Is there a difference of temperature sensitivity between marine phytoplankton and heterotrophs?

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Abstract

The temperature sensitivity of phytoplankton growth rates, parameterized as the activation energy (E_a) in the Boltzmann-Arrhenius equation, is critical to determining how global warming will affect marine ecosystems and the efficiency of the biological pump in the ocean. We applied both linear and nonlinear regression models to two laboratory temperature-growth experimental datasets to estimate the E_a of each taxon of phytoplankton and heterotrophic protists. We found that phytoplankton E_a and normalized growth rates depended strongly on community composition. Diatoms grew more rapidly and had lower E_a values, whereas cyanobacteria grew more slowly and had higher E_a values. The phytoplankton E_a was underestimated by a single OLS regression on the pooled dataset because slowly growing cyanobacteria dominated in warm, oligotrophic ocean gyres, and rapidly growing diatoms dominated in cold, nutrient-rich waters. By contrast, the median E_a values estimated from individual experiments did not differ between phytoplankton and heterotrophic protists. Our results suggest that phytoplankton community composition needs to be considered when trying to predict the effects of ocean warming on ecosystem productivity and metabolism.

Temperature sensitivity of phytoplankton growth rate plays a critical role in determining the response of primary production to ocean warming in global-scale ocean models (Sarmiento et al. 2004; Taucher and Oschlies 2011) as well as the response to seasonal and other temperature changes. The Metabolic Theory of Ecology (MTE) predicts that the mean activation energy (E_a) of metabolism should be around 0.65 eV (Gillooly et al. 2001; Brown et al. 2004). The E_a for photosynthesis, however, is thought to be significantly lower than the value ($\sim$ 0.65 eV) for heterotrophic activities such as community respiration and zooplankton grazing (Allen et al. 2005; López-Urrutia et al. 2006; Rose and Caron 2007; Regaudie-de-Gioux and Duarte 2012). This difference has profound implications, in that rising temperature would tend to preferentially enhance heterotrophy, and with it the release of CO_2, potentially leading to a positive feedback to climatic warming (López-Urrutia et al. 2006). This difference of temperature sensitivity might also be the critical factor causing low carbon export efficiency in low latitude, warm oceans compared to high latitude regions (Laws et al. 2000).

In the literature, estimates of E_a differ as a function of methodologies and datasets. One of the earliest and most widely used temperature coefficients ($Q_{10} = 1.88$, corresponding to an E_a of 0.41 eV) given by Eppley (1972) and later confirmed by Rose and Caron (2007) and Bissinger et al. (2008), was estimated by fitting the upper envelope of phytoplankton growth rate vs. temperature in a pooled laboratory dataset. Some studies have argued that fitting the upper envelope is inappropriate and have instead used ordinary least squares (OLS) regression to fit mean growth rates under optimal conditions, the result being a slightly lower estimate ($\sim$ 0.3 eV) of E_a (Sal and López-Urrutia 2011). An E_a of 0.3 eV is more consistent with the results from terrestrial ecosystems (Allen et al. 2005) and is also more consistent with photosynthesis being less sensitive to temperature than respiration.

While the above estimates of phytoplankton E_a were based on laboratory data, other studies have estimated E_a using field data, which is arguably more representative of in situ plankton communities. Based on changes of oxygen concentrations during light-dark bottle incubations,

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Regaudie-de-Gioux and Duarte (2012) found an E_a of 0.32 eV, similar to the value reported by López-Urrutia et al. (2006). Chen et al. (2012) estimated a similar E_a (0.36 eV) for phytoplankton growth rates based on the dilution technique (Landry and Hassett 1982).

Although the evidence for the lower temperature sensitivity of photosynthesis seems pervasive, there is reason to be concerned about several statistical methods used in previous studies. The approach for calculating E_a has been a regression with temperature as the predictor (X) and the biological rate as the response variable (Y). One important assumption in OLS is residual independence (Faraway 2004). Growth rates of one taxon measured at different temperatures should be more correlated with each other than with the growth rates of different taxa. Thus in a pooled dataset that includes the growth rates of the same taxon at different temperatures and the growth rates of different taxa at different temperatures, the assumption of residual independence is violated. This concern also applies to field datasets that include uneven spatial and temporal distributions of experimental data. For laboratory datasets that consist of a number of independent experimental results, an apparent solution is NOT to pool the data together as in Eppley (1972), López-Urrutia et al. (2006), and Rose and Caron (2007), but instead to run regressions for each taxon separately, as in Dell et al. (2011).

Another well-known problem for OLS regression is the errors in X. When the values of X are controlled by the investigator, OLS can give an unbiased estimate of the regression slope even if the predictor is subject to error. However, when the predictor is merely observed without control by the investigator, the OLS estimate tends to underestimate the regression slope (Ricker 1973). It is noteworthy that the errors associated with X include not only measurement errors but also natural variability, with the later accounting for most of the errors in biological samples (Ricker 1973). The natural variability typically includes the uncertainties caused by various unknown variables, which co-vary with the predictor. Type II regressions such as the geometric mean (GM) regression or ranged major axis (RMA) regression have been recommended for such situations (Ricker 1973; Laws and Archie 1981; Legendre and Legendre 1998).

In laboratory experiments with a single taxon, the temperatures are predetermined by the investigator, so that there is no problem in applying OLS regression. However, in a pooled laboratory dataset, the investigator loses control over the temperatures because the experimental temperatures used for growing the phytoplankters depend on the thermal tolerance of the organisms, which is not under the control of the investigator. Hence it is important to apply OLS regressions to each taxon separately.

Another problem with the estimates of E_a is that most previous studies have used linear regressions, although the temperature response curves are often unimodal (Dell et al. 2011; Thomas et al. 2012; Chen 2015). Pawar et al. (2016)

have shown that deviations from the linear Boltzmann-Arrhenius model can bias estimates of E_a. When the experimental temperatures are biased toward the suboptimal temperature range, E_a tends to be overestimated. And E_a will be underestimated if the experimental temperatures are close to the optimal growth temperature. Although nonlinear models have been applied to analysis of marine phytoplankton data (Thomas et al. 2012), it is unclear whether testing the null hypothesis that the temperature sensitivity of autotrophs and heterotrophs is the same will be unbiased if nonlinear models are used.

Given the above concerns, we used both OLS and nonlinear regression methods to analyze data for each individual taxon in an extensive laboratory phytoplankton dataset and a smaller microzooplankton dataset. We then compared the average E_a with the E_a estimated from a single OLS regression analysis of the pooled datasets. We also tried GM and RMA regressions on the pooled laboratory and field datasets to see whether these Type II regression methods could alleviate the problem in OLS. Our null hypothesis was that the choice of regression methods would not affect estimates of E_a and the relative temperature sensitivity of autotrophic and heterotrophic rates.

