



The Physiological Response of Picophytoplankton to Temperature and Its Model Representation

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Picophytoplankton account for most of the marine (sub-)tropical phytoplankton biomass and primary productivity. The contribution to biomass among plankton functional types (PFTs) could shift with climate warming, in part as a result of different physiological responses to temperature. To model these responses, Eppley's empirical relationships have been well established. However, they have not yet been statistically validated for individual PFTs. Here, we examine the physiological response of nine strains of picophytoplankton to temperature; three strains of picoprokaryotes and six strains of picoeukaryotes. We conduct laboratory experiments at 13 temperatures between -0.5 and 33°C and measure the maximum growth rates and the chlorophyll *a* to carbon ratios. We then statistically validate two hypotheses formulated by Eppley in 1972: The response of maximum growth rates to temperature (1) of individual strains can be represented by an optimum function, and (2) of the whole phytoplankton group can be represented by an exponential function Eppley (1972). We also quantify the temperature-related parameters. We find that the temperature span at which growth is positive is more constrained for picoprokaryotes (13.7 – 27°C), than for picoeukaryotes (2.8 – 32.4°C). However, the modeled temperature tolerance range (ΔT) follows an unimodal function of cell size for the strains examined here. Thus, the temperature tolerance range may act in conjunction with the maximum growth rate to explain the picophytoplankton community size structure in correlation with ocean temperature. The maximum growth rates obtained by a 99th quantile regression for the group of picophytoplankton or picoprokaryotes are generally lower than the rates estimated by Eppley. However, we find temperature-dependencies (Q_{10}) of 2.3 and of 4.9 for the two groups, respectively. Both of these values are higher than the Q_{10} of 1.88 estimated by Eppley and could have substantial influence on the biomass distribution in models, in particular if picoprokaryotes were considered an independent PFT. We also quantify the increase of the chlorophyll *a* to carbon ratios with increasing temperature due to acclimation. These parameters provide essential and validated physiological information to explore the response of marine ecosystems to a warming climate using ocean biogeochemistry models.

Keywords: picophytoplankton, picoeukaryotes, Eppley, phytoplankton growth rates, temperature tolerance, phytoplankton size scaling, physiological parameterization, chlorophyll *a* to carbon ratio

INTRODUCTION

Picophytoplankton contribute 26–56% to the global phytoplankton biomass (Buitenhuis et al., 2013) and about half of the global ocean primary productivity (Grossman et al., 2010). They dominate over wideocean areas, such as the oligotrophic subtropical gyres, and decrease polewards relative to other phytoplankton (Alvain et al., 2008; Buitenhuis et al., 2012). They play a significant role in the recycling of organic matter within the microbial loop of the surface ocean (Azam et al., 1983; Fenchel, 2008), but contribute little to the sinking of particulate matter to the intermediate and deep ocean (Michaels and Silver, 1988). With the projected extension of the oligotrophic subtropical gyres as a consequence of climate warming (Polovina et al., 2008), the recycling of nutrients within the microbial loop and consequently the contribution of picophytoplankton to the phytoplankton community may gain more importance in the marine biogeochemical cycles (Morán et al., 2010).

Temperature is an important environmental variable that determines, directly or indirectly, the biomass, productivity, and cell composition of all phytoplankton groups, single species and even ecotypes (Eppley, 1972; Sarmiento, 2004; Zinser et al., 2007). In particular, temperature directly affects the physiological processes that regulate the growth rates, the temperature span at which growth rates are positive, and the chlorophyll *a* to carbon ratios, among others (Eppley, 1972; Raven and Geider, 1988). In the field, temperature also influences the physical dynamics of the water column and the availability of nutrients and light (Eppley, 1972; Behrenfeld et al., 2006; Johnson et al., 2006), making it difficult to isolate the specific effect of temperature.

The contribution of picophytoplankton to the phytoplankton biomass was shown to correlate with *in situ* temperature (Agawin et al., 2000; Morán et al., 2010). Also a direct effect of temperature on the phytoplankton community size structure was found in the global ocean (Mousing et al., 2014; López-Urrutia and Morán, 2015). However, Marañón et al. (2014) argue that the correlation between temperature and size structure is due to an indirect effect through nutrient supply as they did not find a direct effect of temperature when data from similar nutrient supply regimes were used.

To isolate the specific effect of temperature on the physiology of different phytoplankton groups, representative laboratory strains must be used under controlled nutrient conditions. Furthermore, physiological temperature relevant parameters need to be defined and quantified to identify groups with common traits. It is well established that the maximum growth rate of phytoplankton at optimum conditions is correlated with the cell size and can be represented by a unimodal function of cell size, with decreasing maximum growth rates above and below 2 μm (Chisholm et al., 1992; Bec et al., 2008). This correlation has been shown to be independent of the optimum temperature (Chen et al., 2014) or nutrient supply (Bec et al., 2008), but other temperature-related parameters, such as the temperature tolerance range, have not yet been tested against cell size. It is essential to gain a detailed understanding of the effect of temperature on the physiology to constrain all relevant parameters in ocean biogeochemistry models. These models

explicitly represent different phytoplankton and zooplankton groups with common traits, namely PFTs, to make projections about the implications of a warming climate on the marine ecosystem and its biogeochemical cycles (Le Quéré et al., 2005).

Ocean biogeochemistry models use the generalized equation proposed by Eppley (1972) for modeling the response of maximum growth rates of a phytoplankton community to temperature. Eppley formulated two major hypotheses: First, the maximum growth rates of individual species can be represented by an optimum function in response to temperature, and second, the maximum growth rates of a phytoplankton community can be represented by an exponential function in response to temperature. In addition, he formulated an equation which describes the exponential fit to the upper limit of the maximum growth rates of a phytoplankton community in response to temperature (Equation 1 in Eppley, 1972). Neither of these two hypotheses was statistically verified in Eppley (1972). Montagnes et al. (2003) showed that the maximum growth rates of most individual species are better represented by a linear fit than an exponential fit, but they did not consider an optimum fit, nor did they test the whole phytoplankton community. Bissinger et al. (2008) showed that the upper 99th quantile of the maximum growth rates of a mixed phytoplankton community can be represented by an exponential fit in response to temperature, with a Q_{10} value similar to Eppley (1972), but with a higher maximum growth rate at 0°C. However, Bissinger et al. (2008) did not test other functions.

Temperature also affects the chlorophyll *a* to carbon ratio (θ) of phytoplankton (Geider, 1987). This effect needs to be quantified when using chlorophyll *a* from field observation to estimate biomass, growth rates, or the community composition. For example, its divinyl derivatives are measured by satellites to identify the picoprokaryote *Prochlorococcus* sp. within a phytoplankton assemblage in the field (Chisholm et al., 1992; Alvain et al., 2005). However, the chlorophyll *a* to carbon ratio is a variable component within the cell. Generally, it decreases with temperature due to low temperature chlorosis, slower metabolic reactions or the increase in lipids to maintain membrane fluidity (Geider, 1987). The variability of the chlorophyll *a* to carbon ratio can be amplified by exposure to high light intensities (Geider, 1987). A positive effect of temperature on light-harvesting components and a negative effect on photoprotective components has previously been found between 16 and 24°C for picoprokaryotes and picoeukaryotes (Kulk et al., 2012). However, more data over a wide range of temperatures need to be collected to identify and quantify significant relationships.

