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Temperature effects on the growth rate of marine picoplankton

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ABSTRACT: Marine picophytoplankton are responsible for a significant proportion of primary production in the ocean, and they are most abundant in warm and oligotrophic oceans. We compiled 2 datasets to evaluate 2 hypotheses related to the direct effects of temperature on picophytoplankton growth: (1) the optimal growth temperatures of picophytoplankton are higher than those of other larger phytoplankton; and (2) the activation energies of picophytoplankton growth are higher than those of larger phytoplankton. We found that based on the laboratory data, the optimal temperatures for picophytoplankton growth were not significantly different from those of other phytoplankton after controlling the effect of environmental mean temperature; however, the activation energies of picophytoplankton were marginally significantly higher than those of larger phytoplankton. From the field data, the growth rates of *Synechococcus* and picoeukaryotes increased with increasing temperature and nitrate and chl *a* concentrations, whereas the growth rates of *Prochlorococcus* were not dependent on temperature and decreased with light intensity and nitrate concentrations. When keeping other factors constant, the activation energies of growth rates of *Synechococcus* and picoeukaryotes were 0.53 ± 0.07 (mean ± SE) and 0.62 ± 0.11 eV higher, respectively, than the 0.36 eV estimated for bulk phytoplankton. Our results suggest that *Synechococcus* and picoeukaryotes might benefit from warming in mesotrophic waters, and the growth of *Prochlorococcus* might be retarded by increasing temperature and light levels but might benefit from the increasing oligotrophication in oligotrophic surface oceans.

KEY WORDS: Temperature · Picophytoplankton · Growth rate · Optimal growth temperature · Activation energy

INTRODUCTION

Global warming has both direct and indirect effects on marine organisms and ecosystem functioning. One of the key aspects in predicting the response of primary production to warming is to quantify the direct effects of temperature on phytoplankton growth rate (Sarmiento et al. 2004, Taucher & Oschlies 2011).

Picophytoplankton, usually defined as phytoplankton cells smaller than 2 µm, are an important component of the phytoplankton community, particularly in the oligotrophic open ocean (Sieburth et al. 1978, Liu et al. 1997, Raven 1998). Picophytoplankton are mostly composed of unicellular cyanobacteria (e.g. *Synechococcus* and *Prochlorococcus*) and picoeukaryotes. Picophytoplankton often dominate high-temperature and low-nutrient environments and will likely be favored under ocean warming (Agawin et al. 2000, Li 2002, Finkel et al. 2010, Moran et al. 2010). However, it is still not clear whether high temperature (i.e. direct effects of temperature) or low nutrients (i.e. indirect effects of temperature) primarily leads to the success of picophytoplankton since temperature and nutrient levels are often negatively correlated in the ocean (Finkel et al. 2010).

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In freshwater systems, cyanobacteria tend to grow better at high temperature than other phytoplankton, so it has been argued that that blue-green blooms would occur more frequently under global warming (Jöhnk et al. 2008, Paerl & Huisman 2008, but see Lürling et al. 2013). Will marine cyanobacteria such as *Prochlorococcus* and *Synechococcus* respond to increasing temperature in a fashion similar to freshwater cyanobacteria, presumably because of their shared physiological features? At present, there are ample data on picophytoplankton biomass vs. temperature (Moran et al. 2010, Flombaum et al. 2013), but syntheses of picophytoplankton growth rates versus temperature are scarce. It is important to note that the statistical associations between phytoplankton abundance and temperature cannot be easily translated into temperature-growth rate relationships.

There are 2 possible mechanisms of direct temperature effects that might lead to the relative success of picophytoplankton at high temperatures (Finkel et al. 2010, Dell et al. 2014). The first is that the optimal growth temperatures of picophytoplankton, particularly the marine cyanobacteria *Prochlorococcus* and *Synechococcus*, might be higher than those of larger phytoplankton. The second is that the increasing rate of growth with increasing temperature (i.e. the temperature coefficient of growth rate) of picophytoplankton might be greater than that of larger phytoplankton. For example, Jöhnk et al. (2008) pointed out that the freshwater cyanobacterium *Microcystis* had higher optimal growth temperatures and temperature coefficients of growth than coexisting diatoms and green algae. In modeling exercises, the temperature coefficient of phytoplankton growth rate is often treated as a constant. In actuality, however, it might differ among different groups. A commonly used function describing the temperature dependence of growth rate is the Arrhenius equation, $\mu = \mu_c e^{-E/kT}$, in which μ is the growth rate varying with temperature, μ_c is a normalization constant, E is the activation energy (eV, 1 eV = 96.49 kJ mol^{-1}), k is the Boltzman constant (8.62×10^{-5} eV K^{-1}), and T is absolute temperature (K) (Brown et al. 2004). The activation energy E here is the coefficient describing how fast the growth rate increases with temperature. The activation energy for photosynthesis and phytoplankton growth (~0.3–0.4 eV) can be substantially lower than that of respiration (~0.6–0.7 eV; Eppley 1972, Tang 1995, López-Urrutia et al. 2006, Chen et al. 2012, Regaudie-de-Gioux & Duarte 2012). As the temperature dependence of phytoplankton growth rate and photosynthesis might be largely determined by the thermal kinetics of RuBisCO enzymes, such as the temperature dependence of carboxylation and oxygenation (Farquhar et al. 1980, Davison 1991, Bernacchi et al. 2001, Allen et al. 2005), the activation energy can be species-specific (Tcherkez et al. 2006).