Methods

Laboratory phytoplankton dataset and analysis

Two phytoplankton growth rate datasets were analyzed in this study (Fig. S1 in the Supporting Information). The first dataset consisted of marine phytoplankton specific growth rates (d^{-1}) measured at different temperatures in the laboratory (Supporting Information Fig. S2). This dataset was built upon four published datasets (López-Urrutia et al. 2006; Rose and Caron 2007; Bissinger et al. 2008; Thomas et al. 2012). The measurements were conducted under light- and nutrient-saturated conditions. Only experiments that included at least four temperatures that spanned at least 5°C were included. The cell size in terms of biovolume (μm^3) and the coordinates of the locations where the taxa were isolated were recorded (Supporting Information Fig. S1). The annual mean temperatures of these locations were interpolated from the World Ocean Atlas 2009 (http://www.nodc.noaa.gov/OC5/WOA09/pr_woa09.html) using the method k-nearest neighbor classification in the R package "*class*" (Venables and Ripley 2002). All phytoplankton taxa were classified into five functional types: diatoms, dinoflagellates, green algae, cyanobacteria, and haptophytes. For the OLS regression analysis, we removed data points at temperatures above the optimal growth temperature (T_{opt}) to focus on the "physiological temperature range" of phytoplankton (Pawar et al. 2016). We fit the data from each experiment to a log-transformed linear Boltzmann-Arrhenius model:

$$\ln(\mu) = \ln(\mu_0) + E_a \cdot T_b \qquad (1)$$

in which μ is the growth rate of the plankton at Boltzmann temperature $T_b = \frac{1}{k}\left(\frac{1}{T_0} - \frac{1}{T}\right)$. The parameter k is the Boltzmann

constant (8.62×10^{-5} eV K^{-1}), T_0 is the reference temperature (288 K), T is the experimental temperature (K) and μ_0 is the growth rate constant at temperature T_0. The OLS regression was performed with the function "*lm*" in R. We used the function "*lmodel2*" in the R package "*lmodel2*" to perform GM and RMA regressions (Legendre 2014), which can only be applied to bivariate situations such as Eq. 1 (Legendre and Legendre 1998).

For the nonlinear regression analysis, we included only datasets with five or more experimental temperatures, at least two of which were lower than T_{opt}, and at least one of which was higher than T_{opt}. We used a nonlinear model to fit the phytoplankton growth rate vs. temperature data (Johnson and Lewin 1946; Dell et al. 2011):

$$\mu = \mu_0 \frac{e^{\frac{E_a}{k_b}\left(\frac{1}{T_0}-\frac{1}{T}\right)}}{1+\frac{E_a}{E_h-E_a}e^{\frac{E_h}{k_b}\left(\frac{1}{T_{opt}}-\frac{1}{T}\right)}} \qquad (2)$$

where E_a (eV) is the activation energy of the growth rate without temperature inactivation, E_h (eV) is the parameter indicating how fast the growth rate decreases with increasing temperature due to high temperature inactivation, and T_{opt} (K) is the optimal growth temperature. Although the parameter E_a has a similar meaning in Eqs. 1 and 2, the value should be higher in Eq. 2 because the effect of high temperature inactivation is not taken into account in Eq. 1. Other symbols have the same meaning as in Eq. 1. The nonlinear least squares regression was implemented using the R function "*nls*."

Dilution dataset and analysis

The second dataset was a global dataset of results of dilution experiments expanded from Chen et al. (2012). The dilution technique, which was first used by Landry and Hassett (1982) to measure phytoplankton specific growth rates (d^{-1}) and mortality rates (d^{-1}) due to microzooplankton grazing, is the most widely used method to directly measure phytoplankton specific growth rates in the ocean (Laws 2013). The dilution technique can also give nutrient-replete phytoplankton growth rates (μ_n), and thereby provide an estimate of the extent of nutrient limitation (Marañón et al. 2015). We selected experiments conducted only in surface waters with irradiance levels at least 10% of surface irradiance to minimize the problem of light limitation. The experimental temperature, nitrate concentration, and light level were also recorded from the literature when possible.

The E_a of microzooplankton grazing rates (m, d^{-1}) was also estimated from the dilution dataset. Following Regaudie-de-Gioux and Duarte (2012), we normalized m to the chlorophyll a (Chl a) concentration to obtain a simple estimate of the biomass specific grazing rate.

Laboratory dataset of growth rates of heterotrophic protists (H-Protists)

We also compiled a dataset of growth rates of H-Protists at different temperatures (Supporting Information Fig. S3). Cell volume (μm^3) was also recorded for each taxon. The protists were classified into four groups: ciliates, amoeba, heterotrophic nanoflagellates, and heterotrophic dinoflagellates. Note that there was only one experiment with heterotrophic dinoflagellates (Kimmance et al. 2006). The coordinates of the isolation sites were not recorded due to lack of data.

Results

Laboratory dataset of phytoplankton growth rates

For the pooled laboratory dataset of phytoplankton growth rates, an OLS regression between ln growth rate and temperature gave an E_a of 0.23 ± 0.02 eV (Mean $\pm$ SE, the same below), whereas the GM and RMA regressions gave E_a values of 0.86 ± 0.02 eV and 0.42 ± 0.04 eV, respectively (Table 1; Fig. 1). Including the effect of cell size in the OLS regression, with either a linear or unimodal model (Chen and Liu 2011; Marañón et al. 2013), did not significantly affect E_a.

The OLS and nonlinear regressions applied to each individual experiment are shown in Supporting Information Fig. S2. In contrast to the E_a of 0.23 eV estimated from the OLS regression on the pooled dataset, the median E_a of the individual OLS regressions was 0.66 eV, close to the canonical value of 0.65 eV (Brown et al. 2004). This difference may be explained by the significant changes of community composition along the temperature gradient (Fig. 1). The phytoplankton isolated from warm and offshore waters were mostly cyanobacteria, whereas other taxa, particularly diatoms, were mostly isolated from coastal environments from tropical to polar regions (Fig. 2). When rates were normalized to the same temperature, cyanobacteria dinoflagellates had significantly lower μ_0 values than diatoms, green algae, and haptophytes (Wilcoxon tests, $p < 0.001$; Fig. 3a,b). For example, the median μ_0 normalized to 15°C was 0.75 d^{-1} for diatoms, nearly 3.6 times that of cyanobacteria (0.21 d^{-1}). Thus, the slope of the OLS regression applied to the pooled dataset underestimated the true temperature sensitivity of phytoplankton growth rates because the slowly growing cyanobacteria that dominated the warm environments reduced the magnitude of the regression slope.