The present study will investigate the influence of temperature on the physiology of nine picophytoplankton strains, with the aim of informing the representation of picophytoplankton in ocean biogeochemistry models. It will specifically: (a) quantify the response of maximum growth rates to temperature; (b) evaluate the two hypotheses of Eppley (1972); (c) extract the temperature-related parameters, separately for individual strains and the group of picoprokaryotes, picoeukaryotes, and picophytoplankton; and investigate the relationship (d) between cell size and the temperature-related parameters, and (e) between the chlorophyll *a* to carbon ratio and temperature.

MATERIALS AND METHODS

Cultures and Experimental Setup

Representative strains of picophytoplankton from diverse taxonomic classes were obtained from the Roscoff culture collection (RCC, Vaultot et al., 2004), to investigate the effect of temperature on the maximum growth rates of picophytoplankton. They include three picoprokaryotes, represented by *Synechococcus* sp. (RCC 30), a high light (HL), and a low light (LL) ecotype of *Prochlorococcus* sp. (RCC 296 and 162, respectively), as well as the picoeukaryotes *Bolidomonas pacifica* (RCC 212), which was recently renamed as *Triparma eleuthera* (Ichinomiya et al., 2016), *Micromonas pusilla* (RCC 1677), *Picochlorum* sp. (RCC 289), *Nannochloropsis granulata* (RCC 438), *Imantonia rotunda* (RCC 361), and *Phaeomonas* sp. (RCC 503) (Table 1). All strains were grown in artificial seawater medium (ESAW) (Berges et al., 2001) with ammonium [$882 \mu\text{M} (\text{NH}_4)_2\text{SO}_4$] as the nitrogen source and addition of 10 nM selenium (Na_2SeO_3). The physiological experiments in response to temperature were conducted in 55 ml tubes (Pyrex Brand 9826), which were placed into a temperature gradient bar. The temperature gradient bar was built with space for 65 culture tubes in 13 rows and 5 columns. A temperature gradient is generated by heating one of the short ends and cooling the other end to achieve a gradient between -0.5 and 33°C . Each tube is lighted by an individual ultrabright LED (Winger WEPW1-S1 1W, 95 Lumen, white), achieving a light intensity of up to $480 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ inside the tubes. The LED drivers are connected to mains electricity through a timer in the control unit, running on a 14:10 h light-dark cycle. Light was measured with a Radiometer (Biospherical Instruments Inc. QSL-2101) to be $291 \pm 18 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 8 strains and $81 \pm 5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for the low light *Prochlorococcus* sp. strain. These values are consistent with the average species specific light saturation levels (Stawiarski, 2014). To exclude any effect of light limitation or light inhibition, near optimum light conditions were chosen for each strain. A separate study with incubations at light intensities between 10 and $720 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ has been conducted beforehand. The low light *Prochlorococcus* strain reached its highest growth rates with light saturation between 64 and $120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, but

was light inhibited at light intensities $> 120 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. All other strains reached light saturation between 120 and $330 \text{ m}^{-2} \text{s}^{-1}$. No light inhibition occurred at light intensities $< 330 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Temperatures were measured with a Grant Squirrel 1000. Thanks to the insulation at the sides and top of the temperature gradient bar, the average temperature gradient is linear (linear regression of temperature difference between adjacent sets of tubes, $p = 0.9$). However, the middle tubes in each column tend to be slightly colder at the cold end (up to 0.5°C), and as a consequence the standard deviation of the temperature in the five tubes is higher ($p = 0.002$). To prevent this from biasing the results, measurements are reported at the temperature measured in each tube.

Analyses

For measuring the maximum growth rates, cultures of each strain were acclimated at 13 different temperatures for at least four divisions to reach balanced growth before daily *in vivo* fluorescence measurements were taken with a Turner Design Fluorometer (10 AU) (Anderson, 2005). Samples were placed in the dark prior to measurements and were measured until the signal stabilized. Only acclimated cultures were used within the present study, hence the fluorescence signal is considered as proportional to the low cell densities which were used (Anderson, 2005). The benefit of using this method instead of collecting cell counts was that the culture tube from the temperature gradient bar fits into the sample slot of the Fluorometer. Thus, no volume needed to be removed from the culture tube. The average cell size of the picophytoplankton strains was either provided by the RCC or obtained from the literature for *T. eleuthera* (Guillou et al., 1999).

To obtain chlorophyll *a* to carbon ratios, samples of particulate organic carbon (POC) and chlorophyll *a* were collected while the culture was still in exponential growth phase. POC was sampled on pre-combusted 13 mm GF/F filters for all strains. A layer of 3 filters was used for both *Prochlorococcus* sp. strains, because preliminary tests showed that their cells did not pass through, but were too small to remain on a single filter. Medium blanks were collected for each number of filter

TABLE 1 | Picophytoplankton strains examined within this study, including three strains of picoprokaryotes and six strains of picoeukaryotes, their Roscoff culture collection number (RCC), stain, average cell size (diameter), and location and depth of isolation.

	Species	RCC	Strain	Size (μm)	Location of isolation	Depth of isolation (m)
Picoprokaryotes	<i>Prochlorococcus</i> sp. (HL)	296	GP2	0.6	$8^\circ 32.5' \text{N}, 136^\circ 31.8' \text{E}$	150
	<i>Prochlorococcus</i> sp. (LL)	162	NATL2-M98	0.6	$38^\circ 59' \text{N}, 40^\circ 33' \text{W}$	10
	<i>Synechococcus</i> sp.	30	MAX42Syn	1	$26^\circ 18' \text{N}, 63^\circ 26' \text{W}$	120
Picoeukaryotes	<i>Triparma eleuthera</i>	212	OLI 41 SA-A	1.2	$2^\circ 30' \text{N}, 150^\circ 0' \text{W}$	15
	<i>Micromonas pusilla</i>	1677	MICROVIR 17CR_2	1.5	$54^\circ 24' \text{N}, 4^\circ 3' \text{E}$	10
	<i>Picochlorum</i> sp.	289	OLI 26 SA	2	$7^\circ 0' \text{S}, 150^\circ 0' \text{W}$	15
	<i>Nannochloropsis granulata</i>	438	BL_39	2	$41^\circ 40' \text{N}, 2^\circ 48' \text{E}$	0
	<i>Imantonia rotunda</i>	361	RA000609-17-10	2.5	$48^\circ 45' \text{N}, 3^\circ 57' \text{W}$	0
	<i>Phaeomonas</i> sp.	503	BL_149-10	3	$41^\circ 40' \text{N}, 2^\circ 48' \text{E}$	0

layers. Samples of chlorophyll *a* were collected on pre-combusted 25 mm GF/F filters for 7 strains, but on 25 mm polycarbonate filters (0.2 μm) for both *Prochlorococcus* sp. strains. Both filter types were shown to lead to comparable chlorophyll *a* results using phytoplankton samples (Hashimoto and Shiomoto, 2000). Depending on the cell density of the culture, between 5 and 20 ml per sample were filtered and rinsed with Milli-Q water. After sampling all filters were frozen in liquid nitrogen immediately, and stored at -80°C until analyses. The cell numbers were measured by flow cytometry (BD Biosciences FACSCalibur, flow cytometer) and the flow rate was calibrated using the method of Marie et al. (2005).

