Marine phytoplankton in subtropical coastal waters showing lower thermal sensitivity than microzooplankton

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Abstract

Temperature sensitivity of plankton in terms of activation energy (E_a, eV) in the Arrhenius equation is critical for predicting how marine productivity and carbon export will respond to ocean warming. In this study, we quantified the temperature responses of phytoplankton growth rate and microzooplankton grazing rate by conducting short-term temperature modulation experiments on natural communities at two subtropical sites with contrasting nutrient conditions. Our results showed that the activation energy of phytoplankton growth rate (E_a = 0.36 eV, 95% CI = 0.28 to 0.44 eV) at each station was less than that of microzooplankton grazing rate (E_a = 0.53 eV, 95% CI = 0.47 to 0.59 eV), indicating an increasing grazing pressure on phytoplankton under warming conditions. Although the difference is consistent with that reported in previous studies, it is very likely to arise from another reason, i.e., differential proximities of the optimal temperature (T_{opt} in nonlinear temperature responses of rates) of phytoplankton and microzooplankton to the environmental temperature, as we found that the environmental temperature is closer to the optimal temperature of phytoplankton growth than to that of microzooplankton grazing in this subtropical environment. Our results suggest that

34 nonlinear temperature responses of plankton should be considered when evaluating and

35 predicting the effects of ocean warming on ecosystem productivity and food web

36 dynamics, especially in subtropical and tropical waters.

37

Introduction

Marine phytoplankton plays a vital role in marine food web and global biogeochemical cycling (Field et al. 1998). How marine primary production and the efficiency of marine biological pump will respond to ocean warming strongly depends on the effect of temperature on phytoplankton growth (Sarmiento et al. 2004; Taucher and Oschlies 2011; Cael and Follows 2016). Temperature can affect phytoplankton through both bottom-up and top-down controls. For example, enhanced upper-ocean stratification in a warming ocean reduces nutrient supply, resulting in the decline of primary production and phytoplankton biomass (Behrenfeld et al. 2006; Boyce et al. 2010). Meanwhile, marine zooplankton grazing activities exert a top-down control on phytoplankton, which is also temperature dependent (Rose and Caron 2007). According to the Metabolic Theory of Ecology (MTE; Brown et al. 2004), the temperature sensitivity, in terms of activation energy (E_a, eV), is lower for autotrophic processes (~ 0.32 eV), such as phytoplankton growth, than for heterotrophic processes (~ 0.65 eV), such as zooplankton grazing activity and respiration (Allen et al. 2005; López-Urrutia et

al. 2006; Chen et al. 2012). If this were true, warming may exacerbate the top-down control on phytoplankton biomass, contributing to the decrease of primary production, which ultimately affects the functioning and services of marine ecosystem. This point has been used to explain the common occurrence of phytoplankton blooms in cold waters (Rose and Caron 2007; López-Urrutia 2008) and to predict a more heterotrophic ocean under projected ocean warming because more CO_2 will be released with increasing upper-ocean temperature (Brown et al. 2004; López-Urrutia et al. 2006). However, the difference of temperature sensitivity between autotrophic and heterotrophic rates is still contentious, partly because the estimate of temperature sensitivity is sensitive to the method used.

The widely used temperature sensitivity (Q_{10} = 1.88) is estimated from the Eppley curve by fitting the upper envelope of the maximum growth rate of phytoplankton and temperature in a laboratory dataset including all kinds of species (Eppley 1972; Rose and Carron 2007; Bissinger et al. 2008). Instead of focusing on the envelope relationships across all species, some studies suggested to consider the average rates of species under

68 different temperatures, and applied the ordinary least squares (OLS) regression to fit
69 average growth rate vs. temperature in the laboratory dataset (Sal and López-Urrutia
70 2011). These studies provided evidence for a lower temperature sensitivity of autotrophic
71 processes, which were used to predict warming effect on marine ecosystems. However,
72 the problems hidden in the statistical approach used in the above-mentioned studies when
73 analyzing the dataset should not be ignored (Chen and Laws 2017). The OLS regression
74 used in previous studies was usually applied on a pooled dataset including all data pairs
75 of rates and temperatures to estimate the activation energy without considering the errors
76 in the predictor (X) and the interdependence among the residuals. As a rule of thumb, the
77 rates measured for the same taxa or assemblages at different temperatures are more
78 correlated with each other than with those of different taxa or assemblages at different
79 temperatures. Thus, using a single regression on a pooled dataset, which contains the
80 rates of both the same and different taxa or assemblages, usually violates the assumption
81 of the OLS about the independence of the residuals, resulting in an underestimate of
82 temperature sensitivity (Chen and Laws 2017). For example, a single OLS regression on

a pooled dataset of fast-growing diatoms, which can dominate in cold environments, and of slow-growing cyanobacteria, which tend to dominate in warm environments, will underestimate the regression slope and hence the temperature sensitivity. Thus, a more appropriate method to estimate the temperature sensitivity is to average the responses of individual species to temperatures within a physiologically relevant range (Dell et al. 2011), with the results suggesting that there may be no difference in mean intraspecific temperature sensitivity between phytoplankton and zooplankton (Chen and Liu 2015, Chen and Laws 2017).