When scaling up from the organismal level to the community level, it is important to distinguish the difference in temperature coefficients obtained from laboratory monocultures and field measurements on an assemblage. It has been noted that the within-species temperature coefficient is often higher than that of cross-species (Raven & Geider 1988). Cultured strains could not fully represent the communities in the ocean, and the variations of growth rates associated with different species or ecotypes could be overlooked. While it is impractical to obtain the temperature coefficient of growth rate for every species in the sea, an appropriate average value is desired to simplify this complexity in large-scale modeling exercises. Therefore, it is necessary to extract the information from direct field measurements.

In this paper, we use both laboratory and field data on picophytoplankton growth rates to evaluate 2 hypotheses that address the direct effects of temperature on picophytoplankton growth rate: (1) the optimal growth temperatures of picophytoplankton are higher than those of larger phytoplankton; and (2) the activation energies of picophytoplankton growth are higher than those of larger phytoplankton. We also obtain other co-variables that also influence phytoplankton growth rates and likely affect estimates of optimal growth temperature and activation energy. We compare the optimal growth temperature and activation energy of picophytoplankton to those of other phytoplankton using generalized additive models (GAMs) to incorporate the effects of all selected environmental factors.

MATERIALS AND METHODS

Laboratory data from literature

We extracted data from the literature that measured specific growth rates of marine phytoplankton cultured under light- and nutrient-saturated conditions and constructed a dataset with optimal growth temperature and activation energy of each species (Supplement 1 at www.int-res.com/articles/suppl/m505p037_supp1.xls). When estimating the optimal growth temperature, we only included experiments where both increasing and decreasing parts of the temperature response curves were

available. When calculating the activation energy, we first discarded the data at supraoptimal temperatures (i.e. at temperatures above which μ declines with increasing temperature) so that we dealt with only the increasing part of the temperature response curve. The activation energy was calculated as the positivised slope of the linear regression between ln μ versus $1/kT$. We defined 'picophytoplankton' as cells with equivalent spherical diameters smaller than 2 μm (i.e. cell volume <4.2 μm^3) (Sieburth et al. 1978). Cell size data were obtained from secondary literature if the primary literature did not report the size data. As the annual mean temperature of the locations where the phytoplankton strains were isolated strongly affects the optimal growth temperature (Thomas et al. 2012), we also gathered the longitudes and latitudes of these locations and estimated their annual mean temperatures based on data from the World Ocean Atlas 2009 (www.nodc.noaa.gov/OC5/WOA09/pr_woa09.html) using the function 'knn' in the R package 'class'. The locations where the picophytoplankton strains were originally isolated are shown in Fig. 1.

We constructed GAMs to fit the data on optimal growth temperatures and activation energies with environmental annual mean temperature and size using the function 'gam' in the R package 'mgcv' (Hastie & Tibshirani 1986, Wood 2006, R Development Core Team 2013). GAMs assume that the effects of each predictor on the response variable can be described by smooth functions, and these effects are additive. The advantage of a GAM is that a prior function is not required for data fitting. The model structure for optimal growth temperature or activation energy is:

$$y = s(AnnTemp) + s(lnVol) \quad (1)$$

where y represents optimal growth temperature or activation energy, *AnnTemp* represents the annual mean temperature where the strain was isolated, *lnVol* is the log-transformed cell volume, and s represents a cubic regression spline used for fitting the data. The advantage of using a spline is that it has minimal integrated square second derivatives so that it can achieve the highest smoothness. To minimize overfitting, a penalty is added to the regression to control the smoothness of the fitted curve, and we set the gamma = 1.4 in the 'gam' function to force the effective degrees of freedom to count as 1.4 times the degrees of freedom in the generalized cross-validation score (Wood 2006).