For analysis, the POC samples were dried at 40°C for 24 h, placed into pre-combusted tin capsules and analyzed with an elemental analyser (Exeter Analytical, CE-440 Elemental Analyser), which was calibrated with acetanilide (Exeter Analytical). The chlorophyll *a* samples were extracted in 10 ml acetone (Fisher Scientific, 99.8+ %) in 15 ml centrifuge tubes and disintegrated by shaking and vortexing. The tubes were then wrapped in aluminum foil and stored at 4°C for 24 h. Prior to analysis, the samples were centrifuged and the supernatant was analyzed in a Fluorescence Spectrometer (PerkinElmer LS 45 Luminescence Spectrometer). After reading a sample, 3 drops of 8% HCl were added into the cuvette to measure the background signal caused by chlorophyll degradation products such as phaeopigments. The concentration of the calibration standard (SIGMA product No C5753) was also obtained prior to analyses (Parson et al., 1984).

Calculations

The maximum growth rates of all strains were calculated by linear regression through at least three consecutive measurements of the log-transformed *in vivo* fluorescence measurements during the exponential growth phase. To test for the best representation of the response of the maximum growth rates to temperature, a linear, an exponential, and an optimum fit (Equations 1–3) were applied to the maximum growth rate measurements of each strain, of each of the two groups (picoprokaryotes or picoeukaryotes) and also of all strains combined, representative for a group of picophytoplankton.

$$\text{linear:} \quad \mu_{max} = \mu_{max,0^{\circ}\text{C}} + \text{slope} \times T \quad (1)$$

$$\text{exponential:} \quad \mu_{max} = \mu_{max,0^{\circ}\text{C}} \times Q_{10}^{\frac{T}{10}} \quad (2)$$

$$\text{optimum:} \quad \mu_{max} = \mu_{Opt} \times \exp\left(-\frac{(T-T_{Opt})^2}{\Delta T^2}\right) \quad (3)$$

where μ_{max} is the maximum growth rate, $\mu_{max,0^{\circ}\text{C}}$ is the maximum growth rate at 0°C , T is the temperature, Q_{10} is the temperature dependence, which is a measure for the increase of the maximum growth rate with the increase of temperature by 10°C , μ_{Opt} is the optimum growth rate, T_{Opt} is the optimum temperature, and ΔT is half the width of the temperature range at $\mu_{Opt} \times \exp(-1)$, which will be referred to as temperature tolerance range, not to be confused with $T_{max} - T_{min}$, which will be referred to as the temperature span.

The temperature-related parameters and their standard errors were estimated by minimizing the sum of squares between the fits and the measurements using the Gauss-Newton method in Mstat 12 (Systat software). The obtained parameters were not unique for the optimum fit to the combined data of all strains, because the optimum temperature was indefinite. For this reason, the sum of squares between the model and the data were calculated 15 times with varying starting values and it was found, that there was only a minor variability in the residual sum of squares ($< 0.03\%$) and the parameters.

The relative quality of the three fits to equations 1–3 was compared using the Akaike's Information Criterion (AIC), which compares fits with different numbers of parameters (Equation 4, Burnham and Anderson, 1998).

Akaike's Information Criterion

$$AIC = n_{obs} \log(\sigma^2) + 2n_{param} \quad (4)$$

where n_{obs} is the number of observations, σ^2 is the standard deviation and n_{param} is the number of parameters given in the equation of the fit. The lowest AIC value indicates the best fit. Although there is no formally defined significance level associated with the value of AIC, we have used the definition in Burnham and Anderson (1998), who state that if an AIC differs by less than 2 from the lowest value, this fit is also appropriate.

The data were also compared to the exponential fit presented in Eppley (1972) to the upper limit of the maximum growth rates of a mixed group of phytoplankton, which will be referred to as the absolute maximum growth rates, in response to temperature (Equation 5).

$$\text{Eppley (1972)} \quad \mu_{max} = 0.59 \times 1.88^{\frac{T}{10}} \quad (5)$$

where the first constant is $\mu_{max,0^{\circ}\text{C}}$ and the second constant is the Q_{10} .

To calculate the absolute maximum growth rates for a group of picophytoplankton and picoprokaryotes in response to temperature, we followed the method used by Bissinger et al. (2008). For this, we calculated the upper 99th quantile of the maximum growth rates for both groups by applying a linear quantile regression through the log-transformed maximum growth rates. The Software R with the software package quantreg was used (Koenker, 2006) with a significance level of $p < 0.001$. The resulting coefficients were then exponentially converted and the fit was compared to the fit presented in Eppley (1972). As an alternative means of showing the absolute maximum growth rates of a picophytoplankton community, we also calculated the linear, exponential and optimum fit through the optimum temperatures vs. optimum growth rates of the nine strains only.

Statistical Analysis

To test for significant differences in the maximum growth rates or in the obtained temperature related parameters between the two picophytoplankton groups, the Wilcoxon-Mann-Whitney-U-test was used ($p \leq 0.05$, $df = 1$). To test for cell size related trends of the temperature related parameters, the Mitchell-Olds and Shaw test was used ($p \leq 0.05$) (Mitchell-Olds and Shaw,

1987). It tests for an intermediate maximum, in contrast to a monotonic relationship with extreme values at each end. For the linear trends in the response of the chlorophyll *a* to carbon ratios to temperature, a linear regression was applied and the obtained coefficients were analyzed by one-way ANOVA ($p \leq 0.05$).

RESULTS

Temperature-Response of Individual Picophytoplankton Strains

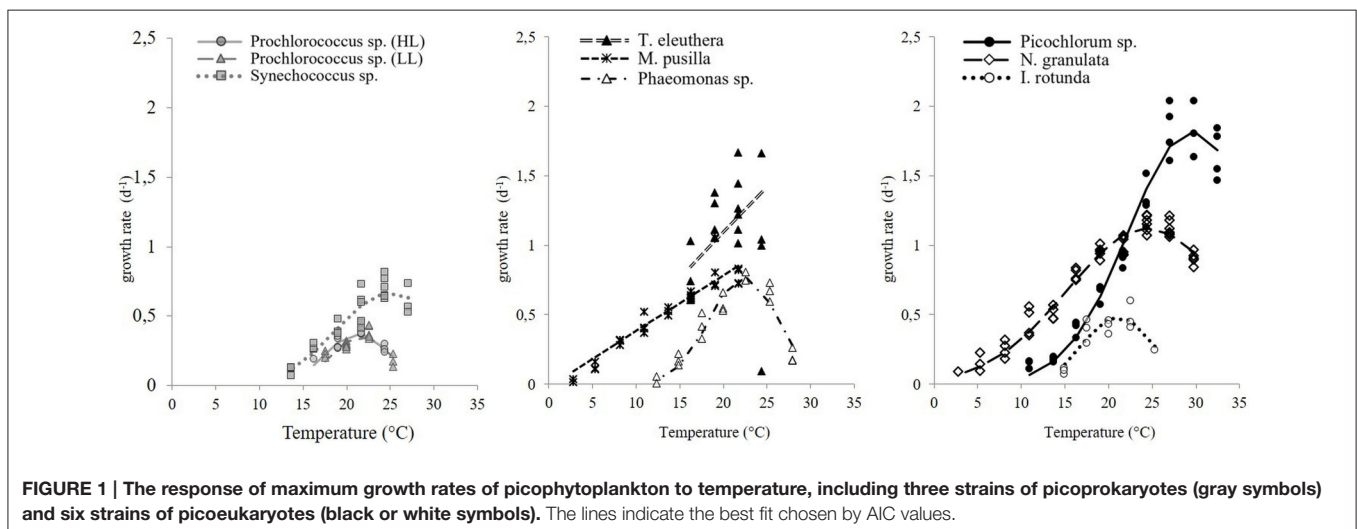
The maximum growth rates of picoprokaryotes range from 0.07 to 0.82 d^{-1} and of picoeukaryotes from 0.005 to 2.04 d^{-1} over the full range of tested temperatures (Figure 1). These rates generally increase with temperature up to an optimum temperature (T_{Opt}), above which they decrease (Figure 1) for all individual picophytoplankton strains. The AIC values are also smallest for the optimum fit (Equation 3) for seven of the nine individual strains (Table 2; Figure 1) compared to the linear (Equation 1) or exponential fit (Equation 2). For the two remaining strains, the AIC values for the optimum fit are within the range of an acceptable representation. *M. pusilla* did not grow at all above the optimum temperature. Therefore, there was no acclimated growth rate above the optimum temperature that would have been needed to get a good fit to the optimum function, and its growth rates are better represented by a linear fit. *T. eleuthera* grew at only four temperatures, thus the available data for this strain were insufficient to distinguish between the fits. The generally best agreement with the optimum fit suggests that three temperature-related parameters need to be quantified for the representation of the response of growth rates of individual strains to temperature: μ_{Opt} , T_{Opt} , and ΔT (Table 3).

