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Differing responses of marine N_2 fixers to warming and consequences for future diazotroph community structure

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ABSTRACT: The globally distributed colonial cyanobacterium *Trichodesmium* and unicellular diazotrophs including *Crocosphaera* together carry out the majority of marine biological nitrogen (N_2) fixation. Future sea surface warming is predicted to influence their abundance and distribution, but temperature reaction norms have been determined for very few representatives of each genus. We compared thermal responses within and between the 2 genera *Trichodesmium* and *Crocosphaera* by measuring reaction norms for growth, N_2 fixation, carbon fixation, and elemental ratios in 7 strains from a global culture collection. Temperature reaction norms of *Trichodesmium* and *Crocosphaera* were remarkably similar for all isolates within each genus, regardless of their geographic origin. Thermal limits of *Trichodesmium* and *Crocosphaera* ranged from 18 to 32°C and 24 to 32°C, and optimum growth temperatures were ~26 and ~30°C, respectively. The highest cellular ratios of nitrogen to phosphorus and carbon to nitrogen were found at optimum growth temperatures, and the lowest ratios near their thermal limits. In a mixed competition experiment, *Trichodesmium* growth rates were ~25% higher than those of *Crocosphaera* at 24°C, while those of *Crocosphaera* were ~50% higher at 28°C. Comparison of these results to current and projected seasonal temperature regimes in the subtropical Atlantic and Pacific Oceans suggests that predicted warmer temperatures may favor *Crocosphaera* over *Trichodesmium*, but that both genera may be excluded where future temperatures consistently exceed 32°C. Sea surface warming could profoundly alter the community structure and stoichiometry of marine N_2-fixing cyanobacteria, thus fundamentally changing the biogeochemical cycling of this globally significant source of new nitrogen.

KEY WORDS: Global change · Warming · Temperature · Nitrogen fixation · *Trichodesmium* · *Crocosphaera*

INTRODUCTION

Climate change is predicted to change the biodiversity of marine pelagic ecosystems by altering competitive interactions within the biological community (Hutchins et al. 2009, Boyd et al. 2010, Boyd & Hutchins 2012). One major physical parameter that is changing due to the greenhouse effects of anthropogenic CO_2 emissions is sea surface temperature, which has increased globally an average of ~0.6°C in the past 100 yr (Hoegh-Guldberg & Bruno 2010). Climate change models predict sea surface temperature to continue to rise into the future (IPCC 2007, Domingues et al. 2008).

Although concern about anthropogenic climate change is relatively recent, researchers have long been interested in how phytoplankton respond to changing temperature, and there have been a num-

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ber of classic laboratory culture studies on this topic. Eppley et al. (1972) demonstrated that algal growth rates and assimilation rates increased with increasing temperature. Raven & Geider (1988) reported that temperature influences algal genotypic and phenotypic variations, chemical transformations, nutrient transport processes, and enzyme kinetics. A recent paper compiled a large dataset from the literature on the temperature responses of cultured phytoplankton, and used these data to model their likely distribution changes in a future warmer ocean (Thomas et al. 2012). Another recent study used a community-wide collective experimental approach to examine the thermal ranges of 25 eukaryotic and prokaryotic species, and concluded that while their optimum temperatures and thermal niche widths were often consistent with prior work, maximum growth rates can vary widely between studies (Boyd et al. 2013).

The influence of temperature on phytoplankton species composition has also been seen in numerous field studies across the globe. Banse (1991) highlighted the role of temperature as a fundamental control on phytoplankton growth rates in the ocean. One of the responses of phytoplankton to climate change may be shifts in assemblage structure (Falkowski et al. 1998). One study found that as a result of sea surface warming in Swedish coastal waters, winters were warmer, ice cover disappeared at a faster rate in the spring, and phytoplankton blooms occurred earlier. Furthermore, organisms such as cyanobacteria and chlorophytes appeared sooner in the year, and dominated the community over a longer period (Weyhenmeyer 2001).

Experimental evidence also suggests that warming causes changes in phytoplankton floristics. In a Bering Sea shipboard manipulative experiment, as temperature increased, the community shifted away from diatoms and toward nanophytoplankton (Hare et al. 2007). Similarly, Rose et al. (2009) found experimental warming of Antarctic waters also resulted in assemblage changes, with the diatom genera *Cylindrotheca* and *Thalassiosira* being dominant at elevated temperatures. In a North Atlantic spring bloom experiment, coccolithophorids outcompeted diatoms at warmer temperatures (Feng et al. 2009). It is evident that particular groups will benefit from increased temperature, but warming is also likely to put other organisms at a competitive disadvantage.

Several groups of nitrogen (N_2)-fixing cyanobacteria are globally distributed in tropical and subtropical oligotrophic oceans. These include the non-heterocystous filamentous genus *Trichodesmium*, and unicellular forms including UCYN-B such as *Crocosphaera watsonii*, UCYN-A, and UCYN-C (Zehr 2011). A variety of studies have demonstrated that these unicellular diazotrophic cyanobacteria, especially *Crocosphaera* and UCYN-A, are abundant and contribute substantial amounts of N in many oligotrophic regions (Langlois et al. 2008, Kitajima et al. 2009, Moisander et al. 2010). *Trichodesmium* and all of the unicellular groups play key roles in the nitrogen cycle, because together they are responsible for the majority of total marine biological N_2 fixation (Sohm et al. 2011a). Since UCYN-A are currently not available in culture, little is known about their physiology other than what has been gleaned from environmental molecular studies. In contrast, *Trichodesmium* and *Crocosphaera* have been extensively studied due to the availability of numerous laboratory culture strains (Falcón et al. 2005, Hutchins et al. 2007, 2013, Fu et al. 2008, Webb et al. 2009).

According to Galloway & Cowling (2002), these groups together fix an average of 110 Tg N yr^{-1} in marine ecosystems. Karl et al. (2002) reported that biological N_2 fixation accounts for an average of 100 to 200 Tg N yr^{-1}. Westberry & Siegel (2006) estimated that globally *Trichodesmium* fixes 42 Tg N yr^{-1} during bloom conditions, and 20 Tg N yr^{-1} during non-bloom conditions. *Crocosphaera watsonii* and other unicellular groups like UNCY-A and UCYN-C together carry out about as much N_2 fixation as *Trichodesmium* spp. (reviewed in Sohm et al. 2011a).

A number of studies have shown that the nutrients iron and phosphorus can control N_2 fixation and growth of these 2 marine N_2 fixers (Sañudo-Wilhelmy et al. 2001, Berman-Frank et al. 2007, Fu et al. 2007, Chappell et al. 2012). Temperature has also long been recognized as a major factor that controls *Trichodesmium* abundance, and 20°C has been considered the minimum temperature for *Trichodesmium* to survive (Carpenter 1983). Sea surface temperature has been used to define the geographic extent of this genus (Capone & Carpenter 1982) and to predict ecosystem N_2-fixation rates (Bissett et al. 1999). Temperature is predicted to influence the abundance and global distribution of *Trichodesmium* and *Crocosphaera* (Breitbarth et al. 2007, Sohm et al. 2011a). Based on *nifH* DNA copy abundance, UCYN-A cyanobacteria (average 10^3–10^5 copies l^{-1}, Church et al. 2008, Moisander et al. 2010) have been observed at temperatures ranging from 15 to 30°C (Needoba et al. 2007, Church et al. 2008, Langlois et al. 2008). Both *Trichodesmium* and *Crocosphaera* may benefit from rising sea surface temperatures because their ranges will expand poleward. In particular, *Trichodesmium* spp. has been predicted to increase its

global distribution by 11%. However, this augmented range in the higher latitudes may be offset by temperature increases at low latitudes that exceed optimal growth limits (Breitbarth et al. 2007). Currently, sea surface temperatures in these areas reach a maximum of 24 to 26°C in summer (August–September), which coincides with documented temperatures for optimum growth rates in *Trichodesmium* and *Crocosphaera*. For example, *Trichodesmium* generally grow optimally at temperatures from 24 to 30°C and *Crocosphaera*'s general temperature range is from 22 to 36°C (Sohm et al. 2011a).

However, these temperature ranges for diazotrophs are based on a very limited set of experiments with only a few representatives of each group. Laboratory studies performed to date have described thermal responses of growth, N_2 fixation, and nutrient uptake in only one strain of *Trichodesmium* (Breitbarth et al. 2007, Hutchins et al. 2007), and a few strains of *Crocosphaera* (Falcón et al. 2005, Webb et al. 2009). Reported temperature effects on growth of *Crocosphaera* seem contradictory, but this appears to be due to certain isolates having more restrictive temperature profiles (e.g. P-7 and WH8501, respectively, Falcón et al. 2005, Webb et al. 2009). However, the commonly cultivated strain *Crocosphaera* WH8501, in particular, may not be an ideal model for the genus as a whole because it appears to be atypical in a number of respects including genome size, transposase abundance, and repeated genomic sequences (Bench et al. 2013).