The effect of phytoplankton functional types (PFTs) on μ_0 seemed much stronger than that of cell size (Fig. 3c,d). Even at the same size, the μ_0 values of diatoms were significantly higher than those of cyanobacteria and dinoflagellates. Although in general a unimodal relationship existed between μ_0 and size and a weak decreasing trend of μ_0 with size existed within diatoms and dinoflagellates, the

Table 1. A summary of estimated E_a (eV; Mean $\pm$ 1 SE) of phytoplankton growth rate (μ, d^{-1}), heterotrophic protist growth rate (μ, d^{-1}), and microzooplankton grazing rate (m, d^{-1}) derived from OLS, GM, and RMA regressions on three datasets. Phyto: phytoplankton. μ_n: nutrient-enriched phytoplankton growth rate (d^{-1}). m: microzooplankton grazing rate (d^{-1}). Chl: Chl a concentration (μg L^{-1}). N: number of observations. The median E_a values estimated from linear and nonlinear (NLS) regressions applied on individual experiments are also shown. The numbers in the parentheses are the number of experiments involved.

	OLS	GM	RMA	N	Linear	NLS
Phyto lab data (μ)	0.24 ± 0.02	0.83 ± 0.02	0.41 ± 0.04	1387	0.66 (234)	0.78 (178)
Protist lab data (μ)	0.42 ± 0.05*	0.77 ± 0.05	0.72 ± 0.04	173	0.66 (41)	1.06 (17)
	0.53 ± 0.05†			172		
Dilution data (μ_n)	0.40 ± 0.02	0.73 ± 0.02	0.53 ± 0.02	1291		
Dilution data (m:Chl)	0.50 ± 0.04	1.39 ± 0.04	0.68 ± 0.05	1291		

*The effect of cell size not considered.
†The effect of cell size has been taken into account.
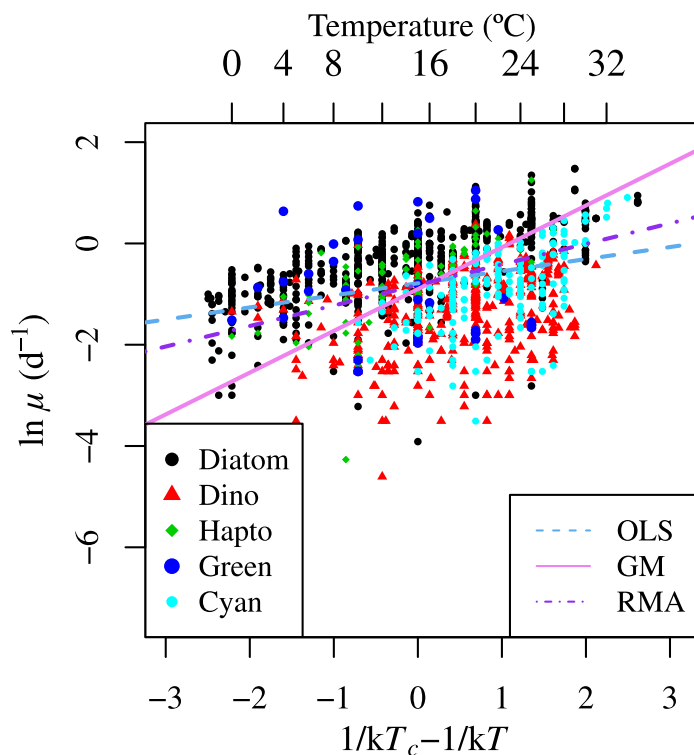
Fig. 1. Regression lines of phytoplankton specific growth rates vs. temperature in the pooled laboratory dataset. OLS: ordinary linear regression ($y = 0.24 \cdot x - 0.79$). RMA: ranged major axis regression ($y = 0.41 \cdot x - 0.82$). GM: geometric mean regression ($y = 0.83 \cdot x - 0.90$). Dino: dinoflagellates. Hapto: haptophytes. Green: green algae. Cyan: cyanobacteria.

variations between PFTs were so large that a size scaling equation seemed meaningless.

There was no universal value of E_a among PFTs (Fig. 3e,f). Based on the linear model, cyanobacteria had significantly higher E_a values (median = 1.0 eV) than diatoms (median = 0.47 eV), green algae (median = 0.63 eV), and haptophytes (median = 0.69 eV) (Wilcoxon tests, $p < 0.01$). This result suggests that at higher temperatures (e.g., 30°C), the

difference of growth rates between cyanobacteria and rapidly growing diatoms and green algae is smaller than at 15°C (Supporting Information Fig. S4; Sal et al. 2015). For example, the median μ_0 normalized to 30°C was 1.25 d^{-1} for cyanobacteria and only 1.94 d^{-1} for diatoms, although the difference was still significant. This pattern, combined with the fact that the optimal temperature tends to be higher for cyanobacteria than for other phytoplankton (Chen 2015), implies that cyanobacteria have a preference for high temperature, and the dominance of cyanobacteria in warm, oligotrophic oceans can be partially attributed to a temperature effect (López-Urrutia and Morán 2015). Interestingly, the median E_a of diatoms is close to the estimate of 0.42 eV from both the RMA regression (Fig. 1) and the Eppley curve, the suggestion being that these two are actually estimating the E_a of diatoms. This suggestion is understandable, because diatoms dominate the pooled dataset and usually have the highest growth rates among all phytoplankton.

We checked whether the use of the nonlinear model affected the above results (Figs. 3, 4). As expected, the E_a values estimated with the nonlinear model, with a median value 0.78 eV, tended to be larger than those from the linear model (Fig. 4a). The differences in μ_0 estimates were less pronounced (Fig. 4b). As a result, the differences of E_a values between cyanobacteria and green algae or haptophytes became insignificant with using the nonlinear model. The difference of E_a values between cyanobacteria and diatoms, however, still persisted.