The derived optimum growth rates (μ_{Opt}) differ significantly ($p = 0.04$, $df = 1$) between the two groups, the average for picoprokaryotes is $0.47 \pm 0.17 d^{-1}$ and for picoeukaryotes it is $1.05 \pm 0.47 d^{-1}$. The average optimum temperature (T_{Opt}) of the individual strains is $23.3 \pm 2.7^\circ C$ and the temperature tolerance

range (ΔT) is $8.2 \pm 3.3^\circ C$, with no significant difference in these two temperature-related parameters ($p_{T_{Opt}} = 0.8$; $p_{\Delta T} = 0.12$, $df = 1$) between picoprokaryotes and picoeukaryotes. None of the three temperature-related parameters was correlated with the latitude of isolation of the strains (Figure 2), i.e., tropical strains did not have a significantly higher T_{Opt} than temperate strains. However, the overall temperature span at which the growth rates are positive is narrower for the three investigated strains of picoprokaryotes (13.7–27°C, Table 3) than for the six strains of picoeukaryotes (2.8–32.4°C). *Nannochloropsis granulata*, *M. pusilla* and *Picochlorum* sp., the three intermediate sized picoeukaryotes, grow at the most measured temperatures spanning up to 27°C, which is reflected in their higher ΔT values. Cell size has an effect on the temperature-related parameters for the individual picophytoplankton strains tested within this study. A significant unimodal relationship was found between the temperature tolerance range (ΔT) and cell size ($R^2 = 0.73$, $p = 0.018$), but not between μ_{Opt} ($R^2 = 0.49$, $p = 0.17$), or T_{Opt} ($R^2 = 0.25$, $p = 0.43$) and cell size. The cell size at which ΔT is maximal is $1.8 \mu m$ (Figure 2). There is also statistical support ($p < 0.01$, Mitchell-Olds and Shaw test) for a maximum of μ_{Opt} at the higher end of measured values of ΔT , although μ_{Opt} does not increase significantly with ΔT ($p = 0.1$, linear regression, one-way ANOVA).

Temperature-Response of Picophytoplankton

The maximum growth rates for the picoprokaryotes in response to temperature can be described equally well by all three fits (similar AIC values; Table 2), but the maximum growth rates for the picoeukaryotes are best described by either the linear or the optimum fit. Finally, the maximum growth rates for the group of picophytoplankton are best described by the exponential fit (Figure 3) and can be quantified by the two temperature-related parameters $\mu_{max,0^\circ C}$ and Q_{10} (Table 3).



In order to compare the temperature response of the group of picophytoplankton or of picoprokaryotes to the parameters obtained by Eppley (1972) and Bissinger et al. (2008), a fit to the upper exponential 99th quantile of the maximum growth rates was calculated (Figure 3). For the group of picophytoplankton (μ_{pic}) the calculated Q_{10} is 2.3 (Equation 6) but of picoprokaryotes (μ_{pro}) the temperature response is stronger and results in a much higher Q_{10} of 4.9 (Equation 7). For the picoeukaryotes (μ_{euk}) the Q_{10} would be 2.8 (Equation 8), but the AIC does not give support for an exponential fit as an acceptable representation of the maximum growth rates in response to temperature for this group (Table 2). Hence we will exclude this fit from the further discussion.

$$\mu_{pic} = 0.22 \times 2.3^{\frac{T}{10}} \tag{6}$$

$$\mu_{pro} = 0.023 \times 4.9^{\frac{T}{10}} \tag{7}$$

$$\mu_{euk} = 0.19 \times 2.8^{\frac{T}{10}} \tag{8}$$

The corresponding coefficients for the linear regression to the logarithmically transformed data are presented in Table 4.

A different method to represent the response of the absolute maximum growth rates of the group of picophytoplankton to temperature is to test the fits through the optimum values of the nine strains (Figures 3, 4). With this method, the AIC value is lowest for the linear fit (-5.41), is also appropriate for the exponential fit (-5.3), but clearly better than for the optimum fit (-2.68).

TABLE 2 | AIC values for the linear, exponential and optimum fits for individual picophytoplankton strains, both groups of picoprokaryotes and picoeukaryotes, and picophytoplankton.

Strain	Linear	Exponential	Optimum
<i>Prochlorococcus</i> sp.(HL)	-33.6	-33.7	-42.7
<i>Prochlorococcus</i> sp.(LL)	-37.7	-37.8	-43.6
<i>Synechococcus</i> sp.	-42.9	-39.2	-44.9
<i>Triparma eleuthera</i>	-19.3	<u>-18.7</u>	<u>-18.6</u>
<i>Micromonas pusilla</i>	-59.4	-49.9	<u>-58.9</u>
<i>Picochlorum</i> sp.	-42.5	-32.2	-52.7
<i>Nannochloropsis granulata</i>	-74.7	-64.1	-107.9
<i>Imantonia rotunda</i>	-18.5	-17.7	-22.0
<i>Phaeomonas</i> sp.	-20.3	-19.8	-37.3
Picoprokaryotes	-95.1	<u>-94.3</u>	<u>-94.0</u>
Picoeukaryotes	-162.6	-158.9	<u>-161.2</u>
Picophytoplankton	-200.8	-205.7	-203.3

The lowest values are shown in bold print, other appropriate values ($\Delta AIC < 2$) are underlined.