It is unknown how much variability there is between individual species and strains of these 2 globally distributed genera, and whether a genetic diversity of temperature tolerances exists that would allow replacement of current strains by more thermally adapted ones as sea surface temperatures increase. A recent study showed that strains of *Crocosphaera watsonii* with similar phenotypes (e.g. cell size) clustered together based on genome size and genetic capabilities; however, similar clustering was not observed in strains isolated in close temporal or spatial proximity to each other (Bench et al. 2013). It is not known how thermal responses compare between *Trichodesmium* spp. and *C. watsonii* populations from geographically separated areas, and whether the few commonly available culture isolates are appropriate models for the temperature responses of these genera as a whole.

The goal of this study was to investigate the influence of temperature on the growth, N_2 fixation, carbon (C) fixation, and elemental ratios in multiple strains of these 2 keystone marine N_2 fixers, and compare the diversity of their responses both within and between the 2 genera. We also aim to understand whether individual strains of these organisms can serve as general representations of the thermal tolerances of their genus or species as a whole, and to provide a framework for making better predictions of how this primary climate change variable will affect the community structure of marine diazotrophs in future warmer oceans.

MATERIALS AND METHODS

Strain origins

Four strains of cultured *Crocosphaera* were used in this study, including WH0005 and WH0003 isolated from the North Pacific Ocean basin, WH0402 from the South Atlantic Ocean basin, and WH0401 obtained from the North Atlantic basin (Webb et al. 2009). The 3 strains of *Trichodesmium erythraeum* that we used were RLI from the Great Barrier Reef (Fu & Bell 2003), KO4-20 from the South Pacific, and 21-75 from the Western Equatorial Atlantic (Hynes et al. 2012).

Culture growth conditions and experimental design

Stock cultures of *Trichodesmium* and *Crocosphaera* were maintained in a modified Aquil medium (Price et al. 1989) without combined nitrogen under a light intensity of 120 µmol photons m^{-2} s^{-1} with a light-dark cycle of 12:12 high: dark (LD) in Percival incubators. For the response curve experiments, 400 ml unialgal cultures were grown in 600 ml polycarbonate bottles across a range of temperatures from 18 to 35°C. Some strains of *Trichodesmium* that were found to be able to grow at 18°C were also then assayed at 16°C. All experiments used 4 replicate bottles at each temperature. Semi-continuous dilution culturing methods were practiced in this experiment because they allow measurement of temperature effects during acclimated, steady-state growth. The advantage of this method over chemostat cultures (where growth rates are fixed by the dilution rate chosen) is that in semi-continuous cultures, dilution volumes can be adjusted to match the intrinsic growth rates in each individual experimental bottle, thus allowing growth rates to be tested as a variable in each treatment.

Semi-continuous cultures (4 replicates of each treatment) were diluted every 2 to 3 d with medium

that was acclimated to the appropriate temperature prior to dilution, using *in vivo* fluorescence as a real time biomass indicator. These growth rates were validated by subsequent preserved cell counts, similar to previous studies (Webb et al. 2009, Fu et al. 2010, Hutchins et al. 2013). Cultures were maintained in exponential growth phase and were optically thin (~50–100 µmol C l^{-1}) to avoid self-shading or nutrient depletion. Light levels were checked with a Licor 1000 light meter. To avoid the cultures becoming nutrient-limited, 10 µM P and 450 nM Fe were added to the Aquil medium (but with no added fixed N). Our stock cultures grown at 28°C were able to maintain exponential growth for at least 7 d without dilution (F. X. Fu unpubl. data), far longer than the 2 d between dilutions in this semi-continuous experiment.

Each bottle was diluted individually based on the growth rate calculated for that bottle, allowing each culture to 'choose' their own growth rate under the experimental conditions. For all experiments, final sampling of 4 replicate bottles for each treatment occurred once steady-state growth was obtained for each growth condition (typically after 8–20 generations). Steady-growth status was defined by no significant difference in cell- or chlorophyll *a* (chl *a*)-specific growth rates for at least 3 consecutive transfers. Cultures were deemed unable to grow at a particular temperature only after 4 independent unsuccessful culturing attempts at that temperature.

In order to test how temperature change could affect competition between the 2 marine N_2 fixers *Crocosphaera* and *Trichodesmium*, a simple batch culture competition experiment was carried out in which the 2 species were inoculated at a 1:1 ratio (based on equivalent levels of cell carbon due to the large differences in their cell sizes) into nutrient-replete medium and grown for 10 d at 2 temperatures representative of present day and possible future sea temperatures (24 and 28°C). Batch cultures were used in the competition experiment, instead of the semi-continuous dilutions employed in the uni-algal experiments, in order to avoid favoring either of the 2 co-cultured species through the choice of a particular dilution rate. *Crocosphaera* WH0003 and *Trichodesmium* RLI cultures were fully acclimated to these 2 temperature conditions before beginning the competition experiment. In order to focus only on the effect of temperature on competition, the competition experiment cultures were grown in medium lacking fixed N, but replete with P, Fe, and other micronutrients. Medium and growth conditions were identical to those used in the uni-algal thermal response growth curve experiments. Growth rates were determined based on cell counts during exponential growth phase of the mixed cultures.

Determination of growth rates and chl *a*

Reported growth rates were based on microscopic cell counts. For chl *a* determination, subsamples of 10 to 20 ml from each triplicate bottle were GF/F filtered, extracted in 6 ml of 90% acetone, stored overnight in the dark at −20°C, and chl *a* concentrations were measured fluorometrically using a Turner 10-AU fluorometer (Welschmeyer 1994). Samples for cell counts and chl *a* were always taken at the same time in the diel cycle (Tuit et al. 2004), between 09:00 and 10:00 h in the morning.

Primary production

After the cultures reached steady state in each treatment, primary production was measured in duplicate using 24 h incubations of 30 ml subsamples with $H^{14}CO_3$ under the appropriate experimental growth conditions for each treatment (Fu et al. 2008). All ^{14}C uptake rates were corrected for dark uptake, and C-fixation rates were calculated using initial experimental dissolved inorganic carbon (DIC) concentrations and chl *a* for each treatment. Primary production was normalized to cellular chl *a* content. For the analysis of total DIC, 25 ml samples from each bottle were preserved with 200 µl 5% $HgCl_2$ and stored at 4°C until analysis in triplicate as in Fu et al. (2007).

Nitrogen fixation

N_2-fixation rates were measured using the Acetylene Reduction Assay (ARA, Capone 1993) by gas chromatography with a Shimadzu gas chromatograph GC-8a (Shimadzu Scientific Instruments) equipped with a flame ionization detector. A theoretical ratio of 3:1 (mol C_2H_2: mol N_2 reduced) was used to convert rates of ethylene production (C_2H_2 reduction) to N_2 fixation (Montoya et al. 1996). Assays were initiated by adding 2 ml of C_2H_2 to the headspace of 28 ml serum vials containing 10 ml of culture. Ethylene production was measured by removing 100 µl of headspace at 2 to 3 h intervals over the entire 12 h light period (*Trichodesmium*) or dark period (*Crocosphaera*) due to their differing diel strategies for N_2 fixation (Tuit et al. 2004). Samples were gently agi-

tated to equilibrate gas concentrations between the headspace and culture samples following injection of acetylene and before measuring ethylene concentrations. For each temperature, 4 replicates were incubated under the same light and temperature conditions as the experimental cultures and each experiment was carried out 2 times. After total N_2-fixation rates were determined, the chl *a* concentration of each sample was measured and used to normalize N_2-fixation rate.

Elemental ratios

Particulate organic nitrogen (PON) and particulate organic carbon (POC) samples were taken in the morning, and filtered onto combusted 25 mm diameter Whatman GF/F filters. POC and PON samples were dried at 60°C for 3 d, compressed into pellets and the molar amounts of C and N were determined using an elemental analyzer (Costech Analytical Technologies). Particulate organic phosphorus (POP) samples were analyzed using the spectrophotometric method described in Fu et al. (2005) at a wavelength of 885 nm. All PON, POC, and POP data were blank corrected using measurements of identically treated filters (equivalent volumes of cell-free filtrate from the cultures passed through GF/F filters).

RESULTS

For both *Trichodesmium* and *Crocosphaera*, growth rate response curves showed expected overall patterns, beginning with a gradual increase with rising temperature, a peak at an optimum temperature, and a decline afterwards (Fig. 1). The shape of all the curves was fairly symmetrical relative to the maximum growth rates. In general, the thermal limits for *Trichodesmium* and *Crocosphaera* growth were remarkably similar within each genus, from 18 to 32°C and from 24 to 32°C, respectively. Although *Crocosphaera* had narrower overall temperature ranges compared to *Trichodesmium*, the unicellular genus generally grew 1.6 to 2 times faster than *Trichodesmium* under all growth conditions, except for the small cell strain *Crocosphaera* WH0401. Optimal growth temperatures for all *Trichodesmium* and *Crocosphaera* isolates were between 24 and 28°C and between 28 and 30°C, respectively (Fig. 1).