Although the median values of the intraspecific E_a between phytoplankton growth rate and zooplankton grazing rate are similar, it does not mean that their E_a should be the same for all communities. Some analysis based on laboratory culture data suggested that there is great variability in the E_a of phytoplankton growth around the median value of 0.65 eV, and it varies among different phytoplankton taxa (Dell et al. 2011; Chen and Laws 2017). Kremer et al. (2017) also found that the estimate of temperature sensitivity was affected by phytoplankton functional groups. Thus, when estimating the temperature

sensitivity of in situ phytoplankton communities, a preferable approach is to measure E_a of natural plankton assemblages instead of laboratory species, to take the community composition into consideration. When quantifying the temperature sensitivity of natural assemblages, it is also inappropriate to run a single regression for a pooled dataset consisting of data pairs collected from different locations at different time points (Rose and Caron 2007; Chen et al. 2012; Regaudie-De-Gioux and Duarte 2012), due to similar statistical problems involved in the analysis of laboratory data (Chen and Laws 2017). The key is that the assemblages used in the analysis should have similar compositions. For now, the best approach might be to run short-term temperature modulation experiments to circumvent the issue of community composition shift as shown in Vaquer-Sunyer & Duarte (2013) and Chen & Liu (2015), although acclimation may be a problem in such experiments. Our previous study at a subtropical coastal site using such approach (Chen and Liu 2015) provided useful insights into thermal response of the natural phytoplankton community dominated by diatoms. However, to generalize these patterns, we need to investigate the temperature sensitivity of plankton in various environments.

One important component of the present study is to examine the responses of different size classes of phytoplankton to temperature. Size is usually regarded as the master trait of phytoplankton (Lichtman and Klausmeier 2008). There are ongoing debates about the role of temperature in affecting phytoplankton size structure. Marañón and his colleagues (Marañón et al. 2013, 2014, 2015) strongly believed that nutrient supply rather than temperature plays the overriding role in determining phytoplankton size structure, while some scientists argued that the effect of temperature cannot be totally neglected (Lopez-Urrutia and Morán 2015; Ward 2015). Nonetheless, all the above-mentioned studies relied on correlations of chlorophyll with temperature and nutrients. The best evidence should come from the comparison between nutrient- and temperature-related growth rates of different size classes. As previous studies have already pointed out that pico-phytoplankton, especially cyanobacteria, has a higher temperature sensitivity than larger phytoplankton (Kulk et al. 2012; Chen et al. 2014; Chen and Laws 2017), we expect that different phytoplankton size classes will respond differently to the same temperature variation.

With the above points in mind, we compared the acute responses of phytoplankton growth rate and microzooplankon grazing rate to short-term temperature modulations at two contrasting subtropical sites in the Hong Kong coastal waters. One site (Western Estuarine Station, or WE) is located in the downstream of the Pearl River and is dominated by large phytoplankton, such as diatoms, due to its eutrophic environment (Chen et al. 2009). The other site (Eastern Oceanic Station, or EO) is jointly affected by the China Coastal Current and oceanic water from the South China Sea, where the phytoplankton community is usually nutrient-limited and is dominated by cyanobacteria *Synechoccocus* in summer. The dilution technique, the most commonly used method to directly measure phytoplankton specific growth rate and microzooplankton grazing rate simultaneously (Landry and Hassett 1982; Calbet and Landry 2004; Laws 2013), was applied to measure phytoplankton growth rate and grazing loss rate of the whole phytoplankton community and three different size classes (micro-, nano-, and pico-phytoplankton) at five different temperatures in a range possibly including the temperature optima of constituent species. We aim to find out the thermal responses of

growth rate and grazing loss rate of natural phytoplankton assemblages, and to test the following hypothesis: cyanobacteria should have a higher temperature sensitivity than other phytoplankton, which can affect the temperature sensitivity of the whole phytoplankton community. Therefore, the temperature sensitivity of phytoplankton community at Station EO, which is dominated by small phytoplankton, should be higher than that at Station WE, which is dominated by large phytoplankton. We expect that at the eutrophic station WE, the average temperature sensitivity should be roughly equal between phytoplankton growth and microzooplankton grazing, while at Station EO dominated by small phytoplankton, the temperature sensitivity of phytoplankton growth should be a little higher than that of microzooplankton grazing, particularly for the small size classes.

Materials and Methods

Study sites and measurements of environmental parameters

We evaluated the impacts of temperature on phytoplankton growth rate and microzooplankton grazing rate at Station WE (22° 21.32'N, 113° 56.78'E) and Station EO

(22° 20.45'N, 114° 17.70'E), both in the Hong Kong coastal waters. These two stations have distinct hydrographic and trophic characteristics (Fig. 1). Experiments were conducted monthly from May 2016 to April 2017. Water temperature and salinity were measured using a YSI EXO2 multi-probe sensor, which was calibrated before each sampling. Surface sea water (*ca.* 30 L) was collected in the polycarbonate carboys from these two stations, and brought back to the laboratory for the following experiments. The samples for inorganic nutrients were collected from the sea water filtered through a 0.2 μm filter capsule, stored in -20ºC freezer, and thawed at room temperature prior to analysis. Inorganic nutrient concentrations including nitrate, nitrite, ammonia, phosphate, and silicate were measured using a Skalar auto-analyzer (San Plus system, Netherlands) in the laboratory according to the JGOFS protocol.