Field data

We obtained only the activation energies of phytoplankton growth from field data, as the field growth rates appear to monotonously increase with temperature, and we were unable to reliably estimate an optimal temperature. The growth rates of field picophytoplankton were estimated using the dilution technique (Landry & Hassett 1982). We merged the data from our own measurements in the China seas with the data collected from the literature (Fig. 1; Supplement 2 at www.int-res.com/articles/suppl/m505p037_supp2.xls). The *in situ* phytoplankton growth

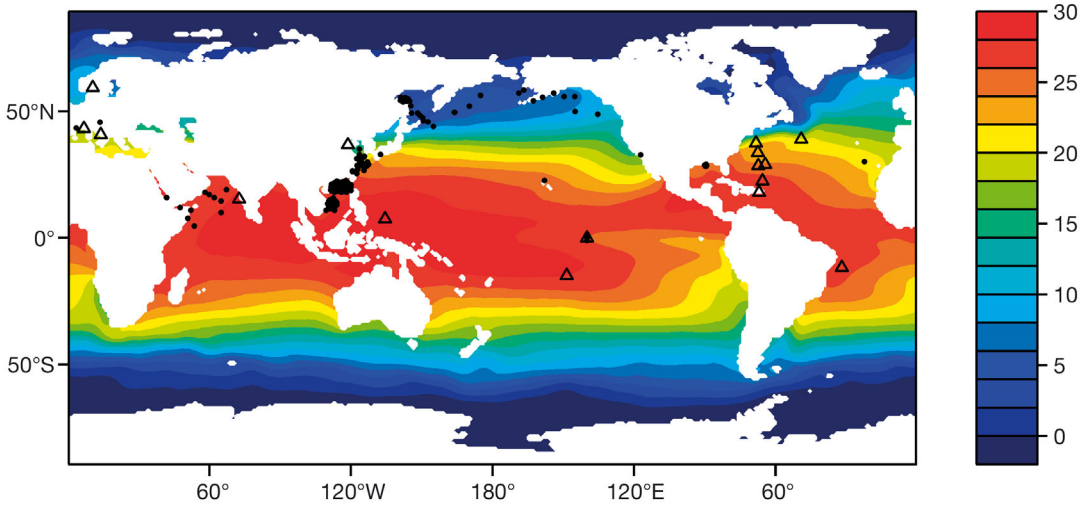

Fig. 1. Global ocean map overlaid with annual mean temperature (°C). Filled circles indicate sites where field picophytoplankton growth rates were measured. Open triangles indicate locations where laboratory picophytoplankton cultures were originally isolated

rates measured by the dilution technique were consistent with estimates by other approaches (e.g. the ^{14}C method; Calbet & Landry 2004, Landry et al. 2011). The dilution technique is also the most widely used method to estimate growth rates of subpopulations of phytoplankton in the field because of its easy manipulation (Laws 2013). In total, we collected 99, 243, and 216 growth rate estimates for *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, respectively.

Here we describe the detailed procedures of the dilution technique for our own experiments. The procedures of other studies were largely similar to ours. We first added measured amounts of particle-free water, prepared by gravity filtering the seawater through a 0.2 µm filter capsule (Pall Corporation) into 1 l polycarbonate bottles. The bottles were then gently filled with whole seawater to full capacity. A gradient of dilution levels of natural seawater were prepared. Sometimes for satisfying the key assumptions of the dilution technique (Landry & Hassett 1982), the bottles were enriched with inorganic nutrients to promote constant phytoplankton growth. The added nutrient recipes were 0.5 µmol l^{-1} NH_4Cl, 0.03 µmol l^{-1} KH_2PO_4, 1 nmol l^{-1} $FeCl_3$, and 0.1 nmol l^{-1} $MnCl_2$ for oceanic stations and 10 µmol l^{-1} $NaNO_3$ and 1 µmol l^{-1} KH_2PO_4 for coastal and estuarine stations. Bottles filled with unfiltered seawater without nutrient addition served as no-nutrient controls. Two additional bottles filled with unfiltered seawater were sacrificed for initial samples for chl *a* and flow cytometric (FCM) analyses. All of the bottles were tightly capped and incubated for 24 h in a deck incubator cooled by running surface seawater and covered with 1 layer of neutral screen to simulate the *in situ* light environment (~50% of surface irradiance). After incubation, samples were taken from each bottle for chl *a* and FCM analyses.

FCM samples were fixed with 0.5% buffered paraformaldehyde and frozen at −80°C until analysis (Vaulot et al. 1989). Three populations of picophytoplankton (i.e. *Prochlorococcus*, *Synechococcus*, and picoeukaryotes) were delineated and enumerated based on light scattering and fluorescence on a Becton-Dickson FACSCalibur flow cytometer (Olson et al. 1993). The exact flow rate was calibrated by weighing a tube filled with distilled water before and after running for certain time intervals, and the flow rate was estimated as the slope of a linear regression curve between elapsed time and weight differences (Li & Dickie 2001). For chl *a* analyses, 100 to 500 ml of seawater was filtered onto 25 mm glass-fibre GF/F filters (Whatman) under low vacuum (<150 mm Hg). The filters were frozen and stored in liquid nitrogen until analysis. Upon return to the lab, the filters were soaked in 90% acetone, and fluorescence was measured using the non-acidification method on a Turner Designs fluorometer (Welschmeyer 1994).