Crocosphaera cultures were unable to grow at 22°C but could be maintained alive at this temperature for up to 3 wk, although biomass progressively decreased with time. In contrast, all 3 *Trichodesmium* isolates maintained positive growth down to 18°C, although none were able to grow at 16°C. At the upper end of their thermal ranges, growth rates of *Trichodesmium* and *Crocosphaera* began to decrease as temperature increased above 30°C. Both genera could still actively grow at 32°C, but when cultures of both *Trichodesmium* and *Crocosphaera* were subjected to the highest temperature (35°C) they were dead after 3 and 7 d, respectively. This observation suggests that these 2 N_2 fixers are able to survive longer near their lower thermal limit than at their upper one.

In general, trends were similar for C fixation, which also exhibited similar temperature optima and upper and lower limits in both *Trichodesmium* and *Crocosphaera* (Fig. 2). C fixation increased with temperature until 30°C for all strains of *Crocosphaera* except for strain WH 0402, which reached an average maximum at 28°C. In contrast to *Crocosphaera*, the optimum temperature for C fixation by *Trichodesmium* was between 24 and 30°C, with a peak at 26°C.

As with the other rate measurements, N_2-fixation rates were significantly affected by temperature for both species and followed nearly identically the relationship observed for growth and C fixation with temperature (Fig. 3). N_2 fixation in all the *Crocosphaera* strains reached an average maximum at 28 to 30°C, and *Trichodesmium* had a temperature optimum between 24 and 28°C, with an average maximum value at 26°C.

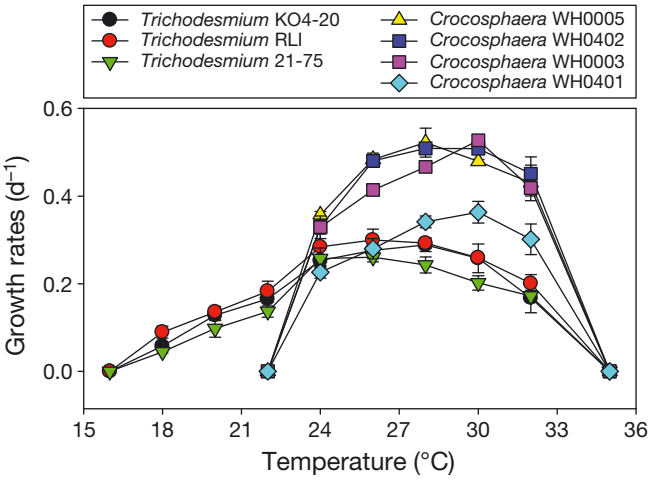

Fig. 1. Cell-specific growth rates (d^{-1}) of *Trichodesmium* strains RLI, 21-75, and KO4-20 and *Crocosphaera* strains WH0005, WH0402, WH0003, and WH0401 versus temperature (°C). The symbols represent the means and error bars are the standard deviations of 4 replicates

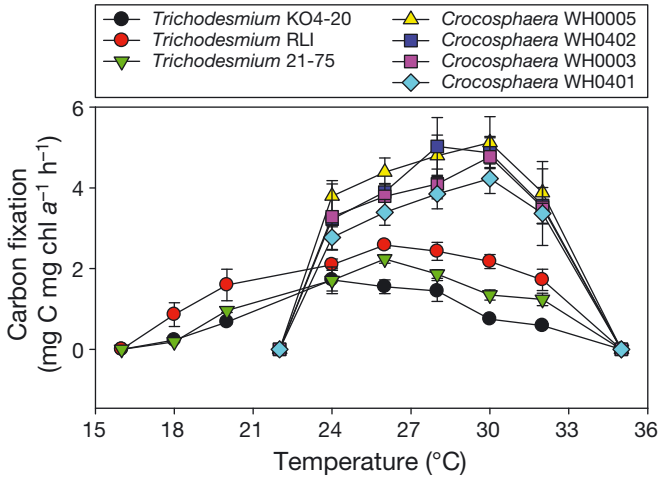

Fig. 2. C-fixation rates (mg C mg chl a^{-1} h^{-1}) of *Trichodesmium* strains RLI, 21-75, and KO4-20 and *Crocosphaera* strains WH0005, WH0402, WH0003, and WH0401 versus temperature (°C). The symbols represent the means and error bars are the standard deviations of 4 replicates

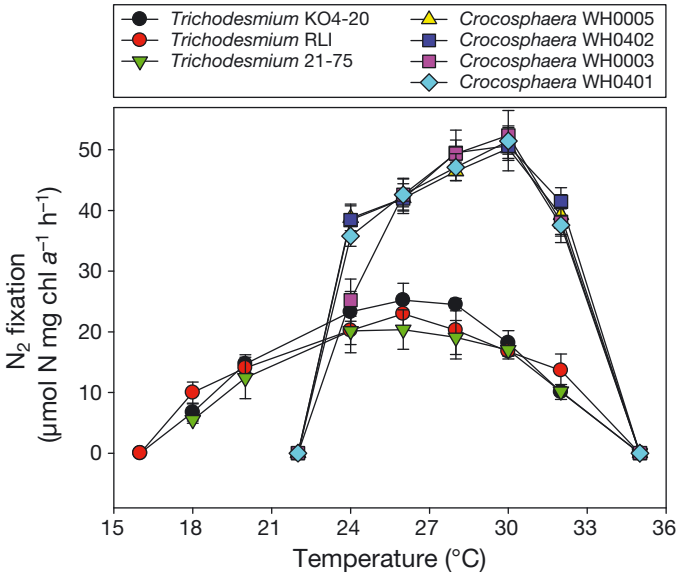

Fig. 3. N_2-fixation rates (µmol N mg chl a^{-1} h^{-1}) of *Trichodesmium* strains RLI, 21-75, and KO4-20 and *Crocosphaera* strains WH0005, WH0402, WH0003, and WH0401 versus temperature (°C). The symbols represent the means and error bars are the standard deviations of 4 replicates

The response of cellular ratios of nitrogen to phosphorus (N:P, mol:mol) in *Crocosphaera* and *Trichodesmium* follows the general trend of the rate measurements (Fig. 4a). *Trichodesmium* N:P ratios increased from ~10 at 18°C to a maximum of ~20 at 28°C. At higher temperatures, the ratios began to decrease again to a minimum value of ~12 at 32°C. There was no significant difference in N:P ratios from 24 to 28°C

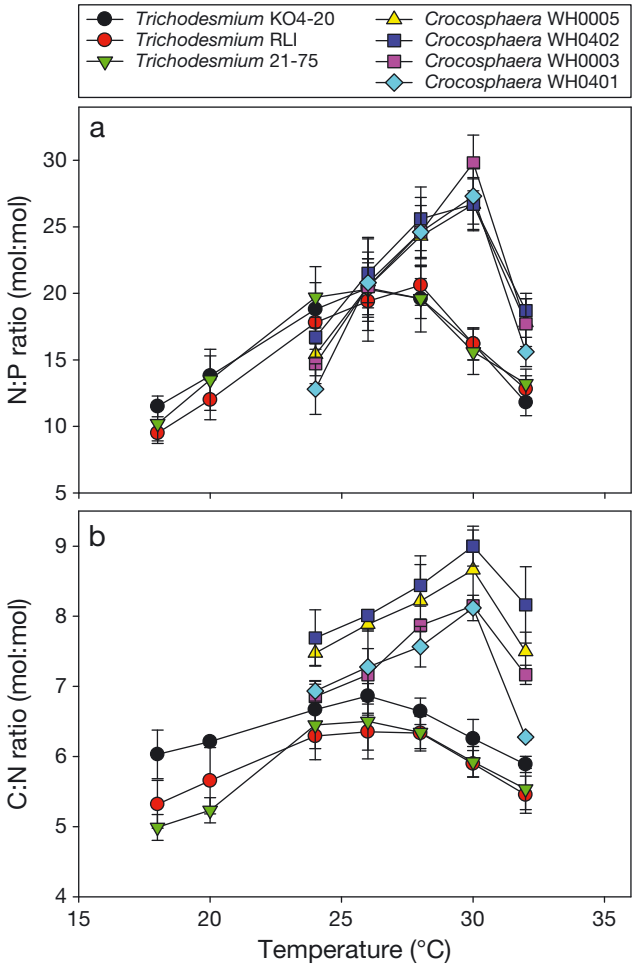

Fig. 4. Cellular ratios of (a) N:P (mol:mol) and (b) C:N (mol:mol) versus temperature for *Trichodesmium* strains RLI, 21-75, and KO4-20 and *Crocosphaera* strains WH0005, WH0402, WH0003, and WH0401. The symbols represent the means and error bars are the standard deviations of 4 replicates

($p > 0.05$). For *Crocosphaera*, the general tendency was an increase in the N:P value with temperature up to 30°C, and a reduction above this point. Although the ratios for all species or strains tested in this study grown at temperature ranges between 24 and 30°C were close to Redfield N:P ratios (16, Redfield 1934), those in *Crocosphaera* generally were somewhat higher than the ones in *Trichodesmium* at equivalent temperatures. This observation could be due to the sampling time ~4 h into the photoperiod for *Crocosphaera*, since the cellular N quotas in another isolate (WH8501) have been shown to be maximal at the beginning of the light period (Dron et al. 2012).

