isolate found carbon fixation rates increased under elevated pCO_2 relative to present day levels (Hutchins et al. 2007). OA-driven increases in growth rate, in the absence of increased carbon fixation, have been explained as a reallocation of energy and resources away from the CCM that is only required under low and ambient pCO_2 (Rost et al. 2008; Hurd et al. 2009). In our study, the response of phylotype A13 to OA is consistent with these observations of elevated growth rates (but not P^{max}). This suggests a reallocation of resources already available to the cell, in particular down-regulation of the CCM, which can be energetically costly (Giordano et al. 2005). Such down-regulation of the CCM (Fig. 3b,c) to instead rely in diffusive uptake of $CO_{2(aq)}$ (Fig. 3a; Hurd et al. 2009), might increase the pool of energy available to invest in the synthesis of new cells.

Marine dinoflagellates *Prorocentrum minimum*, *Heterocapsa triquetra*, *Ceratium lineatum*, and *Protoceratium reticulatum* all appear to regulate their CCM activity through differential expression of carbonic anhydrases (CAs) in response to changes in external CO_2 concentration (Rost et al. 2006; Ratti et al. 2007); such a capacity is clearly beneficial to tolerate large changes of pCO_2, and hence pH in the case of these species, associated with dinoflagellate blooms (Rost et al. 2006; Ratti et al. 2007). Thus, a similar regulatory (buffering) mechanism could exist for phylotype A13. The exact nature of the CCMs in *Symbiodinium* spp. has not been fully characterized, nor is it known whether all *Symbiodinium* phylotypes share a similar CCM design. However, some evidence alludes to the type(s) of CCM mechanisms employed by *Symbiodinium*.

Symbiodinium spp. are known to possess both internal and external CAs that enable the utilization of HCO_3^-, in addition to CO_2, from the external environment for photosynthesis (Yellowlees et al. 1993; Al-Moghrabi et al. 1996; Leggat et al. 1999). Membrane-bound H^+-ATPase, which acts as a proton pump to acidify the external environment and mediate HCO_3^- dehydration into CO_2, has also been identified in *Symbiodinium* phylotypes freshly isolated (theoretically representative of in hospite *Symbiodinium*) from the corals *Galaxea fascicularis* (phylotype unknown) and *Stylophora pistillata* (phylotype A1) (Al-Moghrabi et al. 1996; Bertucci et al. 2010). The expression of the same proton pump was found to be reduced or absent after the isolated *Symbiodinium* had been maintained in cultures (representative of "free-living" *Symbiodinium*; Al-Moghrabi et al. 1996; Bertucci et al. 2010). Furthermore, Al-Moghrabi et al. (1996) demonstrated that the cultured *Symbiodinium* were able to transport HCO_3^- actively and directly across into the cell with the use of a Na^+ and HCO_3^- cotransporter, possibly with the sue of the greater availability of Na^+ available in seawater. The ability to regulate CCM activity has also been identified for an unknown *Symbiodinium* phylotype isolated from the giant clam, *Tridacna gigas* (Leggat et al. 1999). Here, the photosynthesis of freshly isolated *Symbiodinium* appeared to be predominantly supported by CO_2 uptake; however, after 2 d of isolation, the *Symbiodinium* showed a preference for HCO_3^- uptake to support photosynthesis. Such a response appears indicative of an acclimatory response by the algae to the DIC species most available within the two environments (i.e., CO_2 when in hospite and HCO_3^- when free-living) and demonstrates further still the plasticity in CCM regulation by this dinoflagellate (Leggat et al. 1999). Furthermore, the contrasting inorganic carbon species preference of in hospite zooxanthellae isolated from *G. fascicularis* (Al-Moghrabi et al. 1996) and *T. gigas* (Leggat et al. 1999) reflects the predominant inorganic carbon species available within the tissues of each host (i.e., HCO_3^- within the coral and CO_2 within the giant clam; Leggat et al. 1999).