Dilution dataset

In the dilution dataset, an OLS regression between $\ln(\mu_n)$ and temperature yielded an E_a of 0.40 ± 0.02 eV, while the GM and RMA regressions yielded E_a values of 0.73 ± 0.02 eV and 0.53 ± 0.02 eV, respectively (Fig. 5a). Including the effect of light or nitrate in the OLS regression did not improve the goodness of fit or affect the estimation of E_a. The analysis of μ_0 yielded similar results. The E_a of microzooplankton grazing rate normalized to Chl a concentration (m : Chl) estimated by the OLS, GM, and RMA regressions were 0.50 ± 0.04 eV, 1.39 ± 0.04 eV, and 0.68 ± 0.05 eV, respectively (Table 1; Fig. 5b).
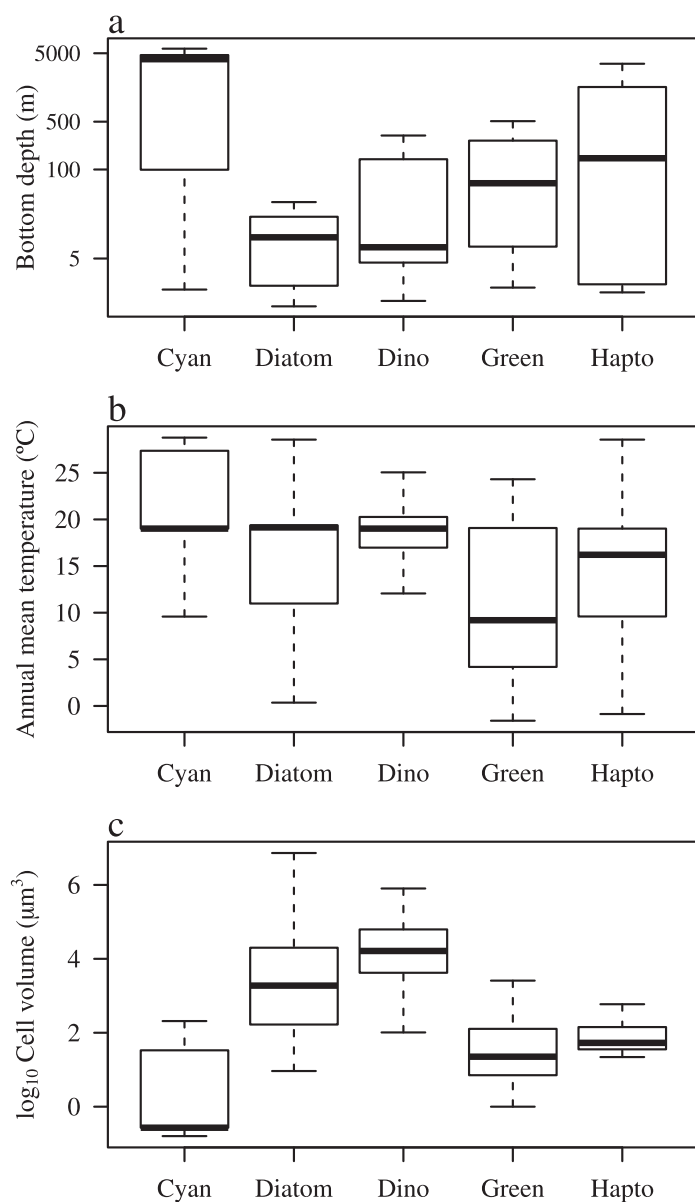
Fig. 2. Box-and-whisker plots showing the relationships of phytoplankton functional types with (**a**) the bottom depth, (**b**) annual mean temperatures of the isolation locations, and (**c**) the $\log_{10}$ cellular volumes.

Laboratory dataset of H-Protists

For the pooled dataset of growth rates of H-Protists, the OLS regression gave an E_a of 0.42 ± 0.05 eV, whereas the GM and RMA regression gave E_a values of 0.77 ± 0.05 eV and 0.72 ± 0.04 eV, respectively (Table 1; Fig. 6). After taking into account the effect of cell size, the E_a estimated with the OLS regression increased to 0.53 ± 0.05 eV, with an estimated allometric exponent of -0.09 ± 0.02 (Fig. 6).

The median E_a of the individual OLS regressions applied to each experiment was 0.66 eV, identical to the median phytoplankton E_a. Heterotrophic nanoflagellates had significantly higher μ_0 values than other organisms (Fig. 7a; Supporting

Information Fig. S4). The differences of the μ_0 values between nanoflagellates and ciliates can be accounted for by cell size, but the differences of μ_0 values between nanoflagellates and amoebae were not due to size (Fig. 7b). The effects of cell size were also evident within groups. The E_a values were not affected by PFT or cell size, and the universal value was about 0.65 eV (Fig. 7c,d). A comparison of the mean E_a value from the pooled dataset and the median value from the individual regressions revealed that the smaller differences among heterotrophic protists compared to phytoplankton may reflect the fact that there were no apparent changes of community structure along temperature gradients for heterotrophic protists (Fig. 6). The median E_a estimated from the nonlinear regressions was 1.06 eV. This value, however, is less robust than that of phytoplankton because only 17 experiments satisfied the conditions for a nonlinear regression.

Phytoplankton E_a vs. H-Protists

A comparison between the E_a histograms of phytoplankton and H-Protists suggests that the differences of E_a values depended on species composition (Fig. 8). The most notable difference was the fact that the E_a values of some diatoms within the Class *Bacillariophyceae* and *Coscinodiscophyceae* were closer to 0.4 eV than 0.65 eV (Supporting Information Fig. S5).