Chlorophyll a to Carbon Ratios

The chlorophyll a to carbon ratio (θ) for the group of picophytoplankton increases significantly with temperature between 0.004 and 0.037 g Chl g⁻¹ C ($R^2 = 0.42, p < 0.001$, Figure 5), and can be described by Equation (9).

$$\theta = 1.01 * 10^{-3} + 9.38 * 10^{-4} T \tag{9}$$

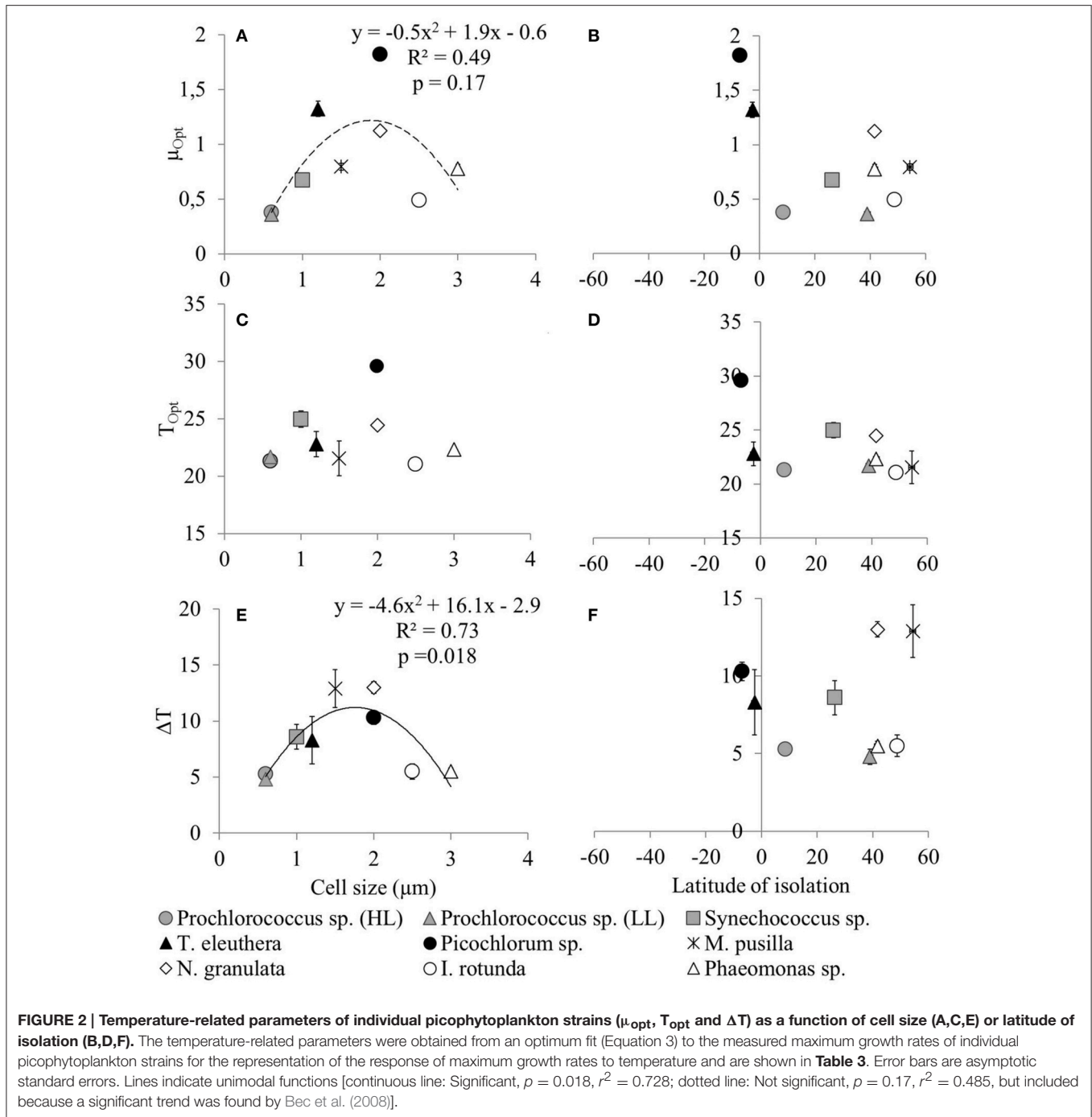
This relationship is also significant ($p \leq 0.05$) for individual strains (see Supplementary Material), unless a strain grew only over a narrow temperature range (both *Prochlorococcus* sp. strains and *Imantonia rotunda*), or there was a high variability in the data over a low range of chlorophyll a to carbon ratios (*Micromonas pusilla*). Four strains show a drop in chlorophyll a to carbon ratio above T_{Opt} (both *Prochlorococcus* sp. strains, *Picochlorum* sp., and *Nannochloropsis granulata*).

The cellular chlorophyll a concentration increases significantly ($p < 0.05$) with temperature for seven strains

TABLE 3 | Temperature-related parameters for a linear, exponential, and optimum fit to represent the response of the maximum growth rates to temperature for individual picophytoplankton strains, both groups of picoprokaryotes and picoeukaryotes, and picophytoplankton.

Strain	n	Linear		Exponential		Optimum			Measured	
		$\mu_{max,0^\circ C}$	Slope	$\mu_{max,0^\circ C}$	Q_{10}	μ_{opt}	T_{Opt}	ΔT	T_{min}	T_{max}
<i>Prochlorococcus</i> sp. (HL)	16	0.30 (± 0.15)	0.001 (± 0.007)	0.30 (± 0.15)	1.02 (± 0.23)	0.38 (± 0.01)	21.3 (± 0.2)	5.3 (± 0.4)	16.3	24.4
<i>Prochlorococcus</i> sp. (LL)	19	0.35 (± 0.17)	-0.003 (± 0.008)	0.35 (± 0.20)	0.92 (± 0.25)	0.36 (± 0.02)	21.7 (± 0.2)	4.8 (± 0.5)	17.5	25.3
<i>Synechococcus</i> sp.	24	-0.38 (± 0.13)	0.042 (± 0.006)	0.10 (± 0.04)	2.08 (± 0.31)	0.67 (± 0.03)	25.0 (± 0.7)	8.6 (± 1.1)	13.7	27.0
<i>Triparma eleuthera</i>	18	-0.22 (± 0.44)	0.066 (± 0.021)	0.38 (± 0.16)	1.70 (± 0.33)	1.32 (± 0.07)	22.8 (± 1.1)	8.3 (± 2.1)	16.3	24.4
<i>Micromonas pusilla</i>	29	-0.02 (± 0.06)	0.040 (± 0.003)	0.21 (± 0.03)	1.92 (± 0.16)	0.80 (± 0.03)	21.5 (± 1.5)	12.9 (± 1.7)	2.8	21.7
<i>Picochlorum</i> sp.	35	-0.90 (± 0.16)	0.087 (± 0.006)	0.22 (± 0.05)	1.96 (± 0.17)	1.82 (± 0.04)	29.6 (± 0.4)	10.3 (± 0.6)	10.9	32.4
<i>Nannochloropsis granulata</i>	50	0.19 (± 0.08)	0.033 (± 0.004)	0.43 (± 0.06)	1.39 (± 0.07)	1.12 (± 0.02)	24.5 (± 0.3)	13.0 (± 0.5)	2.8	29.8
<i>Imantonia rotunda</i>	13	-0.12 (± 0.24)	0.024 (± 0.012)	0.13 (± 0.10)	1.67 (± 0.59)	0.49 (± 0.04)	21.1 (± 0.5)	5.5 (± 0.7)	14.9	25.3
<i>Phaeomonas</i> sp.	20	0.17 (± 0.26)	0.013 (± 0.012)	0.29 (± 0.18)	1.22 (± 0.33)	0.78 (± 0.04)	22.3 (± 0.2)	5.5 (± 0.3)	12.3	27.9
Picoprokaryotes	59	-0.23 (± 0.14)	0.029 (± 0.006)	0.08 (± 0.03)	2.02 (± 0.36)	0.51 (± 0.08)	27.7 (± 5.0)	12.7 (± 5.6)	13.7	27.0
Picoeukaryotes	165	-0.28 (± 0.10)	0.054 (± 0.005)	0.23 (± 0.03)	1.83 (± 0.11)	1.51 (± 0.28)	37.7 (± 6.8)	21.8 (± 5.2)	2.8	32.4
Picophytoplankton	224	-0.35 (± 0.10)	0.050 (± 0.005)	0.14 (± 0.02)	2.09 (± 0.14)	33.93 (± 1.1)	125.5 (± 0.4)	52.7 (± 0.2)	2.8	32.4

The asymptotic standard error is shown in brackets. The number of measured maximum growth rates is n, the measured minimum temperature (T_{min}), and maximum temperature (T_{max}), define the temperature span at which growth rates were positive.