The cellular carbon to nitrogen ratios (C:N, mol:mol) of all 3 strains of *Trichodesmium* increased from 5–6 at 18°C to 6.3–6.7 at 24°C (Fig 4b). At temperatures above 30°C, the ratios of C:N decreased signif-

icantly to around 5.5. In general, C:N ratios of *Crocosphaera* were approximately 20% higher than the those of *Trichodesmium*, regardless of the temperature. Similar to the trends in growth rates, *Crocosphaera* C:N ratios increased from 6.9–7.7 at 24°C up to 8.1–9.0 at 30°C, and then decreased again at higher temperatures.

In the mixed culture competition experiment, growth rates of *Trichodesmium* RLI were ~25% higher than those of *Crocosphaera* WH0003 at 24°C, while those of *Crocosphaera* were nearly 50% higher at 28°C (Fig. 5a). Thus, the growth rates of the unicellular cyanobacterium responded positively to increasing temperature across this 4°C range, whereas those of *Trichodesmium* RLI did not. These observations confirmed that the differences in growth rates we observed in their uni-algal semi-continuous cultures as a function of temperature (Fig. 1) were reflected in competitive success, as measured by relative cell abundance. At the end of the experiment, the ratios of *Trichodesmium* to *Crocosphaera* (cell:cell) at 24°C had increased dramatically to ~1, while at 28°C they were close to the initial value of 0.4 (Fig. 5b).

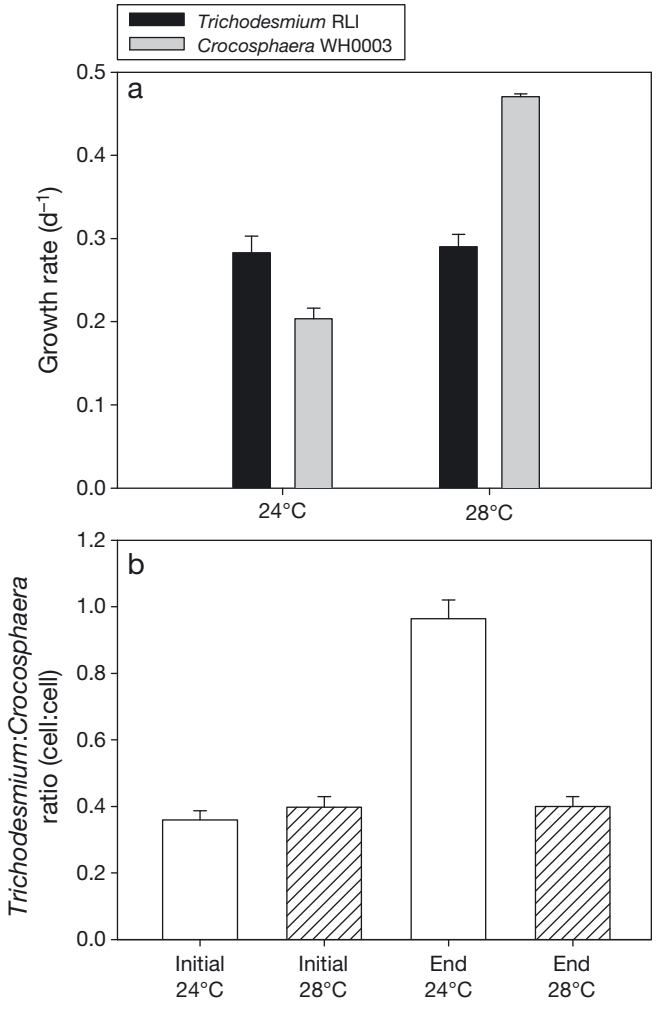

Fig. 5. Outcome of an interspecific competition experiment at 24 and 28°C using mixed cultures of *Trichodesmium* RLI and *Crocosphaera* WH0003. (a) Mean cell-specific growth rates (d^{-1}) of *Trichodesmium* RLI (black bars) and *Crocosphaera* WH0003 (grey bars) (d^{-1}) during the last 10 d of the experiment, and (b) ratios of *Trichodesmium* to *Crocosphaera* abundance (cell:cell) at the beginning and end of the experiment. Values are the means and error bars are the standard deviations of triplicate samples

DISCUSSION

Very few strains of marine N_2-fixing cyanobacteria have been examined in previous temperature response studies, so our knowledge of how projected sea surface warming may influence their biology on a global scale has been limited. In this study, we documented how growth rates, C-fixation rates, N_2-fixation rates, and elemental ratios were affected by temperature in 4 strains of *Crocosphaera* and 3 strains of *Trichodesmium* from 2 major ocean basins. Our most striking result in this study was the strong overall similarity in temperature response curves of all the strains tested within each genus. Despite being isolated from widely separated regions of the Atlantic and Pacific, all 3 *Trichodesmium* isolates had very congruent curves with nearly identical maximum and minimum temperature limits. The same was true for the 4 equally geographically dispersed *Crocosphaera* isolates. Thus, any one of these individual strains can serve as a general model for the thermal reaction norms of all of the members of its genus that we tested. This uniformity of responses to temperature is in striking contrast to the broad diversity of functional response curves that many of these same *Trichodesmium* and *Crocosphaera* isolates exhibit relative to another concurrent global change variable, CO_2 concentration (Hutchins et al. 2013). These strains of *Crocosphaera* and *Trichodesmium* have been in culture for periods ranging from 4 to 10 yr, suggesting that the duration of adaptation to culture conditions was not a determining factor in their physiological responses.

This observation does not seem to support a recent modeling paper that suggests the biogeography of diazotrophs is proximately controlled by iron and nitrogen distributions. This model suggested that the fact that global distributions of these nutrients covary to some extent with temperature either positively (iron) or negatively (nitrogen) is indirectly responsible for the apparent thermal limits of N_2 fixers (Mon-

teiro et al. 2011). In fact, our results suggest that each of the 2 diazotroph genera we examined has relatively rigid, genus-specific temperature tolerances that are likely an inherent property of their genetics and physiology. Thermal reaction norms in our isolates were nearly uniform regardless of their geographic provenance; since the relationship between, for instance, iron supply and temperature varies tremendously between the Atlantic and Pacific basins, this argues that diazotroph thermal limits may represent an intrinsic biological response rather than being an incidental result of local iron availability. In their examination of 81 previous studies, Thomas et al. (2012) found that phytoplankton optimum growth temperatures at the genus level are tightly correlated to the mean temperatures in their native habitats, with the implication that temperature has been a strong selective agent in phytoplankton evolution.

Growth, C fixation, N_2 fixation, and elemental ratios of the unicellular N_2 fixer *Crocosphaera* increased with increasing temperature up to 30°C, but further temperature increases were inhibitory. These results confirm the previous study of Webb et al. (2009), which found that the maximum growth rate of *Crocosphaera* spp. was attained between 26 and 30°C. However, growth in the isolate they used in this previous study continued at 34°C, slightly higher than the upper thermal limit for growth of 32°C for all 4 of the isolates we tested. These differing observations could be due to variability in the accuracy of temperature measurements, or the different culturing methods applied in each study, particularly low-light grown batch cultures (25–50 µmol m^{-2} s^{-1}, Webb et al. 2009) versus the semi-continuous and light-saturated ones used in our study (>120 µmol m^{-2} s^{-1}). One laboratory study with the cyanobacterium *Planktothrix agardhii* showed that temperature had a strong influence on light-saturated growth, but only a weak effect on light-limited growth (Nicklisch et al. 2008). These somewhat different responses to experimental conditions by the same species or strains suggest a need to apply uniform protocols in laboratory studies (Boyd et al. 2013).

The correlation of *Crocosphaera* growth and biomass with elevated temperature is consistent with the observed distributions of natural populations of this genus (Sohm et al. 2011a). Copies of the nitrogenase-coding gene *nifH* per liter for *Crocosphaera* in the Pacific Ocean have been shown to increase dramatically as temperature increases from 22 to 30°C (Moisander et al. 2010). Another Pacific study found that copy numbers of *nifH* phylotypes of UCYN-B (*Crocosphaera*) were highest when the surface temperature ranged from 24 to 27°C (Church et al. 2008). Similarly, a latitudinal transect in the eastern Atlantic Ocean revealed that active N_2 fixation (most likely by unicellular N_2 fixers) was associated with water temperatures ~28°C (Staal et al. 2007). Copies per liter of UCYN-B *nifH* genes (i.e. *Crocosphaera*) did not vary in the tropical Atlantic Ocean between 25 and 29°C (Goebel et al. 2010). Likewise, the biomass of *Crocosphaera* in the Arabian Sea was found to be preferentially located in waters between 27 and 31°C (Mazard et al. 2004). Our culture results, together with these field observations, suggest that temperature is an important factor affecting the distribution, abundance, and N_2-fixation rates of *Crocosphaera* in the subtropical oceans.