The growth rates of picophytoplankton were estimated as follows. Assuming an exponential growth model, we calculated the net growth rate (k_i) of phytoplankton in each dilution treatment according to the formula $k_i = \ln[C_i/(D_i{}^*C_o)]$, where C_i was phytoplankton abundance in the i^{th} treatment bottle at 24 h, D_i was the dilution factor (proportion of unfiltered seawater) of the i^{th} treatment, and C_o was the initial phytoplankton abundance. Phytoplankton growth rate was estimated as the intercept of a linear regression of net growth rate against the dilution factor (Landry & Hassett 1982). In cases where nutrients were added into the bottles, both nutrient-enriched growth rate (μ_n) and non-enriched growth rates (μ_o) were obtained.

Nitrate concentrations were measured following the standard protocol (Parsons et al. 1984). The data for photosynthetically active radiation (PAR) were obtained from the Goddard Earth Sciences Data and Information Services Center (http://disc.sci.gsfc.nasa.gov/). To minimize the problem of nutrient limitation or nutrient inhibition (Worden & Binder 2003), we chose the higher value of μ_o and μ_n when both estimates were available.

We also used GAMs to estimate the activation energy of picophytoplankton growth rate with the effects of other environmental factors (i.e. chl *a*, PAR, and nitrate) taken into consideration. We considered the effect of chl *a* because picophytoplankton community compositions likely vary with the trophic status of the environment that can be represented by chl *a*. The values 'chl *a*' and 'nitrate' were log-transformed before GAM analysis. The GAM model structure is:

$$\ln \mu = s(lnChl) + s(PAR) + s(lnNitrate) + E(1/kT_c - 1/kT) \quad (2)$$

where µ is the growth rate of picophytoplankton measured in the field, *lnChl* is the natural log-transformed chl *a* concentration, *lnNitrate* is the log-transformed nitrate concentration, E is the activation energy to be estimated, k is the Boltzman constant, and T_c and T are the reference temperature (288 K) and *in situ* absolute temperature (K), respectively. As data on the growth rates of larger phytoplankton are very scarce in field experiments, we compared the activation energy of picophytoplankton to that of bulk phytoplankton that include both small and large phytoplankton. The activation energy of bulk phyto-

Table 1. Temperature-growth rate laboratory experiments of marine picophytoplankton species

Species	Optimal temperature (°C)	Activation energy (eV, mean ± SE)	Reference
Prochlorococcus marinus MED4	24	1.07 ± 0.13	Moore et al. (1995)
P. marinus SS120	24	1.02 ± 0.24	Moore et al. (1995)
P. marinus MED4	24	0.75 ± 0.12	Johnson et al. (2006)
P. marinus MIT9215	25	1.07 ± 0.07	Johnson et al. (2006)
P. marinus MIT9312	25	0.81 ± 0.07	Johnson et al. (2006)
P. marinus MED4	≥24	0.93 ± 0.02	Kulk et al. (2012)
P. marinus NATL2A	24	0.69 ± 0.07	Zinser et al. (2007)
P. marinus MIT9313	28	0.57 ± 0.06	Zinser et al. (2007)
Prochlorococcus sp. MIT9313	≥24	1.18[a]	Kulk et al. (2012)
Synechococcus CCFWC502	20	2.29[a]	Boyd et al. (2013)
Synechococcus WH8103	28	0.70 ± 0.10	Moore et al. (1995)
Synechococcus CCMP837	18	Not available	Mackey et al. (2013)
Synechococcus CCMP1183	24	1.15 ± 0.53	Mackey et al. (2013)
Synechococcus CCMP1379	≥27	1.02 ± 0.20	Mackey et al. (2013)
Synechococcus CCMP1630	≥27	1.08 ± 0.16	Mackey et al. (2013)
Synechococcus CCMP1632	21	2.42[a]	Mackey et al. (2013)
Synechococcus WH8102	21	2.91[a]	Mackey et al. (2013)
Synechococcus CCMP2606	≥27	1.67 ± 0.21	Mackey et al. (2013)
Synechococcus PCC7002	≥27	0.91 ± 0.23	Mackey et al. (2013)
Micromonas pusilla	20	0.21 ± 0.21	Throndsen (1976)
Nannochloris oculata	25	0.18 ± 0.06	Cho et al. (2007)
Ostreococcus sp.	20	0.83[a]	Kulk et al. (2012)

[a]Standard error cannot be calculated because of only 2 data points

plankton has been estimated in an earlier report using similar approaches (Chen et al. 2012).

RESULTS

Laboratory data from literature

Our data compilation of laboratory cultures consists of 111 microphytoplankton strains (>20 µm), 224 nanophytoplankton strains (~2–20 µm), and 22 picophytoplankton strains (<2 µm), with the details of picophytoplankton species listed in Table 1. The optimal growth temperatures of *Prochlorococcus* ranged from 24 to 28°C. The optimal growth temperatures of *Synechococcus* were mostly between 18 and 28°C. The optimal growth temperatures of 3 picoeukaryote strains ranged from 20 to 25°C. The median optimal temperature of picophytoplankton was 24°C with the 95% CI from 19 to 28°C (Fig. 2A). Comparatively, the median optimal temperatures of nano- and microphytoplankton were 22 and 20°C, respectively (Fig. 2A). Therefore, the optimal growth temperatures of marine picophytoplankton were within the range of those of other larger phytoplankton, confirmed by the nonparametric rank Wilcoxon test ($p > 0.05$). The GAM with annual mean temperature and cell volume as