(see Supplementary Material). For *M. pusilla* it becomes significant ($p = 0.005$) if the four highest outliers over the whole temperature range are excluded. For *I. rotunda* a significant ($p = 0.038$) increase in chlorophyll *a* is found up to its optimum temperature. There is also a stronger significance ($p < 0.001$) for *N. granulata* up to its optimum and a decrease, however not significant above its optimum temperature. No significant trend was found for the high light *Prochlorococcus* sp. strain.

Phaeomonas sp. shows a significantly ($p = 0.037$) decreasing trend with increasing temperature.

The cellular carbon concentration increases significantly with temperature for the low light *Prochlorococcus* sp. strain ($p = 0.004$). It decreases significantly for the three picoeukaryotes *N. granulata* ($p < 0.001$), *Phaeomonas* sp. ($p = 0.001$), and for *Picochlorum* sp. between 14 and 27°C ($p = 0.016$). No significant trends were established for the other strains.

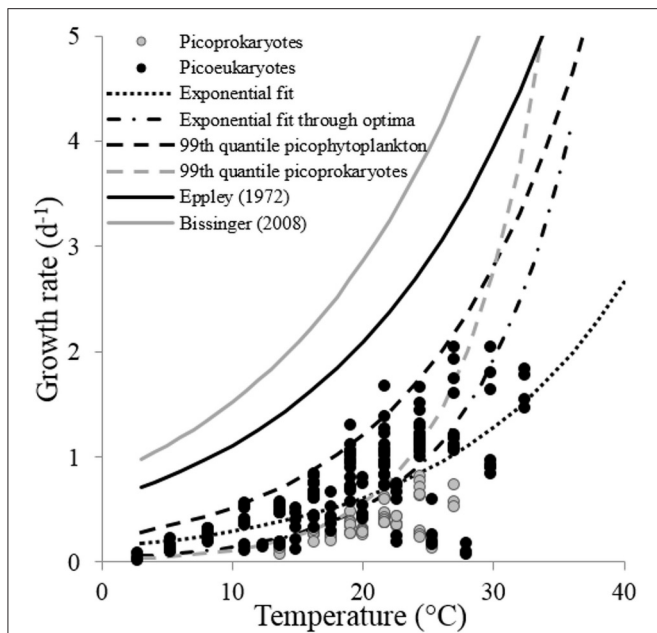


FIGURE 3 | The response of the maximum growth rates of a group of picophytoplankton to temperature. The lines indicate different fits discussed within this study: The exponential fit obtained from Equation (2) (exponential fit), the exponential fit through optimum growth rates at optimum temperatures of individual strains (exponential fit through optima), the exponential fit obtained from a 99th quantile regression to maximum growth rates of both, a group of picophytoplankton (99th quantile picophytoplankton), and a group of picoprokaryotes (99th quantile picoprokaryotes) and the fits presented in Eppley (1972) and Bissinger et al. (2008).

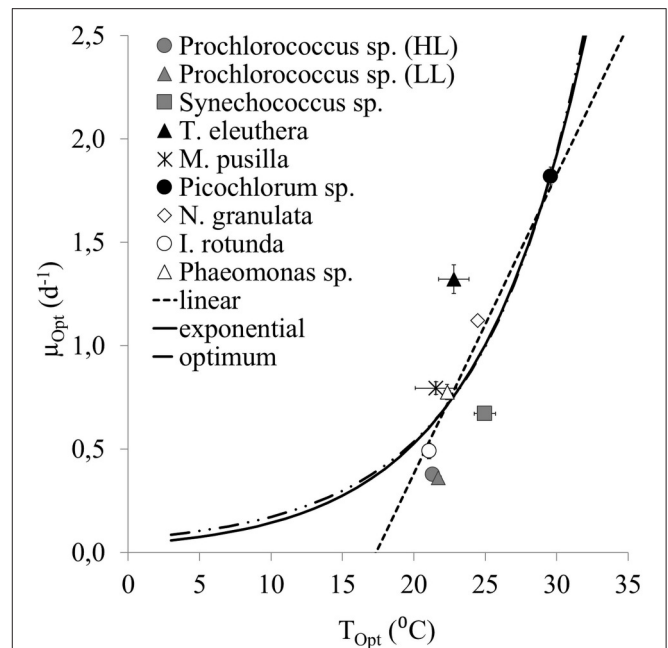


FIGURE 4 | A linear, exponential and optimum fit through optimum growth rates at optimum temperatures of the individual strains examined within this study. Error bars represent the standard error.

TABLE 4 | Coefficients obtained from a linear 99th quantile regression to the log-transformed maximum growth rates of a group consisting of picoprokaryotes, and of picophytoplankton, using strains examined within this study with standard errors.

	Intercept	Standard error	Slope	Standard error
Picoprokaryotes	-3.774	0.076	0.160	0.004
Picophytoplankton	-1.496	0.012	0.084	0.001
Picoeukaryotes	(-1.680)	(0.186)	(0.101)	(0.009)

Coefficients for picoeukaryotes are provided for completeness.

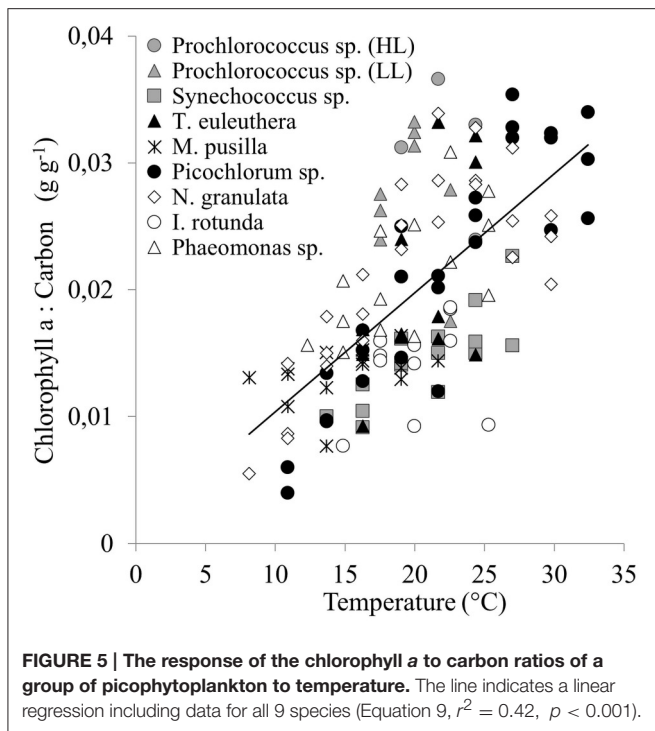
DISCUSSION

Temperature-Response of Individual Picophytoplankton Strains

In agreement with the first hypothesis formulated by Eppley (1972), our results show strong evidence that the maximum growth rates of individual picophytoplankton strains in response to temperature are best represented by an optimum function. Thus, the best way to parameterize this response is to describe their optimum growth rates, optimum temperatures, and temperature tolerance ranges.