Observational evidence also suggests that temperature is a major constraint on *Trichodesmium* distributions and growth. Most reported *Trichodesmium* blooms occur in subtropical and tropical oceans where surface temperatures are >25°C (Capone et al. 1997), and maximum abundances often occur at temperatures of 28 to 29°C. This general trend has been supported by observations in the tropical Atlantic Ocean (Goebel et al. 2010), the Indian Ocean (Lugomela et al. 2002), the East China Sea (Chang et al. 2000), and the Great Barrier Reef (Bell et al. 1999). *Trichodesmium* abundances are dramatically decreased where temperatures are <22°C (Tyrrell et al. 2003), although natural populations have been observed to fix nitrogen actively at depths of at least 75 m, corresponding to a temperature of 21°C (Letelier & Karl 1998). *Trichodesmium nifH* phylotypes are present in the North Atlantic Ocean throughout a temperature range from 19 to 30°C, but they are most abundant in waters warmer than 26°C (Langlois et al. 2005, Langlois et al. 2008).

Experimental culture work (Breitbarth et al. 2007) and theoretical evidence (Stal 2009) also suggest temperature is an important control on N_2 fixation and abundance of *Trichodesmium*. Our results are in agreement with several previous laboratory studies with a single North Atlantic isolate (IMS 101) showing that its optimal growth temperature range lies between 24 and 28–30°C (Chen et al. 1998, Mulholland & Bernhardt 2005, Breitbarth et al. 2007). Staal et al. (2003) suggest that high temperature may help non-heterocystous cyanobacteria like *Trichodesmium* to fix nitrogen by lowering dissolved O_2 solubility and increasing respiration rates, both of which tend to protect nitrogenase from inactivation by O_2. However, Hutchins et al. (2007) found no significant difference in N_2- and CO_2-fixation rates by IMS 101 and another *Trichodesmium* strain from the Pacific at

25 and 29°C. A 3 to 4°C temperature decrease results in an increase in dissolved O_2 concentrations of only ~7%, suggesting the changing degree of O_2 inactivation may be relatively minor across this thermal range. Temperature may also affect O_2 and CO_2 diffusion rates to and from active sites in the cell, and potentially cellular fixed nitrogen losses. Such temperature-related diffusion effects may affect the 2 genera differently due to the significant differences in their cell sizes.

The general shapes of the thermal functional response curves for each genus have environmental and ecological implications. To predict phytoplankton population distributions in the ocean, it is important to understand their upper and lower limits of temperature to maintain growth (Goldman & Carpenter 1974). For instance, it is obvious that *Trichodesmium* as a group have lower minimum temperature limits than *Crocosphaera*, and so have the potential to extend their ranges to significantly higher latitudes. *Trichodesmium* grows at least down to 18°C, while all of our *Crocosphaera* isolates were unable to grow below 22°C.

However, upper temperature limits were virtually identical for all 7 isolates from both genera, with growth ceasing at 35°C. This suggests that neither genus may have an inherent competitive advantage in regions that reach or exceed this temperature in a future warmer ocean. Nevertheless, the relative abundance of these 2 groups of marine N_2 fixers in the ocean may well be affected by an increase of 4°C, within the range predicted for sea surface temperatures within this century (IPCC 2007). Our *Trichodesmium* and *Crocosphaera* artificial community competition experiment (Fig. 5) suggests that temperature may be one factor (among many) determining the outcome of interspecific competition in mixed natural marine N_2-fixer assemblages. Due to the strong congruence in thermal response curves for all of the isolates within both genera (Figs. 1 to 3), we assume that the 2 strains used in our competition experiment (*Trichodesmium* RLI and *Crocosphaera* WH0003) are good general models for their respective genera. It is notable that in nature competition between *Crocosphaera* and *Trichodesmium* is undoubtedly also affected by the availability of resources like phosphate, iron, and light. Considering the effects of temperature in isolation though, future warming may favor the dominance of *Crocosphaera* relative to *Trichodesmium*, and the opposite appears to be true for cooler temperatures.

To illustrate the implications of these observations for diazotroph community structure, we plotted growth rates from our culture isolates versus monthly sea surface temperature data from the Hawaii Ocean Time-series station (HOT, http://hahana.soest.hawaii.edu/hot/) and the Bermuda Atlantic Time-Series station (BATS, http://bats.bios.edu/). Annual temperature trends were graphed for both the current ocean (2009/2010 data, Fig. 6a,b), and under a hypothetical 3 to 4°C warming scenario (Fig. 6c,d), likely a realistic projection for the future subtropical ocean (IPCC 2007). These simulations represent potential maximum growth rates for these 2 genera as determined only by temperature, neglecting the influence of other factors such as the availability of limiting nutrients or inhibition of N_2 fixation by inputs of fixed nitrogen.

Our data predict that currently *Crocosphaera* potential maximum growth rates exceed those of *Trichodesmium* at the BATS Atlantic location throughout the summer and fall, while *Trichodesmium* has much higher potential growth rates in the winter and spring months when cooler water temperatures virtually preclude growth of *Crocosphaera* (Fig. 6a). At the Pacific HOT station, *Crocosphaera* potential growth rates are higher throughout the year, except during January and February when water temperatures are below minimum limits for this genus (Fig. 6b).

With simulated future increasing water temperature, *Crocosphaera* gains a large advantage as its potential growth rates are no longer constrained by winter temperatures below its minimum threshold, and its potential growth rates are predicted to exceed those of *Trichodesmium* throughout the annual cycle in both oceans (Fig. 6c,d). However, summer temperatures may exceed optimum levels at BATS and result in a seasonal depression of growth rates in both genera (Fig. 6c). Potential N_2- and CO_2-fixation rates of *Trichodesmium* and *Crocosphaera* will follow these same trends, subject of course to other constraints such as the availability of iron, phosphorus, CO_2, and NO_3^- pulses from mixing events. Thus, the temperature-dependence of growth, N_2 fixation, and primary production reported here may help to predict both spatial and seasonal patterns in the distribution of *Crocosphaera* and *Trichodesmium* in the future ocean.

Thomas et al. (2012) examined thermal tolerance curves from the literature for 194 cultured strains of phytoplankton, and found that in general their optimum growth temperatures were well correlated to the mean temperatures in the regimes where they were isolated. Many tropical species, though, originated in environments with mean temperatures that were equal to or higher than their optimum growth temperatures. They suggested that tropical phyto-