Together, these various studies demonstrate the plasticity of CCM activity within the *Symbiodinium* genus and that CCM regulation is greatly affected by the characteristics of the available inorganic carbon pool. Our data for A13 therefore suggests that the change in carbon speciation via the increase in $CO_{2(aq)}$ (Table 1) enabled phylotype A13 to down-regulate expression of CAs and CCM-related structures (such as H^+-ATPase and Na^+ and HCO_3^- cotransporters) and invest the resources and energy into growth instead. The lack of a similar response for A1 and B1 further suggests that some *Symbiodinium* phylotypes are perhaps not able to reduce their dependence on active HCO_3^- uptake in favor of the less costly diffusive uptake of CO_2. Clearly, further study is required to evaluate these proposed mechanisms.

In summary, the present study demonstrates that the effect of OA on the dinoflagellate genus *Symbiodinium* will be phylotype-specific and, in many cases, could be specific for a particular clonal line within a phylotype classification, such as the ITS2 divisions used here (i.e., a different B1 line might respond differently to OA). Both the lack of sensitivity observed for phylotypes A1 and B1 and the differential selection for growth (A13) and productivity (A2) under OA are clearly important as to how this important dinoflagellate group will respond to future changes of seawater pCO_2, at least in the "free-living" state. Our data suggest that OA may select among *Symbiodinium* phylotypes while free-living and, ultimately, the symbiont pool available to symbiotic cnidarians that acquire their symbionts by horizontal transmission during the larval settlement phase (reviewed in Sheppard et al. 2009). Some symbioses between *Symbiodinium* and cnidarians can be flexible, in that a number of coral species are able to associate with a variety of different *Symbiodinium*

phylotypes (symbiont generalists), whereas other coral species will only ever form a symbiosis with specific phylotypes (symbiont specialists) (Baker 2003; Warner et al. 2006). Thus, OA selection among the free-living *Symbiodinium* pool could have fundamental implications for reef-building corals, favoring the symbiont generalists over the symbiont specialists and potentially reducing coral diversity and community structure on a reef.

While we have focused on the free-living state of *Symbiodinium*, and focus should continue on how OA affects the viability and photosynthetic performance in hospite (Langdon and Atkinson 2005; Crawley et al. 2010). Much more information is needed concerning the dynamics of CCM initiation, expression, and maintenance in both the alga and the host because our observations do suggest that in hospite carbon delivery and pH could substantially affect algal physiology and possibly population structure (Weiss and Reynolds 1999; Furla et al. 2000). However, a more focused study of the in hospite interactions between host and symbiont is required.

The change in seawater pCO_2 expected for this century will also be accompanied by increases in sea surface temperatures (Solomon et al. 2007). To fully understand the ecological response of *Symbiodinium* to OA, the interactive effect of temperature and pCO_2 on their physiology should also be considered. Previous studies have shown that the different phylotypes of *Symbiodinium* exhibit a range of tolerances to elevated temperatures (Robison and Warner 2006; Suggett et al. 2008). In particular, phylotype A13 is considered extremely sensitive to increases in growth temperature from 26°C to 32°C (Robison and Warner 2006). This potential for sensitivity to rising sea surface temperatures might negate the increase in growth rate observed for this phylotype under elevated pCO_2. This highlights the need for future OA studies to also consider and incorporate the synergistic effects of temperature and pCO_2 into the design of the experiment.

Although we have evaluated the effect of OA on four ecologically relevant phylotypes here, our observations suggest that the choice of algal species for OA experiments could determine whether, and to what extent, an OA-driven response is observed. Further evaluating other widespread *Symbiodinium* species, such as those within clades B, C, and D, might be important to contextualize the diversity of responses within the vast phylogenetic diversity of this genus. Furthermore, it would be informative to establish whether isolates of the same phylotype, but from different hosts, behave similarly under elevated pCO_2 in that isolate-specific differences in physiology have been observed (Rogers and Davis 2006; Hennige et al. 2009) and could elucidate how the same phylotype has adapted to the carbon environment of different hosts. Our data suggest that CCM activity is reduced in at least one *Symbiodinium* phylotype under elevated pCO_2; however, a mechanistic approach will be required to determine whether or not this is the case. Fundamentally, the present study demonstrates the importance of a physiological-based approach to better understand how key phototrophs respond to OA conditions and, in turn, determine likely effects on ecosystem (and biogeochemical) function.

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