The optimum growth rates, which were obtained for the individual strains of picoprokaryotes are lower than

of picoeukaryotes. This confirms theoretical assumptions concerning the deviation of picophytoplankton from the classical allometric relationship with decreasing maximum growth rate with cell size in this group (Raven, 1998; Bec et al., 2008). The optimum temperatures, are slightly lower for *Prochlorococcus* sp. than for *Synechococcus* sp., which is also in agreement with previous studies (Moore et al., 1995; Johnson et al., 2006; Zinser et al., 2007). However, our estimated values are both lower than previously reported values of 24–25 and 28°C, respectively for the two species. There are different reasons which may cause this discrepancy. A possible reason is that none of these studies applied an optimum fit to their results. Instead T_{Opt} was only described as the temperature at which the highest growth rate (μ_{Opt}) was measured, even though T_{Opt} and μ_{Opt} may be achieved between the tested temperatures. We have shown that the optimum function gives the best fit of the response of growth rates of individual picophytoplankton strains to temperature. We therefore conclude that our technique is more accurate in defining T_{Opt} , because it is able to interpolate between data points and provide error intervals. Another potential reason could be the change of photophysiological properties with temperature. We show that the increase in chlorophyll *a* to carbon ratio is due to the significant increase in cellular chlorophyll *a* concentration for most strains. This is in agreement with the expected increase of light harvesting compounds with increasing temperature and is also associated with the decrease of photoprotective compounds (Geider, 1987). This effect may also contribute to the shift of the optimum temperature with light intensity (Geider, 1987). The strains used by Moore et al. (1995) and those in the present study were



grown under light saturation. For this an extensive series of light experiments has been conducted for the strains examined here beforehand (Stawiarski, 2014). However, these different strains also have different light optima. Hence, the differences in temperature optima may also be attributed to the natural variability of ecotypes, which may be linked to the adaptation to different light conditions (Johnson et al., 2006; Zinser et al., 2007). The optimum temperatures for picoeukaryotes obtained within the present study are similar to those presented in previous studies (20–25°C) (Thronsdon, 1976; Cho et al., 2007). A full description of the light response at constant temperature of some of these strains will be published in a separate paper (Stawiarski et al., in prep.).

T_{Opt} is a common temperature-related parameter, which is used for modeling the distribution of different phytoplankton groups. Our results demonstrate that there are no significant differences in T_{Opt} between picokaryotes and picoeukaryotes. Also there is no relationship between optimum temperature or latitude of isolation for the strains examined here, but both groups have been shown to occupy different thermal niches in the field (Buitenhuis et al., 2012). One could argue that culturing conditions may have led to a genetic adaptation and a shift in T_{Opt} , but we found that *Picochlorum* sp., the tropical strain which has been in culture the longest, shows the strongest deviation ($>9^\circ\text{C}$) from its permanent culturing temperature. Contrary to our results, a study, in which an optimum function was used to obtain temperature-related parameters for 194 different strains of phytoplankton, found that T_{Opt} follows a unimodal function of latitude and annual mean temperature of isolation (Thomas et al., 2012). We may not have found this trend in our data because of the much smaller number of data points we could obtain. However, they also find that T_{Opt} shows considerable deviations

from the annual mean temperature in polar and temperate waters, which suggests that T_{Opt} is not the ultimate parameter controlling the distribution. Peak *in situ* abundances of different phytoplankton groups are not found at optimum temperatures, because of the combination of fluctuations in local temperature and the sharp drop in growth rates above T_{Opt} .

The temperature span at which growth was positive for individual strains in our study is comparable to their *in situ* distribution. Peak *in situ* abundances of *Prochlorococcus* sp. were reported at both lower (19°C) and higher temperatures (25–28°C) than their optimum temperature (Zinser et al., 2007) with strong inhibition above 28°C (Moore et al., 1995). The upper limit of the temperature span for the *Prochlorococcus* sp. strains presented here is consistent with those results, and the lower limit of the temperature span is consistent with the results of Kulk et al. (2012). We show that some picoeukaryotes grow over a wider span of temperatures than the smaller picokaryotes. However, we do not find a direct correlation between ΔT and latitude of isolation, which is in agreement with the study by Thomas et al. (2012). Instead, we find evidence that ΔT is significantly correlated with picophytoplankton cell size and can be represented by a unimodal function. The bigger picokaryote *Synechococcus* sp. grew over a wider span of temperatures than the smaller *Prochlorococcus* sp., consistent with earlier studies (Moore et al., 1995; Malinsky-Rushansky et al., 2002; Mackey et al., 2013). Also, the picoeukaryotes of an intermediate size ($\sim 2\ \mu\text{m}$) had a higher ΔT than other-sized members of the group. Together with the higher maximum growth rates of this intermediate size class (Bec et al., 2008) we suggest that their relatively high temperature tolerance range may contribute to their ubiquitous distribution. However, cell size and its variability would explicitly need to be measured over the full range of temperatures to gain a better understanding of this relationship.

We also find that a higher optimum growth rate is achieved by picophytoplankton strains with a high temperature tolerance range. In practice this would favor generalists in the field rather than allowing the coexistence of several specialist strains in different niche spaces. A field study has shown that 90% of analyzed gene sequences of picoeukaryotes can be attributed to Prasinophyceae, of which *M. pusilla*, an intermediate sized picoeukaryote, is an important member, with higher contributions in temperate and polar areas (Vaulot et al., 2008). In agreement with these results, we show that *M. pusilla* has a relatively high temperature tolerance range and a relatively high optimum growth rate. However, the study by Vaulot et al. (2008) was biased toward coastal areas and other factors such as light, nutrients, and water column stratification also need to be considered, especially in the open and oligotrophic ocean when investigating the community structure (Johnson et al., 2006; Bouman et al., 2011).

Temperature-Response of Picophytoplankton

In agreement with the second hypothesis formulated by Eppley (1972), our results show that the maximum growth rates for the group of picophytoplankton in response to temperature are best represented by an exponential function (Table 2,

Figure 3). This representation is also appropriate for the group of picoprokaryotes alone (**Table 2**). Hence, for the calculation of the absolute maximum growth rates of these two groups we follow the approach by Bissinger et al. (2008), who confirmed the Q_{10} (1.88) estimated by Eppley (1972) for a mixed phytoplankton group. Our results show that for a group of picophytoplankton the temperature-dependence is higher and for a group of picoprokaryotes more than twice as high (**Table 4**, **Figure 3**, Equations 6, 7) as for this group of mixed phytoplankton. These results are in agreement with recent studies on picophytoplankton, which found a higher Q_{10} value for picophytoplankton compared to larger species (Chen et al., 2014) and also higher values for picoprokaryotes (3.6–4.4) than for picoeukaryotes (1.7–2) (Kulk et al., 2012). It should be noted that the variance in the data increases with temperature, which could bias the statistical results at small sample size. We found that the main contributor to the increase in variance is the interspecific variation of μ_{opt} . However, the average squared residuals have a quite similar distribution as a function of temperature for the three functions. In addition, our sample size of 224 growth rates is large, so that the increase in variance would not bias the results, and we conclude that the comparison of Delta AIC to decide which function fits the data best seems valid despite the increase in variance with temperature in the observations.