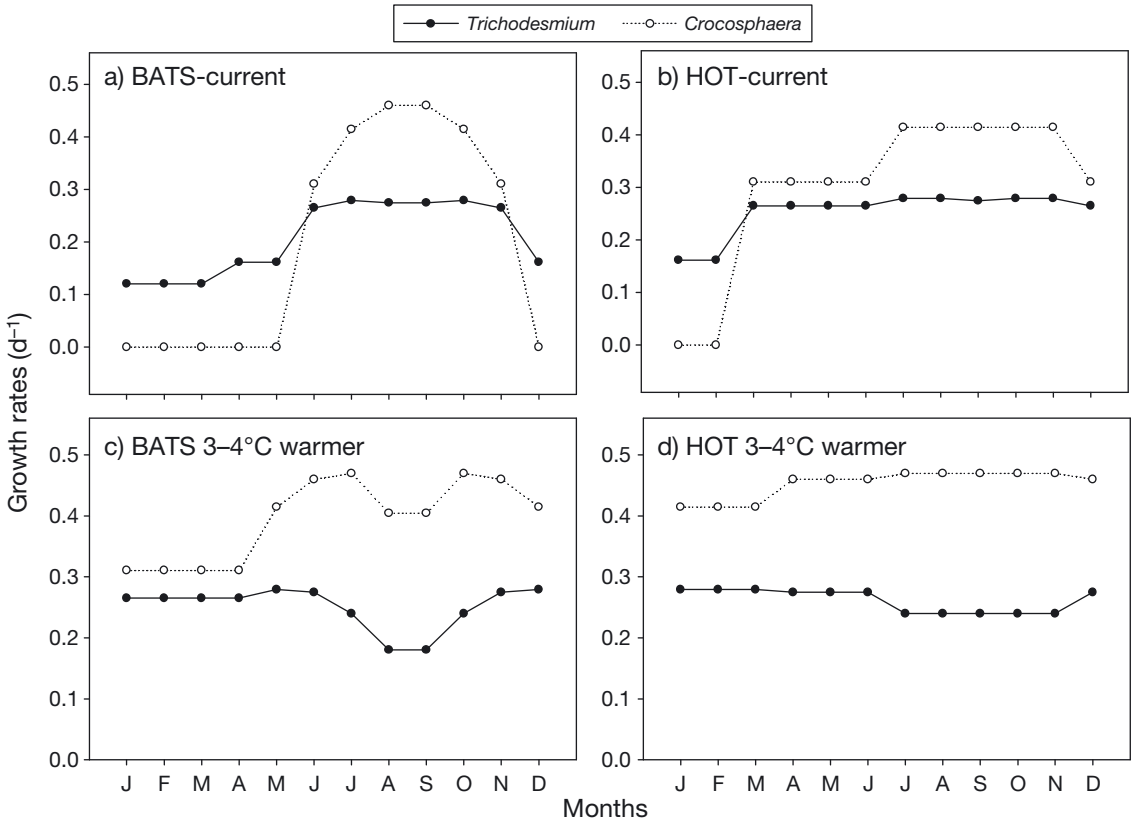

Fig. 6. Potential maximum growth rates based on our experimental results with *Trichodesmium* and *Crocosphera* across a complete seasonal cycle at (a) the current Bermuda Atlantic Time Series station (BATS) in the North Atlantic Ocean and (b) the current Hawaii Ocean Time Series station (HOT) in the North Pacific Ocean, compared to potential maximum growth rates subsequent to hypothetical future warming of 3 to 4°C at (c) BATS and (d) HOT

plankton communities may, therefore, be vulnerable to biodiversity losses as a consequence of future sea surface warming. Indeed, many regions near the equator are already at or approaching the temperature limits where maximum growth and N_2-fixation rates were observed in our cyanobacteria strains. In the future, the equatorial oceans could potentially exceed their maximum thermal limits in the summer, but blooms might still occur in the winter. As the oceans continue to grow warmer, *Trichodesmium* spp. and *Crocosphaera* spp. also have the potential to move poleward.

Both thermal exclusion of N_2-fixers from low latitudes, and biogeographic range extensions into higher latitudes, could have major implications for basin-scale nitrogen cycle processes and the overall productivity and composition of marine food webs. An ecosystem model suggests that N_2 fixation in the subtropical gyres will increase by 27% due to warming and stratification, and that physical factors are likely to be the main cause of this elevated N_2 fixation instead of changing supplies of subsurface nutrients (Boyd & Doney 2002). However, many studies have demonstrated that either iron or phosphorous largely limit N_2 fixation (Mahaffey et al. 2005). More recent field studies with natural populations of N_2 fixers have confirmed the importance of nutrient limitation of N_2 fixers (Hynes et al. 2009, Kitajima et al. 2009, Moore et al. 2009, Chappell et al. 2012). Although their results suggest that the distribution and N_2 fixation of microplanktonic diazotrophs including *Trichodesmium* spp. were controlled by the supply of iron and phosphorus, it is noteworthy that the temperatures in these regions during these studies ranged from 24 to 29°C, which fall into the optimal ranges for their growth and N_2 fixation. However, in areas where temperature was below 23 to 24°C, N_2-fixation rates were significantly reduced, despite relatively high availability of phosphorus and iron. Similarly, the highest concentrations of *nifH* filamentous phylotypes in the northern Atlantic Ocean were detected between 25 and 30°C when these regions received dust deposition (a source of iron) exceeding 2 g m^{-2} yr^{-1} (Langlois et al. 2008). This suggests that iron- and phosphorus-limited *Trichodesmium* N_2-fixation rates can

be further constrained when temperatures fall outside of optimal thermal ranges for growth.

In our study, cellular N:P ratios in both genera responded with temperature-related trends similar to those of growth, C fixation rates, and N_2-fixation rates. These thermal variations in N:P ratios could have resulted from changes in either cellular P or N quotas. Cellular P quotas in *Trichodesmium* have been shown not to change across 2 temperatures (18 and 25°C) (Fu et al. 2005). That study suggests that temperature does not directly control phosphate uptake rates, but does exert an indirect influence by controlling long-term growth rates. N:P ratios co-varied with N_2-fixation rates across our experimental temperature ranges in *Trichodesmium* and *Crocosphaera*, so this stoichiometric variability may be due to changes in cellular nitrogen content. Rates of enzymatically-mediated processes such as N_2 fixation are recognized to be dependent on temperature from basic metabolic principles (Raven & Geider 1988). We also observed a strong temperature dependence of RUBISCO-mediated C-fixation rates, consistent with this explanation.

Temperature also affected the cellular C:N ratios in all our strains of *Trichodesmium* and *Crocosphaera*, and like N:P ratios these trends closely followed those of growth rates. Maximum C:N ratios were attained at optimum growth temperatures, even though both C fixation and N_2 fixation peaked at about these same temperatures. Comparison of the magnitude of changes in C:N ratios with C-fixation and N_2-fixation changes across the same temperature ranges suggest that the reasons for this common response could differ between the 2 genera. For instance, in our 3 *Trichodesmium* isolates, C:N ratios increased an average of ~15% between 20 and 26°C; across this same temperature range, C-fixation rates increased an average of 97%, but N_2-fixation rates increased an average of only ~67%. The greater stimulation of C fixation than of N_2 fixation by rising temperature is, thus, roughly consistent with the observed rise in C:N ratios in this genus. In contrast, C:N ratios also increased between 24 and 30°C in our 4 *Crocosphaera* strains by a similar amount (~17%), but increases in C-fixation rates (~45%) and N_2-fixation rates (~48%) were roughly in balance. One possible cause for such increased C:N ratios when C-fixation and N_2-fixation changes are roughly synchronized could be preferential loss of fixed N relative to C, as indirect evidence suggests that some diazotrophs can lose substantial amounts of both elements (Mulholland 2007, Garcia et al. 2011, 2013). However, when the temperature exceeds 30°C, the C:N and N:P ratios of both *Trichodesmium* and *Crocosphaera* drop quickly, suggesting that as with growth and fixation rates, this temperature represents a critical tipping point for the elemental ratios of these 2 groups.

These temperature-mediated shifts in N:P and C:N ratios of key functional groups like N_2-fixing cyanobacteria could have large biogeochemical consequences. Major global nutrient and carbon cycle processes are affected by stoichiometric shifts in phytoplankton, including the relative degree of N versus P limitation of primary producers in the ocean, and the amount of C stored in the deep ocean by the 'biological pump' (Hutchins et al. 2009). Much of the carbon and nitrogen fixed by *Trichodesmium* and *Crocosphaera* is ultimately transferred to the rest of the plankton community (Mulholland & Bernhardt 2005), so altered C:N ratios under future warmer conditions could have broad implications for both primary and secondary production. Because the C:N and N:P ratios of *Crocosphaera* are considerably higher than those of *Trichodesmium*, community composition shifts between the 2 genera at elevated temperatures (Figs. 5 & 6) will also likely have large consequences for the ocean biogeochemical cycles of C, N, and P. One study shows that the C:N ratios of *Crocosphaera* increased and decreased during the light and dark period, respectively (Dron et al. 2012). Because we sampled during the early light period, C:N ratios of our *Crocosphaera* cultures should not be greatly overestimated. Another possible cause for the relatively higher C:N ratios of *Crocosphaera* could be production of carbon-rich extracellular polysaccharide (Sohm et al. 2011b).

Ocean warming will have other impacts on marine primary producers besides direct effects on growth rates. For instance, warming-enhanced water column stratification is likely to lower primary production by reducing vertical nutrient fluxes (Boyd et al. 2010, Hoegh-Guldberg & Bruno 2010). By examining a decade of ocean color data, Behrenfeld et al. (2006) showed that phytoplankton biomass in the tropical Pacific has declined, which they attributed to increased stratification and reduced vertical mixing. In the future, the effects of warming on the growth rates of N_2 fixers may be contrary to the impacts of reduced supplies of nutrients like P. Increases in N_2 fixation (and leakage of fixed N to other organisms) at higher temperature may partly counter the decreased vertical supply of nitrogen in the future. However, a temperature-driven increase in N_2 fixation may also increase P and iron utilization, leading to limitation of diazotrophy. Ocean acidification may need to be considered as well, since growth and N_2-

fixation rates of both *Trichodesmium* and *Crocosphaera* can be stimulated by increased CO_2 (Hutchins et al. 2007, 2009, 2013, Fu et al. 2008). All of these consequences of global change processes will directly or indirectly affect the physiological responses of N_2 fixers and other phytoplankton in the future ocean (Boyd & Hutchins 2012, Fu et al. 2012, Gao et al. 2012), making overall predictions problematic.