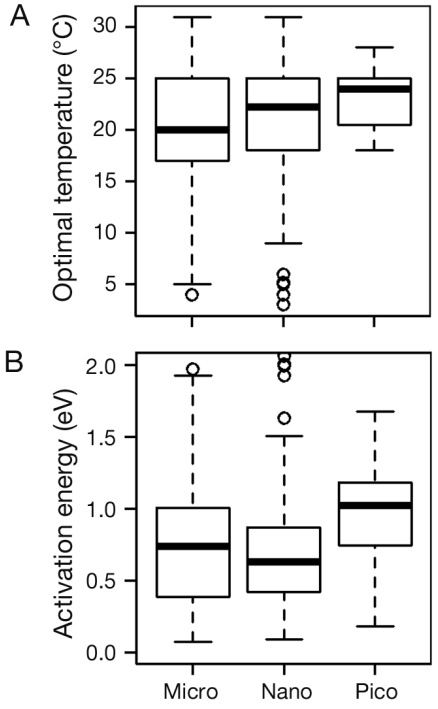

Fig. 2. (A) Optimal growth temperatures and (B) within-species activation energies of 109 strains of microphytoplankton (Micro, >20 µm), 225 strains of nanophytoplankton (Nano, ~2–20 µm), and 22 strains of picophytoplankton (Pico, <2 µm). Thick line: median; box: 25th–75th percentiles; whiskers: 10th–90th percentiles; dots: extreme values

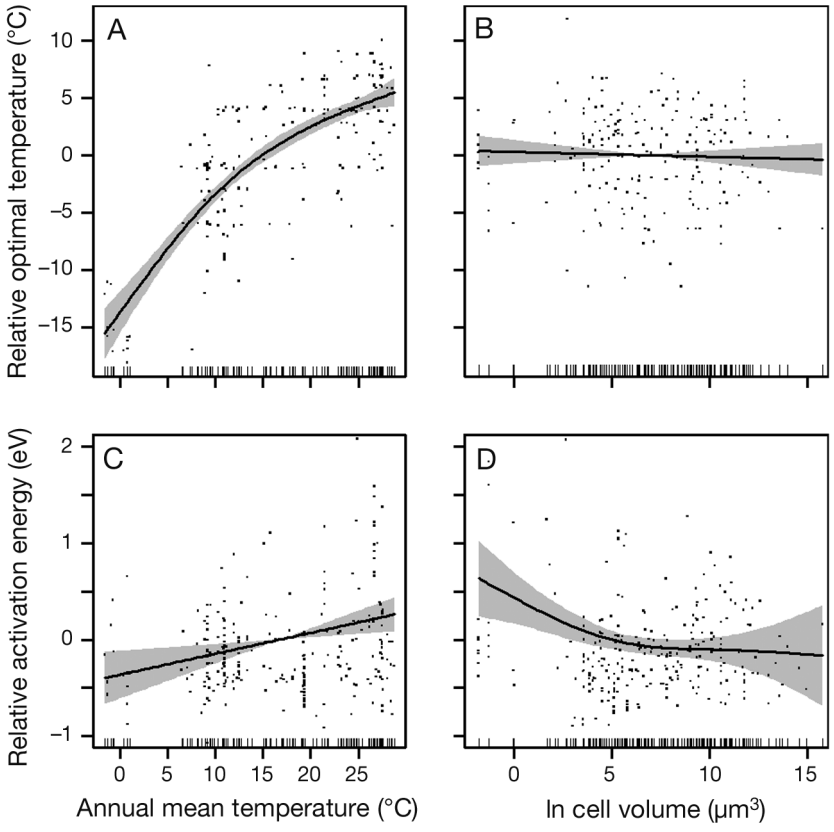

Fig. 3. Effects of environmental annual mean temperature and cell volume on (A,B) optimal growth temperatures and (C,D) activation energies of phytoplankton estimated from generalized additive models. Shaded areas denote 95% CI

predictors explained 62% of the variability of the optimal growth temperature. As predicted, environmental mean temperature is a strong predictor for optimal growth temperature (Fig. 3A). After controlling the effect of the environmental annual mean temperature, there was no effect of cell size on the optimal growth temperature (Fig. 3B).

The within-species activation energies of all *Prochlorococcus* and *Synechococcus* strains were higher than or insignificantly different from 0.65 eV, while the activation energies of 2 of 3 picoeukaryote strains were significantly lower than 0.65 eV (Table 1). The high activation energy of *Ostreococcus* sp. was estimated from only 2 data points and was subject to high uncertainty. The median within-species activation energies of pico-, nano-, and micro-phytoplankton strains were 1.02, 0.63, and 0.74 eV, respectively (Fig. 2B). The GAM explained only 8% of the variability of activation energy. Activation energies increased with annual mean temperature and decreased with increasing size, although the effect of size was marginal ($p = 0.02$; Fig. 3C,D).