Discussion

Bias in the OLS regression for a pooled dataset

We have shown that a single OLS regression on a pooled dataset of laboratory phytoplankton growth rates, which has been widely used in the literature, generates a much lower E_a (~ 0.3 eV) than the median E_a from individual OLS regressions for each experiment (Allen et al. 2005; López-Urrutia et al. 2006). This bias can be attributed to changes of the phytoplankton community composition along a temperature gradient (i.e., slowly growing cyanobacteria tend to dominate at high temperatures). If we simply compare the median E_a values between phytoplankton and H-Protists, there is no apparent difference. Although we only investigated the E_a of H-Protists in this study, the E_a values of other heterotrophic plankton such as mesozooplankton and heterotrophic bacteria are not expected to deviate substantially from the canonical value 0.65 eV predicted by MTE (Huntley and Lopez 1992; López-Urrutia and Morán 2007). The major cause of the difference of E_a values between autotrophs and heterotrophs has been long believed to be the abnormally low E_a of autotrophs (Allen et al. 2005). Since we believe that the previously reported E_a values of phytoplankton are likely underestimates, previous reasoning that warming will drive the plankton ecosystem toward heterotrophy due to the lower E_a of phytoplankton, may be problematic (López-Urrutia et al. 2006; Rose and Caron 2007). With increasing temperature, the marine plankton ecosystem might still be more heterotrophic, not because of the lower E_a of phytoplankton, but because of the changes in phytoplankton
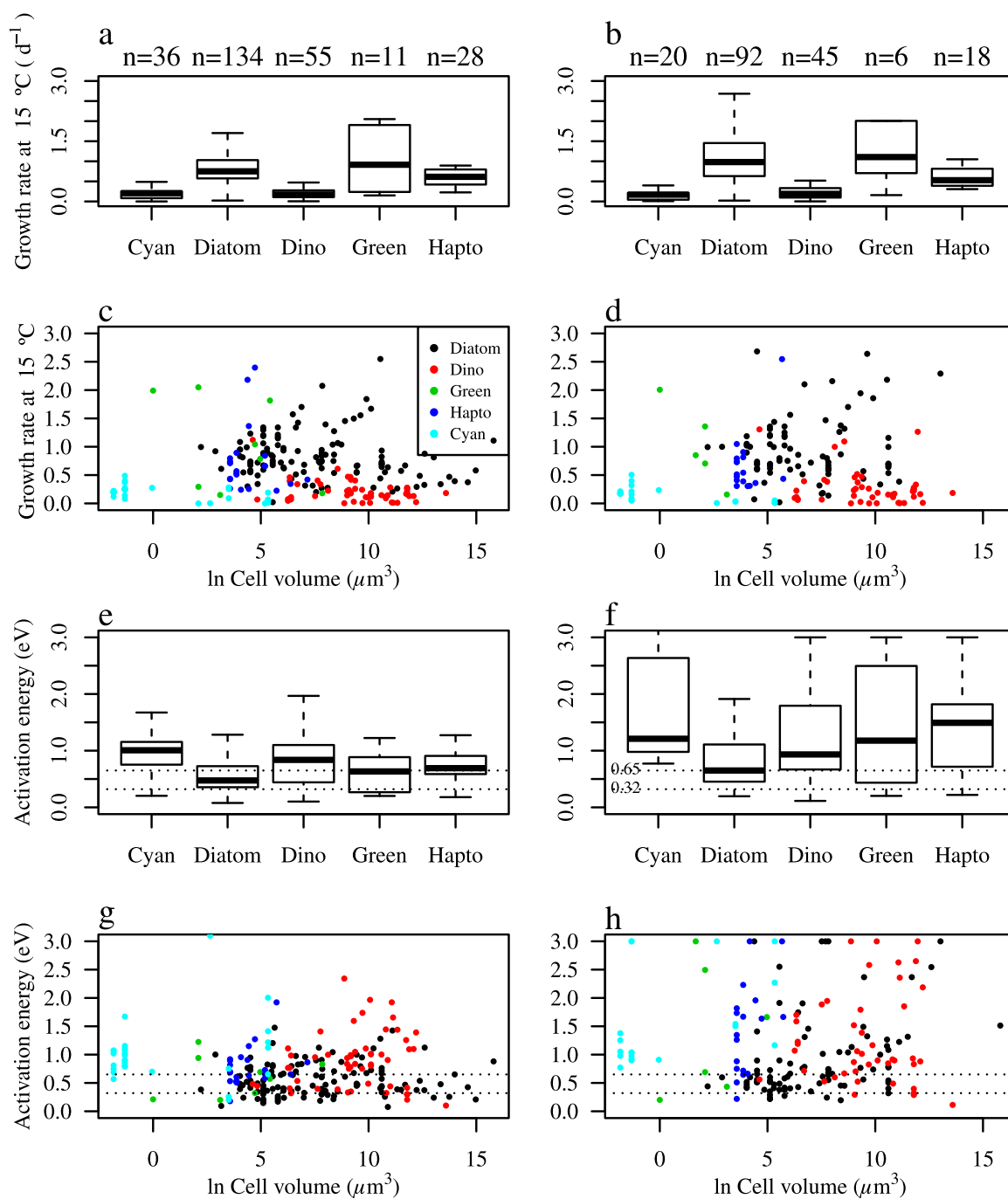
Fig. 3. Relationships of growth rates normalized to 15°C with (**a**, **b**) phytoplankton functional type and (**c**, **d**) cell size. (**e**) and (**f**) show the relationships between activation energies and phytoplankton functional type. (**g**) and (**h**) represent the relationships between activation energies and cell size. (**a**), (**c**), (**e**) and (**g**) are the results from the linear model. (**b**), (**d**), (**f**), and (**h**) are the results from the nonlinear model.

community composition (i.e., the slow-growing cyanobacteria will have a tendency to dominate the community).

Type II regressions may partially alleviate the problem of OLS regression bias by taking into account the errors in the predictors. However, because of the uncertainties in the error structures of both predictors and response variables, Type II regressions may also bias the regression slopes of the pooled

dataset, and different variants of the Type II regression models yield inconsistent results (Table 1; Legendre and Legendre 1998).

This problem becomes intractable when dealing with field datasets for which only a pooled dataset is available. Although there have been many attempts to estimate E_a from field datasets (Chen et al. 2012; Regaudie-de-Gioux and Duarte 2012;
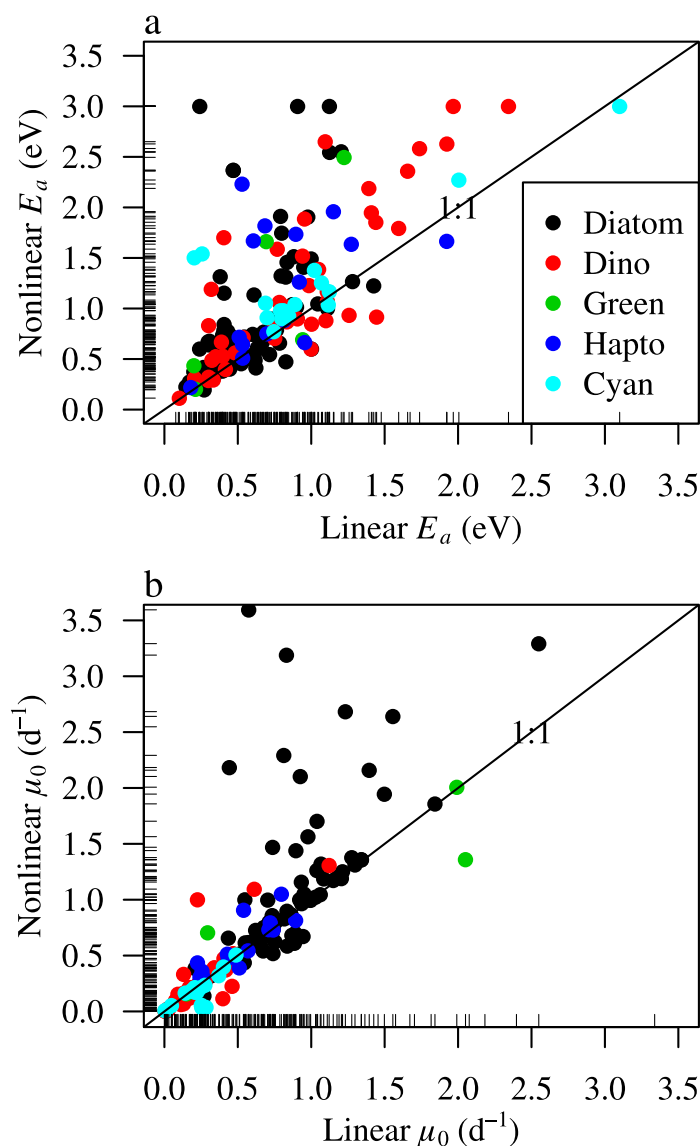
Fig. 4. Comparisons of estimates of (**a**) growth rates normalized to 15°C and (**b**) activation energies between the linear and nonlinear models.