Our study only focused on 2 groups of N_2-fixing cyanobacteria: the unicellular group B (UCYN-B) and *Trichodesmium*. It is noteworthy that the uncultivated unicellular diazotrophic cyanobacteria group UCYN-A has been found at temperatures ranging from 15 to 30°C, with a peak biomass at 24°C (Moisander et al. 2010). Assuming an optimal temperature range of 23 to 24°C (Le Moal & Biegala 2009, Moisander et al. 2010), an increase of 3 to 4°C may not favor their growth and N_2 fixation in the North Atlantic and the North Pacific, since the temperature during most of the year in these areas is above 22°C. Although the actual thermal tolerance curves of UCYN-A remain to be determined, when and if cultures of this symbiotic group become available (Thompson et al. 2012), they do not seem to be likely candidates to fill the N_2-fixation niche in regions where future warming exceeds the tolerances of *Trichodesmium* and *Crocosphaera*.

It is possible that temperature will become even more important in dictating the distribution or controlling the N_2-fixation rates of diazotrophs in the future than it is today. However, predictions based on the results of our study do not consider other interacting climate change factors. Even more important is the question of whether long-term selection by warmer temperatures will result in adaptive changes in diazotroph thermal tolerance curves. A study examining 12 species of eukaryotic phytoplankton found large differences in their ability to adapt to warming, but cyanobacteria were not tested (Huertas et al. 2011). If future rising temperature leads to dispersal of *Crocosphaera* and *Trichodesmium* to higher latitudes, altered light regimes could be problematic. A modeling study showed that the optimal light period for N and C fixation in *Crocosphaera* is ~14 h (Grimaud et al. 2013). Longer dark periods at higher latitudes seem likely to favor N_2 fixation by *Crocosphaera* over *Trichodesmium*. However, increasing light intensities due to shallow stratification in the future ocean may also come into play, since increasing light intensities may decrease the optimal photoperiod for *Crocosphaera* N_2 fixation (Grimaud et al. 2013). Improving our knowledge of the capacity of N_2-fixing cyanobacteria to adapt to the interactive effects of warmer temperatures, increased CO_2, reduced nutrient supplies, and altered irradiance will be essential if we are to make accurate predictions of how these biogeochemically-critical organisms will respond to a rapidly changing ocean environment.

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LITERATURE CITED

Bankse K (1991) Rates of phytoplankton cell devision in the field and in iron enrichment experiments. Limnol Oceanogr 36:1886–1898

Behrenfeld MJ, O'Malley RT, Siegel DJ McClain CR and others (2006) Climate-driven trends in contemporary ocean productivity. Nature 444:752–755

Bell PRF, Elmetri I, Uwins P (1999) Nitrogen fixation by *Trichodesmium* spp. in the central and Northern Great Barrier Reef Lagoon: relative importance of the fixed-nitrogen load. Mar Ecol Prog Ser 186:119–126

Bench SR, Heller P, Frank I, Arciniega M, Shilova IN, Zehr JP (2013) Whole genome comparisons of 6 *Crocosphaera watsonii* strains with differing phenotypes. J Phycol 49: 786–801

Berman-Frank I, Quigg A, Finkel ZV, Irwin AJ, Haramaty L (2007) Nitrogen-fixation strategies and Fe requirements in cyanobacteria. Limnol Oceanogr 52:2260–2269

Bissett WP, Walsh JJ, Dieterle DA, Carder KL (1999) Carbon cycling in the upper waters of the Sargasso Sea: I. Numerical simulation of differential carbon and nitrogen fluxes. Deep-Sea Res I 46:205–269

Boyd PW, Doney SC (2002) Modelling regional responses by marine pelagic ecosystems to global climate change. Geophys Res Lett 29, 1806, doi:10.1029/2001GL014130

Boyd PW, Hutchins DA (2012) Understanding the responses of ocean biota to a complex matrix of cumulative anthropogenic change. Mar Ecol Prog Ser 470:125–135

Boyd PW, Strzepek R, Fu FX, Hutchins DA (2010) Environmental control of open ocean phytoplankton groups: now and in the future. Limnol Oceanogr 55:1353–1376

Boyd PW, Rynearson TA, Armstrong EA, Fu FX and others (2013) Marine phytoplankton temperature versus growth responses from polar to tropical waters – outcome of a scientific community-wide study. PLoS ONE 8:e63091

Breitbarth E, Oschilles A, LaRoche L (2007) Physiological constraints on the global distribution of *Trichodesmium*-effect of temperature on diazotrophy. Biogeosciences 4: 53–61

Capone DG (1993) Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In Kemp PF, Sherr BF, Sherr EB, and Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, FL, p 621–631

Capone DG, Carpenter EJ (1982) Nitrogen fixation in the marine environment. Science 217:1140–1142

Capone DG, Zehr JP, Paerl HW, Bergman B, Carpenter EJ (1997) *Trichodesmium*, a globally significant marine cyanobacterium. Science 276:1221–1229

Carpenter EJ (1983) Physiology and ecology of marine

planktonic Oscillatoria (*Trichodesmium*). Mar Biol Lett 4: 69–85

Chang J, Chiang KP, Gong GC (2000) Seasonal variation and cross-shelf distribution of the nitrogen-fixing cyanobacterium, *Trichodesmium*, in southern East China Sea. Cont Shelf Res 20:479–492

Chappell PD, Moffett JW, Hynes AM, Webb EA (2012) Molecular evidence of iron limitation and availability in the global diazotroph *Trichodesmium.* ISME J 6: 1728–1739

Chen YB, Dominic B, Mellon MT, Zehr JP (1998) Circadian rhythm of nitrogenase gene expression in the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. strain IMS 101. J Bacteriol 180: 3598–3605

Church MJ, Bjorkman KM, Karl DM, Saito MA, Zehr JP (2008) Regional distributions of nitrogen-fixing bacteria in the Pacific Ocean. Limnol Oceanogr 53:63–77

Domingues CM, Church JA, White NJ, Glecker PJ, Wijffels SE, Barker PM, Dunn JR (2008) Improved estimates of upper-ocean warming and multi-decadal sea-level rise. Nature 453:1090–1093

Dron A, Rabouille S, Claquin P, Le Roy B, Talec A, Sclandra A (2012) Light-dark (12: 12) cycle of carbon and nitrogen metabolism in *Crocosphaera watsonii* WH8501: relation to the cell cycle. Environ Microbiol 14:967–981

Eppley RW (1972) Temperature and phytoplankton growth in the sea. Fish Bull 70:1063–1085

Falcón LI, Pluvinage S, Carpenter EJ (2005) Growth kinetics of marine unicellular N_2-fixing cyanobacterial isolates in continuous culture in relation to phosphorus and temperature. Mar Ecol Prog Ser 285:3–9

Falkowski PG, Barber RT, Smetacek V (1998) Biogeochemical controls and feedbacks on ocean primary production. Science 281:200–206

Feng Y, Hare CE, Leblanc K, Rose JM and others (2009) Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. Mar Ecol Prog Ser 388: 13–25

Fu FX, Bell PRF (2003) Factors affecting N_2 fixation by the cyanobacterium *Trichodesmium* sp. GBRTRLI101. FEMS Microbiol Ecol 45:203–209

Fu FX, Zhang Y, Bell PRF, Hutchins DA (2005) Phosphate uptake and growth kinetics of *Trichodesmium* (Cyanobacteria) isolates from the North Atlantic Oceans and the Great Barrier Reef, Australia. J Phycol 41:62–73

Fu FX, Warner ME, Zhang Y, Feng Y, Hutchins DA (2007) Effects of increased temperature and CO_2 on photosynthesis, growth and elemental ratios of marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). J Phycol 43:485–496

Fu FX, Mulholland MR, Garcia NS, Beck A and others (2008) Interactions between changing pCO_2, N_2 fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera.* Limnol Oceanogr 53:2472–2484

Fu FX, Place AR, Garcia NS, Hutchins DA (2010) CO_2 and phosphate availability control the toxicity of the harmful bloom dinoflagellate *Karlodinium veneficum.* Aquat Microb Ecol 59:55–65

Fu FX, Tatters AO, Hutchins DA (2012) Global change and the future of harmful algal blooms in the ocean. Mar Ecol Prog Ser 470:207–233

Galloway JN, Cowling EB (2002) Reactive nitrogen and the world: 200 years of change. Ambio 31: 64–71

Gao K, Helbling EW, Häder DP, Hutchins DA (2012) Responses of marine primary producers to interactions between ocean acidification, solar radiation, and warming. Mar Ecol Prog Ser 470:167–189

Garcia NS, Fu FX, Breene CL, Bernhardt PW, Mulholland MR, Sohm JA, Hutchins DA (2011) Interactive effects of irradiance and CO_2 on CO_2- and N_2 fixation in the diazotroph *Trichodesmium erythraeum* (Cyanobacteria). J Phycol 47:1292–1303