Field data

The field experiments were mostly conducted in the northern hemisphere, particularly in the China seas (Fig. 1). The median ratios (and 95% CI) of μ_o/μ_n were 1.06 (0.52, 5.22), 1.01 (0.18, 1.74), and 0.94 (0.07, 1.45) for *Prochlorococcus*, *Synechococcus*, and picoeukaryotes, respectively. The GAMs explained 31, 29, and 16% of the growth rate variability of *Synechococcus*, picoeukaryotes, and *Prochlorococcus*, respectively. In the field dataset, the activation energies of the growth rates of *Synechococcus* and picoeukaryotes, after controlling the effects of light, nitrate, and chl *a*, were insignificantly different from 0.6 eV but were significantly higher than 0.36 eV estimated for bulk phytoplankton (Chen et al. 2012, Regaudie-de-Gioux & Duarte 2012) ($p < 0.05$; Fig. 4A,E). The growth rates of both *Synechococcus* and picoeukaryotes increased with chl *a* concentrations and also marginally increased with nitrate concentrations but not related to PAR (Fig. 4). For *Prochlorococcus*, the estimate of activation energy was insignificantly different from zero (Fig. 4I). The growth rate of *Prochlorococcus* declined with increasing PAR and nitrate concentration but was relatively invariant with chl *a* concentration except at extremely low chlorophyll levels (Fig. 4J–L).

DISCUSSION

Predicting the responses of phytoplankton community structure and biogeochemical cycles to climate change entails knowledge of both direct and indirect effects of temperature on phytoplankton growth. Our analysis has added knowledge on the direct effects of temperature on marine picophytoplankton growth, with implications relevant to how picophytoplankton will respond to future climate change. Optimal growth temperature and temperature coefficient of growth rate are 2 important thermal traits of phytoplankton growth. As of now, our results suggest that in general, the optimal temperature is not significantly different from that of larger phytoplankton,