Marañón et al. 2014), we do not recommend estimating E_a from field data because of the confounding effects of covariates such as community structure, thermal acclimation, and nutrient and light levels that potentially bias the estimation of the true temperature sensitivity. Here, it is important to reiterate that temperature sensitivity reflects the direct effect of temperature on biological rates, excluding indirect effects of temperature such as temperature–induced water column stratification. The direct effect of temperature deserves investigation because, in ecosystem models, the effects of each environmental factor on phytoplankton growth need to be treated explicitly and separately. In this context, the best estimates of E_a values come from laboratory experiments in which factors other than temperature are optimal.
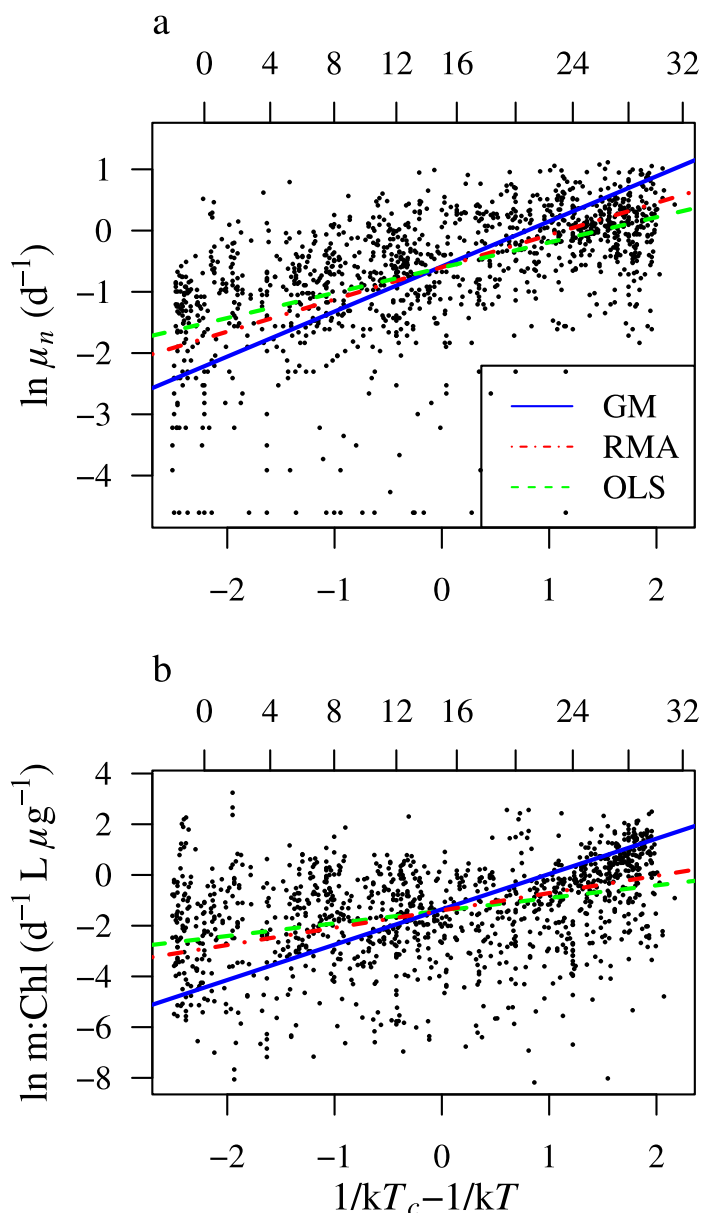
Fig. 5. Data from dilution experiments. (**a**) Nutrient-enriched growth rates (μ_n) and (**b**) microzooplankton grazing rates normalized to Chl *a* concentrations ($m : Chl$) vs. temperature with three linear regression lines shown. OLS regression equation for μ_n: $y = 0.41 \cdot x - 0.61$. GM for μ_n: $y = 0.74 \cdot x - 0.59$. RMA for μ_n: $y = 0.53 \cdot x - 0.60$. OLS regression for $m : Chl$: $y = 0.50 \cdot x - 1.41$. GM for $m : Chl$: $y = 1.39 \cdot x - 1.36$. RMA for $m : Chl$: $y = 0.68 \cdot x - 1.40$.

Limitations of the laboratory data

Conversely, it might be argued that laboratory cultures may not provide a good representation of the behavior of real communities in the ocean. Laboratory experiments are biased toward those species that can be easily isolated and cultured, a fact that is particularly evident from the dominance of coastal taxa in the experiments with diatoms, dinoflagellates, and H-Protists (Fig. 2). It is well known
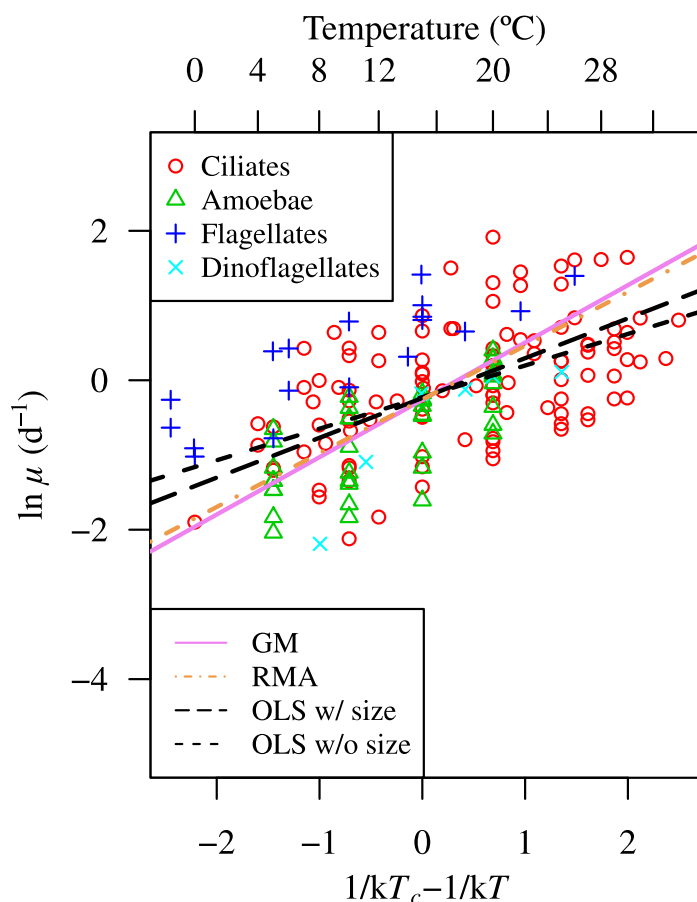
Fig. 6. Regression lines of specific growth rates of heterotrophic protists vs. temperature in the pooled laboratory dataset. OLS: ordinary linear regression ($y = 0.42 \cdot x - 0.23$ without size; $y = 0.53 \cdot x + 0.54 - 0.09 \cdot \ln V$ with size, where $\ln V$ is the log-transformed cell volume). RMA: ranged major axis regression ($y = 0.72 \cdot x - 0.26$). GM: geometric mean regression ($y = 0.76 \cdot x - 0.26$).