The fit to the absolute maximum growth rates of the group of picophytoplankton in response to temperature presented in our study is lower than the fit presented in Eppley (1972). This can be explained by the generally lower maximum growth rates of picophytoplankton compared to those of other phytoplankton groups, e.g., diatoms (Furnas, 1990). The study by Eppley (1972) contained various groups of faster-growing phytoplankton and a substantial number of diatoms (43%). However, Bissinger et al. (2008) showed that a higher proportion of diatoms (68%) would not affect the fit. It is unclear, though, how high the proportion of picophytoplankton was in the database used in Bissinger et al. (2008). The lower absolute maximum growth rates and the higher Q_{10} of the picophytoplankton examined here compared to mixed phytoplankton highlight the importance of quantifying the response of different phytoplankton groups to temperature individually. Especially in ocean regions where picoprokaryotes dominate the phytoplankton biomass, the influence of their higher Q_{10} must be considered when modeling the response of the phytoplankton community to increased temperature as a consequence of climate warming.

We find that the group of picoeukaryotes grow over a wider span of temperatures than the smaller picoprokaryotes. Although our sample size of picoeukaryote species is larger than of picoprokaryote species, and we therefore have to be cautious about the interpretation of the wider temperature span of the picoeukaryotes as a group, these sample sizes reflect the diversity in the ocean of the two groups of picophytoplankton. Picoeukaryotes are spread across 12 classes in 4 divisions while there is only one class of picoprokaryotes (Vaulot et al., 2008). In addition, it is well established that picoeukaryotes dominate picophytoplankton biomass in colder waters at latitudes above 40° and have similar biomass at lower latitudes, but the smaller picoprokaryotes are more restricted

toward warmer (sub-)tropical ocean waters (Buitenhuis et al., 2012). We therefore suggest that the difference in temperature span between the two groups could be real.

Our results further show that the exponential fit to the optimum growth rates only of all examined strains is lower than the fit to the upper 99th quantile of their maximum growth rates. This is because the maximum growth rates of faster-growing species at sub-optimum temperatures are higher than the optima of slower growing species. The fit through the optima was initially presented as an alternative method for representing the response of the absolute maximum growth rates of a phytoplankton community to temperature by Eppley (1972, Figure 2). However, more data of picophytoplankton strains with optima at lower temperatures would need to be included to distinguish better between these two methods of deriving the absolute maximum growth rates of a phytoplankton community.

Temperature and the Chlorophyll *a* to Carbon Ratio

Phytoplankton acclimate to the prevailing environmental conditions by changing their cell composition. The chlorophyll *a* to carbon ratio is an important variable for measuring biomass and primary production and varies between different phytoplankton groups, e.g., diatoms have higher chlorophyll *a* to carbon ratios compared to picophytoplankton (Geider et al., 1997). In agreement with previous studies (Eppley, 1972; Geider, 1987) we show that with increasing temperature the chlorophyll *a* to carbon ratio also increases for the group of picophytoplankton. We also show that this effect on the chlorophyll *a* to carbon ratio is caused by the increase in chlorophyll *a* concentration with temperature, rather than by a potential decrease of cellular carbon.

We also indicate a drop above the optimum temperature for some individual strains. This reduction in photosynthetic machinery at supra-optimal temperatures is comparable to the effect caused by photoinhibition at high light levels to reduce damage (Geider, 1987) and is thus consistent with the photosynthetic model of Baumert and Petzoldt (2008), which attributes the decrease of growth rate above T_{Opt} to an increase in light inhibition with temperature.

Picophytoplankton and Climate Warming

Picophytoplankton, including both groups of picoprokaryotes and picoeukaryotes, is treated as a single plankton functional type in ocean biogeochemical models (Le Quéré et al., 2015). Hence, the assumption is that it can be represented with a common set of physiological traits. Generally, there is some support for this assumption, as both groups of picophytoplankton are adapted to low nutrient and light conditions because of their high nutrient uptake and light harvesting efficiency compared to other phytoplankton groups (Raven, 1998). Both adaptations could help to explain their better success in oligotrophic (Alvain et al., 2008) and deep mixed water columns (Veldhuis et al., 2005). However, the distribution of picoprokaryotes is inversely related to that of picoeukaryotes in the natural environment (Buitenhuis et al., 2012), and these distributions are correlated with nitrogen concentration and depth of the euphotic layer

(Bouman et al., 2011). In addition, temperature was also shown to be an important predictor for the realized ecological niche space of diverse phytoplankton groups (Brun et al., 2015). The temperature span at which growth was positive for *Prochlorococcus* sp. presented in our study is consistent with the quartile temperature span of the realized ecological niche (16–25°C) in the study by Brun et al. (2015) which uses observations from MAREDAT (Buitenhuis et al., 2013). Unfortunately, they were not yet able to specifically separate the realized ecological niche of picophytoplankton due to the lack of available data on a broader range of species. Our study highlights the importance of quantifying the direct impact of each temperature-related parameter for a large variety of phytoplankton strains to define fundamental ecological niches, which are required for the formulation of ocean biogeochemistry models (Le Quéré et al., 2005), and which aim to represent realized ecological niches as emergent properties (Follows et al., 2007).

With ongoing climate warming, the biomass and productivity of picophytoplankton relative to other phytoplankton could increase due to enhanced water column stratification and lower nutrient availability (Behrenfeld et al., 2006; Morán et al., 2010). The results presented here support a potential advantage for picophytoplankton as a consequence of the higher temperature dependence of their maximum growth rates compared to other phytoplankton groups. Picophytoplankton shows a stronger increase in absolute maximum growth rates with temperature with a Q_{10} of 2.3 compared to coccolithophores with a Q_{10} of 1.7 (Buitenhuis et al., 2008), and a mixed phytoplankton community with a Q_{10} of 1.88 (Eppley, 1972). However, the relative advantage of the temperature dependence of the absolute maximum growth rates of picophytoplankton also needs to be considered within an ecosystem with trophic interactions in a warming climate, where the effect of temperature on the top down control by zooplankton may also alter the phytoplankton community structure.

In addition, we suggest that climate warming may also change the composition of the picophytoplankton community itself.

Even though picoprokaryotes may show a stronger increase in biomass in specific regions due to their higher Q_{10} , they are restricted by a narrower temperature tolerance range. The sharp decrease of maximum growth rates above the optimum temperature suggest that the temperature tolerance range is also an influential parameter for the distribution of phytoplankton species and its change with climate warming. We therefore assume that picoprokaryotes will be shifted to higher latitudes or depth. This shift has already been suggested using a neural network model which defines niches of two picoprokaryotes based on temperature, PAR and nutrient availability (Flombaum et al., 2013). However, we also suggest that picoeukaryotes, in particular those of an intermediate size around 2 μm , will be able to increase their contribution to phytoplankton biomass over a wider temperature span.

AUTHORS CONTRIBUTIONS

BS conducted the laboratory experiments, analyzed the data, and is the lead author on this paper. EB and CL wrote the project proposal, acquired funding, and put forward some of the hypotheses. All authors co-wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <http://journal.frontiersin.org/article/10.3389/fmars.2016.00164>

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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