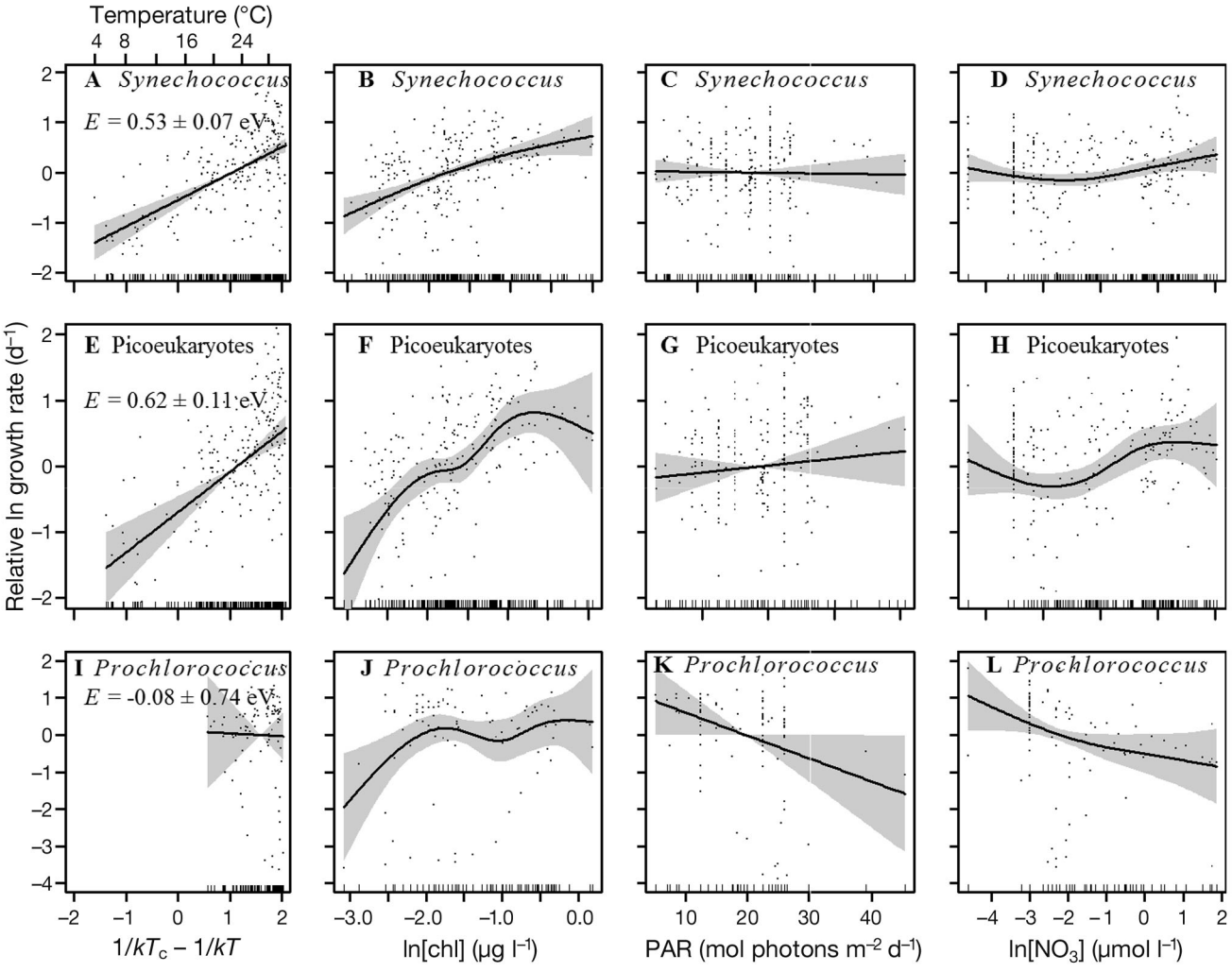

Fig. 4. Effects of temperature, chl *a* (chl) concentration, photosynthetically active radiation (PAR), and nitrate (NO_3) concentration on the growth rates of (A–D) *Synechococcus*, (E–H) picoeukaryotes, and (I–L) *Prochlorococcus*. Solid lines represent smoothing lines from generalized additive models, and shaded areas indicate 95% CI. Estimated activation energies (*E*) and associated standard errors are also shown

but the activation energy of the growth rate of some (but not all) picophytoplankton seems higher than that of larger phytoplankton, although there are uncertainties discussed below.

Optimal growth temperature

One problem in comparing optimal growth temperatures lies in the scarcity of laboratory picophytoplankton data, especially for cold-water strains. One may speculate that as the optimal growth temperatures of most picophytoplankton species were above 20°C in Fig. 2A, with more data available, the mean optimal temperatures of picophytoplankton could be higher than those of larger phytoplankton. We contend that at least some species of *Synechococcus* and picoeukaryotes can be found or even flourish in cold waters (Li et al. 2009). One may argue that based on the strong correlation between optimal growth temperature and the mean temperature of the ambient environment (Fig. 3A; Thomas et al. 2012), the picophytoplankton species living in cold waters should also have a low optimal growth temperature, except for *Prochlorococcus* that are absent at low temperatures (<8°C) (Johnson et al. 2006, Flombaum et al. 2013). However, Tang et al. (1997) pointed out that some freshwater cyanobacteria in polar freshwater systems may grow best at intermediate temperatures (mean ± SE: 20 ± 4°C), leading to the speculation that these organisms were dispersed into polar environments from temperate regions. We need more data on the temperature-growth curves of marine picophytoplankton in cold environments.

Analogously, in freshwater systems, although Jöhnk et al. (2008) showed that the optimal growth temperature of cyanobacteria was higher than that of diatoms and green algae, Lürling et al. (2013) conducted a more comprehensive survey showing that there was no difference in optimal temperatures between cyanobacteria and green algae.

Activation energy

Both the lab and field data suggest that at least for some picophytoplankton groups, the activation energy is higher than that of larger phytoplankton (Fig. 3D, Fig. 4A,E). For lab cultures, the high activation energy of picophytoplankton is mainly contributed by the cyanobacteria *Prochlorococcus* and *Synechococcus*, while 2 of the 3 green algae have low activation energy (Table 1). For field experiments, the activation energies of *Synechococcus* and picoeukaryotes are also higher than the estimate (~0.3–0.4 eV) of the bulk phytoplankton (Chen et al. 2012, Regaudie-de-Gioux & Duarte 2012). Thus, *Synechococcus* appears to be a group with high activation energy, which is consistent with the observations that the abundances of *Synechococcus* peak in the summertime in coastal waters (Waterbury et al. 1986, Agawin et al. 1998, Chen et al. 2009, Liu et al. 2014), and the annual mean abundance of *Synechococcus* increases with annual mean temperature when nutrients are not limiting (Li 1998). However, why do the activation energies of *Prochlorococcus* and picoeukaryotes differ between lab and field data?

A major difference in the activation energy between field picophytoplankton assemblages and lab cultures is that other factors besides temperature that vary with each experiment can also affect growth rates of field assemblages. Although we have considered the effects of light, nutrients, and chl *a*, we might still overlook some other temperature-dependent factors that can affect phytoplankton growth rate. One important factor, the community composition of picophytoplankton assemblages, has not been directly considered because we have limited information on the thermal traits of particular picophytoplankton ecotypes *in situ* (Johnson et al. 2006, Zinser et al. 2007, Mackey et al. 2013), and we do not have the community composition data directly accompanying the growth rate measurements. All of these may contribute to the difference in activation energies estimated from lab and field data. In general, the activation energies estimated for single strains tend to be higher than those estimated in the field that involve changes in community composition (Raven & Geider 1988, Clarke & Johnston 1999), possibly because the cold-water species have evolved the capability to adapt to the cold, allowing them to grow faster than warm-water species under the same temperature, an example of 'countergradient variation' (Conover & Schultz 1995, Clarke 2004). Another difference is that for individual species, growth rate is typically a unimodal function of temperature, while for field phytoplankton assemblages, we do not clearly see a reduction of growth rate at high temperature limits. Thus, it is still challenging to scale up from individual temperature-growth curves to a community temperature-growth model that can be applied to an ecosystem. Unlike the temperature-growth curve of an individual taxon, which has some mechanistic basis (e.g. Schoolfield et al. 1981), the simplified Arrhenius function describing the temperature-growth of phytoplankton assemblages *in situ* is just a statistical approximation (Clarke & Fraser 2004).