that it is difficult to culture many marine planktonic protists, and for this reason we did not attempt to analyze the temperature sensitivity of marine bacteria cultured in the lab.

Another problem is the large variability of E_a (Fig. 8). Applying the median E_a as a universal constant in a global model can certainly be expected to cause errors in localized areas where the plankton community composition differs from the species pool in the compiled laboratory dataset. A better approach might be to apply one E_a value for each PFT and also take into account the random variations of E_a within each PFT. An alternative approach to this problem might be to conduct in situ temperature-modulated experiments to estimate E_a (Vaquer-Sunyer and Duarte 2013; Chen and Liu 2015). A criticism of such short-term experiments is their inability to simulate the effect of temperature acclimation (H. Liu pers. comm.). The dilemma is that neither laboratory nor field data are perfect. Considering the fact that temperature–growth rate relationships have probably been most

extensively studied for plankton, similar problems undoubtedly exist in the estimation of other temperature-rate relationships.

Size vs. PFT effects on plankton traits

There are two main approaches to reducing biological complexity and computational demands while simulating the effects of phytoplankton diversity. One strategy is to aggregate species into a few functional types (Le Quéré et al. 2005). Another strategy is to treat size as a master variable and apply a size-scaling allometric equation to model the effect of the distribution of sizes, with the hope that most of the differences of traits among PFTs can be explained by size (Moloney and Field 1991; Smith et al. 2015). Some studies have combined both approaches, but have suffered from greater computational demands (Ward et al. 2012). We expect that the results of this study will facilitate selection of the right strategy. The phytoplankton μ_0, which is the maximum growth rate at a reference temperature in the model, seems more dependent on PFTs than on size (Fig. 3a). The large amount of scatter in the plot of μ_0 vs. cell size (Fig. 3c,d) means that obtaining a simple size scaling equation, either linear or unimodal, to account for the variations of μ_0 is problematic (López-Urrutia et al. 2006; Chen and Liu 2011). Given the large impact of PFTs on μ_0 values, inclusion of PFTs in phytoplankton models seems necessary (Irwin et al. 2006; Ward et al. 2012). This conclusion also applies to E_a, for which significant differences have been found among PFTs, but no size effect. It should also be noted that obtaining a size-scaling equation requires appropriate approaches to correct for temperature effects because there are substantial variations of E_a among taxa (Marañón et al. 2013; Sal et al. 2015).

The idea of simplifying simulation of the planktonic system by using a general size-scaling equation is similar to MTE, which attempts to model the metabolism of most organisms based on a simple model. Although this idea sounds appealing, the complex biochemical cycles and feedbacks within the seemingly simple unicellular phytoplankton cannot be overlooked; a diversity of growth responses to temperature is very likely (Mackey et al. 2013; Pittera et al. 2014). Any modeler should bear in mind that the simple models commonly used are just emulators of the much more complicated biological systems within plankton cells.

Physiological mechanism responsible for phytoplankton E_a

To ascertain the correct value of the E_a for phytoplankton growth, it is essential to understand the physiological mechanisms underpinning the growth response to temperature. To our knowledge, the first quantitative hypothesis aimed at explaining the abnormally low E_a of photosynthesis was proposed by Allen et al. (2005). Based on the data for a transgenic tobacco, Allen et al. (2005) have estimated an E_a of 0.32 eV for terrestrial plants and have proposed
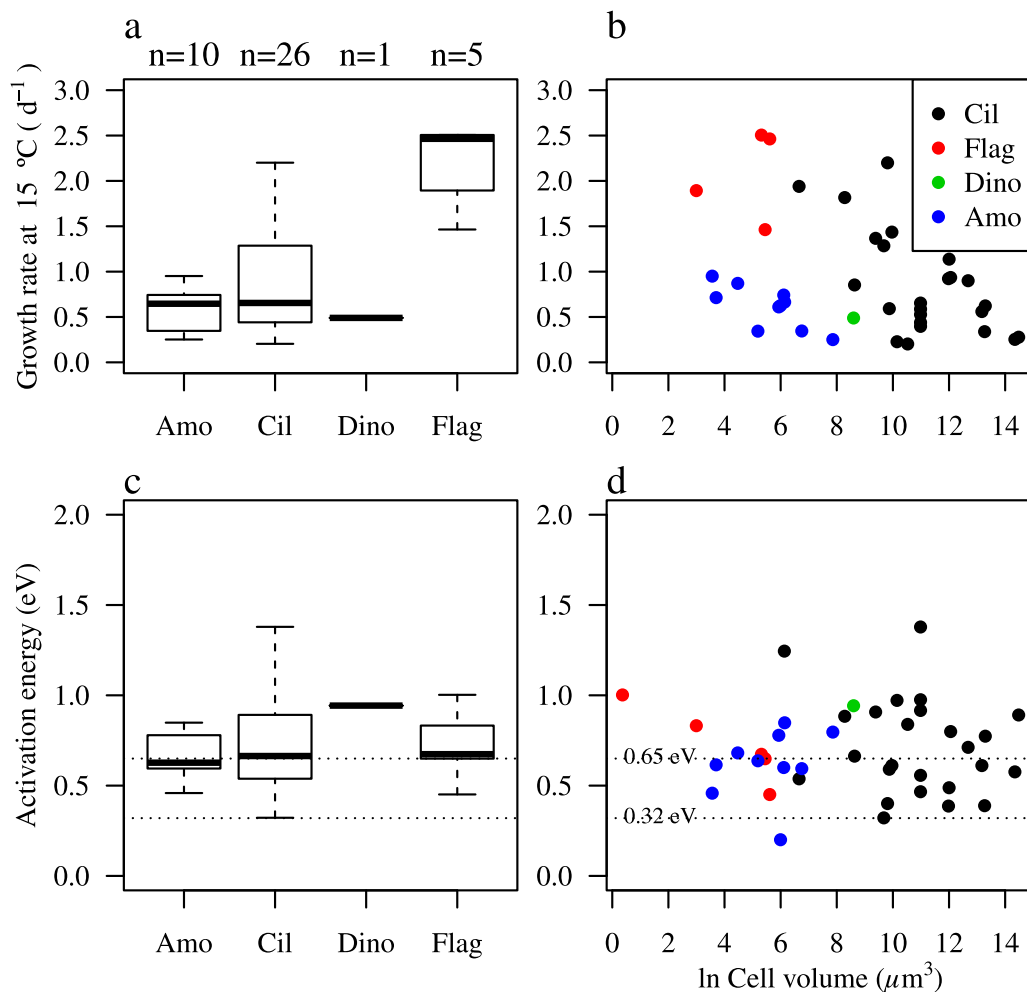
Fig. 7. Growth rates normalized to 15°C vs. (**a**) functional groups and (**b**) cell size of heterotrophic protists. (**c**) Activation energies vs. functional groups. (**d**) Activation energies vs. cell size. Amo: amoebae. Cil: ciliates. Dino: heterotrophic dinoflagellates. Flag: heterotrophic flagellates.