Garcia NS, Fu FX, Breene CL, Yu E, Bernhardt PW, Mulholland MR, Hutchins DA (2013) Combined effects of CO_2 and irradiance on the unicellular N_2-fixing cyanobacterium *Crocosphaera watsonii*: a comparison of 2 isolates from the Western Tropical Atlantic Ocean. Eur J Phycol 48:128–139

Goebel NL, Turk KA, Achilles KM, Montoya JP, Edwards CA, Zehr JP (2010) Abundance and distribution of major groups of diazotrophic cyanobacteria and their potential contribution to N_2 fixation in the tropical Atlantic ocean. Environ Microbiol 12:3272–3289

Goldman JC, Carpenter EJ (1974) A kinetic approach to the effect of temperature on algal growth. Limnol Oceanogr 19:756–766

Grimaud GM, Dron A, Rabouille S, Sciandra A, Bernard O (2013) Modeling light-dark regime influence on the carbon-nitrogen metabolism in a unicellular diazotrophic cyanobacterium. HAL-INRIA 00854479, http://hal.inria.fr/hal-00854479

Hare CE, Leblanc K, DiTullio GR, Kudela RM and others (2007) Consequences of increased temperature and CO_2 for phytoplankton community structure in the Bering Sea. Mar Ecol Prog Ser 352:9–16

Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world's marine ecosystems. Science 328: 1523–1528

Huertas IE, Rouco M, López-Rodas V, Costas E (2011) Warming will affect phytoplankton differently: evidence through a mechanistic approach. Proc Biol Sci 278: 3534–3543

Hutchins DA, Fu FX, Zhang Y, Warner ME and others (2007) CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry. Limnol Oceanogr 52:1293–1304

Hutchins DA, Mulholland MR, Fu FX (2009) Nutrient cycles and marine microbes in a CO_2-enriched ocean. Oceanography 22:128–145

Hutchins DA, Fu FX, Webb EA, Walworth N, Tagliabue A (2013) Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations. Nat Geosci 6: 790–795

Hynes AM, Chappell P, Dyhrman ST, Doney SD, Webb EA (2009) Cross-basin comparison of phosphorus stress and nitrogen fixation in *Trichodesmium.* Limnol Oceanogr 54:1438–1448

Hynes AM, Webb EA, Doney SC, Waterbury JB (2012) Comparison of cultured *Trichodesmium* (Cyanophyceae) with species characterized from the field. J Phycol 48:196–210

IPCC (Intergovernmental Panel on Climate Change) (2007) Climate change 2007: the physical science basis: working group I. Contribution to the fourth assessment report of the IPCC, Cambridge University Press, Cambridge, p 7–22

Karl DM, Michaels A, Bergman B, Capone D and others (2002) Dinitrogen fixation in the world's oceans. Biogeochemistry 57/58:47–98

Kitajima S, Furuya K, Hashihama F, Takeda S, Kanda J (2009) Latitudinal distribution of diazotrophs and their nitrogen fixation in the tropical and subtropical western North Pacific. Limnol Oceanogr 54:537–547

Langlois RJ, LaRoche J, Raab PA (2005) Diazotrophic diversity and distribution in the tropical and subtropical Atlantic ocean. Appl Environ Microbiol 71:7910–7919

Langlois RJ, Hummer D, LaRoche J (2008) Abundances and distributions of the dominant *nifH* phylotypes in the Northern Atlantic Ocean. Appl Environ Microbiol 74: 1922–1931

Le Moal M, Biegala I (2009) Diazotrophic unicellular cyanobacteria in the northwestern Mediterranean Sea: a seasonal cycle. Limnol Oceanogr 54:845–855

Letelier RM, Karl DM (1998) *Trichodesmium* spp. physiology and nutrient fluxes in the North Pacific subtropical gyre. Aquat Microb Ecol 15:265–276

Lugomela C, Lyimo TJ, Bryceson I, Semesi AK (2002) *Trichodesmium* in coastal waters of Tanzania: diversity, seasonality, nitrogen and carbon fixation. Hydrobiologia 477:1–13

Mahaffey C, Michaels AF, Capone DG (2005) The conundrum of marine N_2 fixation. Am J Sci 305:546–595

Mazard SL, Fuller NJ, Orcutt KM, Bridle O, Scanlan DJ (2004) PCR analysis of the distribution of unicellular cyanobacterial diazotrophs in the Arabian Sea. Appl Environ Microbiol 70:7355–7364

Moisander PH, Beinart RA, Hewson I, White AE and others (2010) Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain. Science 327:1512–1514

Monteiro F, Dutkiewicz MS, Follows MJ (2011) Biogeographical controls on the marine nitrogen fixers. Global Biogeochem Cycles 25, doi:10.1029/2010GB003902

Montoya JP, Voss M, Kaehler P, Capone DG (1996) A simple, high precision tracer assay for dinitrogen fixation. Appl Environ Microbiol 62:986–993

Moore CM, Mills MM, Achterberg EP, Geider RJ and others (2009) Large-scale distribution of Atlantic nitrogen fixation controlled by iron availability. Nat Geosci 2:867–871

Mulholland MR (2007) The fate of nitrogen fixed by diazotrophs in the ocean. Biogeosciences 4:37–51

Mulholland MR, Bernhardt PW (2005) The effect of growth rate, phosphorous concentration, and temperature on N_2 fixation, carbon fixation, and nitrogen release in continuous cultures of *Trichodesmium* IMS101. Limnol Oceanogr 50:839–849

Needoba JA, Foster RA, Sakamoto C, Zehr JP, Johnson KS (2007) Nitrogen fixation by unicellular diazotrophic cyanobacteria in the temperate oligotrophic North Pacific Ocean Limnol Oceanogr 52:1317–1327

Nicklisch A, Shatwell T, Köhler J (2008) Analysis and modeling of the interactive effects of temperature and light on phytoplankton growth and relevance for the spring bloom. J Plankton Res 30:75–91

Price NM, Harrison GI, Hering JG, Hudson RJM and others (1989) Preparation and chemistry of the artificial algal culture medium Aquil. Biol Oceanogr 6:443–461

Raven J, Geider RJ (1988) Temperature and algal growth. New Phytol 110:441–461

Redfield AC (1934) On the proportions of organic derivations in sea water and their relation to the composition of plankton. In Daniel RJ (ed) James Johnstone memorial Vol. University Press of Liverpool, Liverpool, p 177–192

Rose JM, Feng Y, Gobler CJ Gutierrez R, Hare CE, Leblanc K, Hutchins DA (2009) Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. Mar Ecol Prog Ser 388:27–40

Sañudo-Wilhelmy SA, Kustka A, Gobler C, Hutchins DA and others (2001) Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central Atlantic Ocean. Nature 411:66–69

Sohm JA, Webb EA, Capone DG (2011a) Emerging patterns of marine nitrogen fixation. Nat Rev Microbiol 9:499–508

Sohm JA, Edwards BA, Wilson BG, Webb EA (2011b) Constitutive extracellular polysaccharide (EPS) production by specific isolates of *Crocosphaera watsonii*. Frontiers Microbiol 2, 00229, doi:10.3389/fmicb.2011.00229

Staal M, Meysman FJR, Stal LJ (2003) Temperature excludes N_2-fixing heterocystous cyanobacteria in the tropical oceans. Nature 425: 504–507

Staal M, Hekkert STL, Brummer GJ, Veldhuis M, Sikkens C, Persijn S, Stal LJ (2007) Nitrogen fixation along a north-south transect in the eastern Atlantic Ocean. Limnol Oceanogr 52:1305–1316

Stal LJ (2009) Is the distribution of nitrogen-fixing cyanobacteria in the oceans related to temperature? Environ Microbiol 11:1632–1645

Thomas MK, Kremer CT, Klausmeier CA, Litchman E (2012) A global pattern of thermal adaptation in marine phytoplankton. Science 338:1085–1088

Thompson AW, Foster RA, Krupke A, Carter BJ and others (2012) Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. Science 337:1546–1550

Tuit C, Waterbury J, Ravizza G (2004) Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. Limnol Oceanogr 49:978–990

Tyrrell T, Marñón E, Poulton AJ, Bowie AR, Harbour DS, Woodward EMS (2003) Large-scale latitudinal distribution of *Trichodesmium* spp. in the Atlantic Ocean. J Plankton Res 25:405–416

Webb EA, Ehrenreich IM, Brown SL, Valois FW, Waterbury JB (2009) Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocosphaera watsonii*, isolated from the open ocean. Environ Microbiol 11:338–348

Welschmeyer NA (1994) Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol Oceanogr 39:1985–1992

Westberry TK, Siegel DA (2006) Spatial and temporal distribution of *Trichodesmium* blooms in the world's oceans. Global Biogeochem Cycles 20, GB4016, doi:10.1029/2005GB002673

Weyhenmeyer GA (2001) Warmer winters: are planktonic algal populations in Sweden's largest lakes affected? Ambio30: 565–571

Zehr JP (2011) Nitrogen fixation by marine cyanobacteria. Trends Microbiol 19:162–173

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