Other factors affecting picophytoplankton growth rate

We find that other environmental factors besides temperature are also important for picophytoplankton growth rates. We have shown that growth rates of *Synechococcus* and picoeukaryotes, but not *Prochlorococcus*, increase with chl *a* concentrations (Fig. 4). This pattern is consistent with the observations that *Synechococcus* and picoeukaryotes are usually more abundant in mesotrophic waters, and *Prochlorococcus* are more abundant in oligotrophic waters (Zubkov et al. 2000, Liu et al. 2007). As light and nutrients have been controlled in the model, the increasing trend of growth rates of *Synechococcus* and picoeukaryotes with chl *a* mostly reflects the changes of community structure along chlorophyll gradients, which suggests that coastal strains generally grow faster than oceanic strains. Palenik et al. (2006) have found notable differences of genes between a coastal and an oceanic strain of *Synechococcus*. Although the genomic data cannot directly prove that coastal strains grow faster than oceanic strains, the findings that the coastal *Synechococcus* strain CC9311 possessed greater capacity of transporting, storing, and utilizing nitrate and metals than the oceanic strain WH8102 imply that the coastal strain might have higher growth potential.

By contrast, the factors affecting the growth rate of *Prochlorococcus* seem to be different with *Synecho-*

coccus and picoeukaryotes. *Prochlorococcus* growth rate decreases with increasing light level, suggesting that photoinhibition on *Prochlorococcus* growth rate is probably prevalent in surface oceans. Other scientists also found that the percentage of viable cells of *Prochlorococcus* decreases from the surface to the depth and decreases with increasing light and UV levels (Agustí 2004, Llabres & Agustí 2006), suggesting that high light level indeed adversely affects the growth rate of *Prochlorococcus*. Moreover, the percentage of viable cells of *Prochlorococcus* is also lower than that of *Synechococcus* and picoeukaryotes in high-light surface waters (Agustí 2004, Llabres & Agusti 2006), consistent with our results that only *Prochlorococcus* growth rate is negatively correlated with light level. Six et al. (2007) also found that *Prochlorococcus* cells have a limited capacity for repairing photosystem II compared to *Synechococcus* when exposed to high light, probably owing to the high concentrations of D1 protein and large light-harvesting antennae in *Prochlorococcus*.

In general, the effects of nutrient additions on the growth rate of picophytoplankton, especially *Prochlorococcus* and *Synechococcus*, is marginal or even negative based on the difference between nutrient-enriched and control bottles. Because of their large surface-to-volume ratios, the growth rates of picophytoplankton are less likely nutrient limited (Raven 1998). Nonetheless, nutrients still play a role in affecting growth rates of picophytoplankton. We observed positive correlations between ambient nitrate concentrations and growth rates of *Synechococcus* and picoeukaryotes and negative correlations between nitrate concentrations and *Prochlorococcus* growth rate (Fig. 4), which is also consistent with the pattern that *Prochlorococcus* dominate in oligotrophic waters and the other 2 picoplankters flourish in mesotrophic waters (Zubkov et al. 2000, Liu et al. 2007).

Implications of ocean warming on picophytoplankton

In summary, our ultimate purpose of elucidating the functional relationship between picophytoplankton growth rate and temperature and other factors serves to predict how picophytoplankton community structure and productivity will respond to ocean warming. Besides increases of temperature, the repercussions of ocean warming include shallowing mixed-layer depth, increasing light level, enhanced stratification and oligotrophication, etc. Picophytoplankton, particularly *Prochlorococcus*, dominate the tropical and subtropical oceans. Further temperature increases in these areas might impose a heat shock to these organisms and reduce their growth rates, as most optimal temperatures are not higher than 28°C (cf. Thomas et al. 2012). Additionally, the increased light level in the surface mixed layer might also further decrease the growth rate of *Prochlorococcus.* The oligotrophication in the tropical and subtropical oceans (Irwin & Oliver 2009) might benefit *Prochlorococcus*, as other groups of phytoplankton could grow slower as nutrient supply diminishes. By contrast, *Synechococcus* and picoeukaryotes could be favored by warming and eutrophication in coastal and mesotrophic waters, as the activation energies of both of them are higher than those of the bulk phytoplankton, and their growth rates increase with chl *a* and nutrient concentrations.

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