that the increasing role of Rubisco oxygenation with increasing temperature reduces the overall temperature sensitivity of net photosynthesis. However, one important distinction between marine phytoplankton and terrestrial plants is the widespread CO_2 concentrating mechanisms (CCMs) in cyanobacteria and eukaryotic algae (Giordano et al. 2005; Yvon-Durocher et al. 2010; Raven et al. 2011). CCMs allow phytoplankton to elevate the internal CO_2 concentration within the plastid to levels orders of magnitude higher than in the external medium. The CCM thereby attenuates the antagonistic effect of O_2 against CO_2 at the binding site of Rubisco (i.e., photorespiration) and causes the temperature sensitivity of net photosynthesis to approach that of the maximal rate of Rubisco carboxylation, which is close to 0.65 eV (Bernacchi et al. 2001; Tcherkez et al. 2006). Thus, the temperature sensitivity of marine phytoplankton can be affected, inter alia, by the capacity of the CCM as well as by the temperature sensitivity of Rubisco carboxylation (Tcherkez et al. 2006).

It is also worth noting that some studies (e.g., Marañón et al. 2013) seem to suggest that the balance between nutrient uptake and growth requirements, instead of Rubisco carboxylation rate, may be the key factor that ultimately determines the growth rate of phytoplankton even under nutrient-replete conditions. This scenario would imply that understanding the E_a of phytoplankton growth requires a focus on nutrient acquisition instead of photosynthesis.

The difference of the temperature sensitivity between phytoplankton and heterotrophs depends on community composition

Given the significant differences of E_a values among phytoplankton PFTs, any discrepancy between the temperature dependence of autotrophic and heterotrophic rates will depend at least partially on phytoplankton community composition. In areas such as the subtropical and tropical oceans, where cyanobacteria dominate, the difference between the temperature sensitivity of autotrophic and
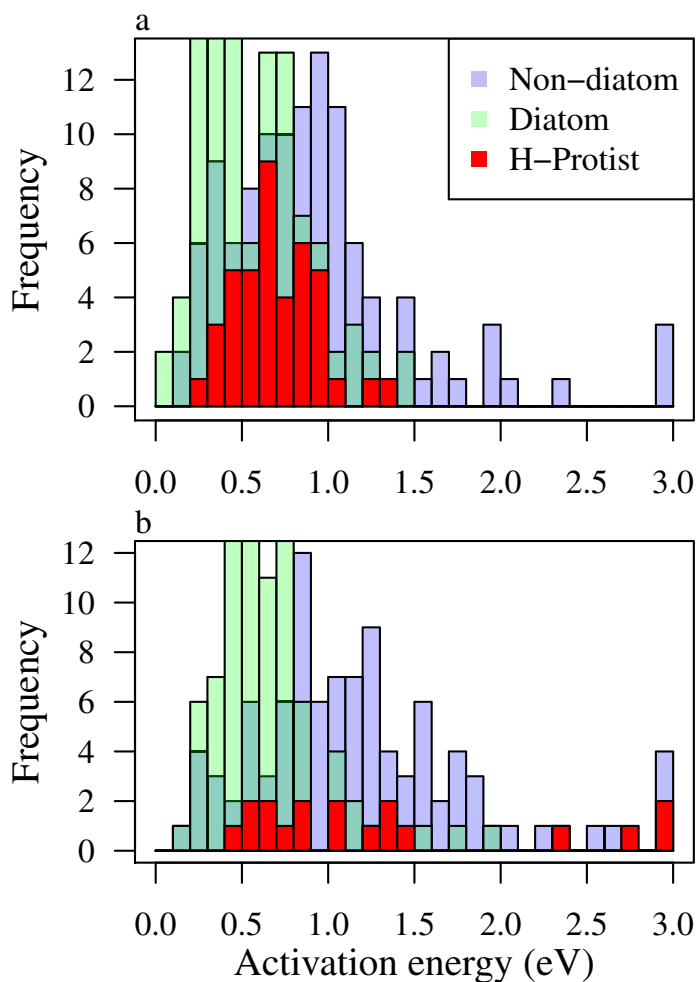
Fig. 8. Frequency distributions of E_a of diatoms, non-diatoms, and heterotrophic protists (H-Protists). (**a**) Linear models. (**b**) Nonlinear models.

heterotrophic activities is expected to be smaller than in areas where diatoms dominate, at least if indirect effects such as stratification are small.

Conclusion

Our results provide important information about the temperature sensitivity of marine plankton, information that is essential for modeling how marine plankton may respond to climate change (Sarmiento et al. 2004; Taucher and Oschlies 2011). In particular, our analysis questions the widespread belief that the temperature sensitivity of phytoplankton is lower than that of heterotrophs. Given the significant variations of μ_0 and E_a among phytoplankton PFTs, an important implication of our results is that it would be preferable to have key PFTs explicitly represented in Earth system models (Le Quéré et al. 2005). Whereas temperature traits are among the most extensively measured characteristics of phytoplankton (Thomas et al. 2012; Chen 2015), careful statistical analyses and a mechanistic understanding are still needed to

provide useful guidance for modeling and predicting how marine ecosystems will respond to climate change.

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Acknowledgments

Conflict of Interest

None declared.