Phytoplankton size structure measurements

Phytoplankton were divided into three size classes by filtering 250 mL sea water sequentially through 20, 2, and 0.2 μm polycarbonate membrane filters (GVS Corporation) using a vacuum pump under low pressure. The phytoplankton retained on

the 20, 2, and 0.2 μm filters were defined as micro-, nano-, and pico-phytoplankton, respectively (Sieburth et al. 1978; Marañón et al. 2001). The biomass of each size class was represented by Chlorophyll *a* (Chl *a*) concentration, which was measured following the JGOFS protocol. After filtration, each filter was immediately stored in a freezer at -80ºC until further treatment. For pigment extraction, the filters were soaked in 5 mL 90% acetone at -20ºC in the darkness for 20 hours. After the extraction, the samples were centrifuged to remove detritus, and the suspensions were then used for measuring fluorescence using a Turner Designs Model 7200 fluorometer with a non-acidification module. The fluorometer was checked against a solid standard each time before measurement (Strickland and Parsons 1972; Ducklow and Dickson 1994). The total phytoplankton biomass was the sum of Chl *a* concentrations of the three size classes.

Short-term temperature modulation experiments

Phytoplankton growth rate and microzooplankton grazing rate were estimated at five different temperatures using the dilution technique (Landry and Hassett 1982). The temperatures for the experiments were set up according to *in situ* ambient temperature T,

188 which was measured using a YSI EXO2 multi-probe sensor when collecting sea water: T
189 – 5ºC, T – 3ºC, TºC, T + 3ºC, and T + 5ºC. In summer, T – 7ºC instead of T + 5ºC was
190 used to minimize the problem of high temperature inhibition. In the dilution experiments,
191 through diluting the natural sea water with filtered sea water at the same site to several
192 proportions and incubating the bottles for 24 hours, the net growth rate of phytoplankton
193 can be calculated. Assuming that phytoplankton growth rate is unaffected by the dilution,
194 and microzooplankton grazing rate is proportional to the fraction of natural sea water,
195 both rates can be estimated through the linear regression of net growth rate against the
196 dilution factors (the proportion of the original unfiltered sea water). In this study, we used
197 two dilution treatments (15% and 100% of natural sea water) in duplicates of 1.2 L PC
198 bottles, known as "two-point" dilution technique, which was modified from the original
199 dilution approach and has been shown to be as accurate as the standard dilution approach
200 (Landry et al. 1984; Strom and Fredrickson 2008; Sherr et al. 2013; Chen 2015a).
201 In each set of dilution experiments (five sets for five temperatures in total), sea
202 water was filtered using a 0.2 μm filter capsule (Pall Corporation) to obtain particle-free

sea water, and was added into two 1.2 L polycarbonate bottles to a prescribed volume. These bottles were then filled with unfiltered sea water to full capacity to get a mixture of 85% particle-free sea water and 15% unfiltered sea water that contained natural plankton. Duplicate 1.2 L polycarbonate bottles filled with unfiltered sea water were prepared for the 100% dilution treatment (100% natural seawater). During this process, unfiltered sea water in carboy was gently stirred occasionally and distributed to bottles as evenly as possible. To ensure sufficient nutrients for phytoplankton growth, especially under higher temperature incubation conditions, nutrients (NO_3^-: 10 μmol L^{-1}, PO_4^{3-}: 1 μmol L^{-1} in final concentration) were added into all experimental bottles. Extra duplicated bottles of 100% unfiltered seawater without nutrient amendment were also prepared and incubated under *in situ* temperatures to evaluate the influence of adding nutrients. Then, all the bottles were capped tightly and placed in an incubator that has five independent enclosed shelves with different temperatures (FIRSTEK) for 24 hours. All five shelves shared the same light intensity of approximately 100 μmol photons m^{-2} s^{-1}, which simulated the average *in situ* light intensity experienced by phytoplankton in the nature, and a light:

dark cycle of 14:10. The samples for determining Chl *a* concentrations of three size classes (size - fractionated Chl *a*) were collected from the initial unfiltered seawater, as well as from each bottle after incubation as described above. Samples for determining cell abundances of pico-phytoplankton including *Synechococcus* and pico-eukaryotes were also collected before and after incubation, and analyzed using a Becton-Dickson FACSCalibur flow cytometer (details are given in the Supplementary Information). Experimental equipment including carboys, filters, bottles, and tubing was acid-washed with 10% HCl, followed by Milli-Q and *in situ* sea water rinses before each experiment.

Estimates of growth rate and grazing rate

Growth rates and grazing mortality rates of the total phytoplankton and three size classes were estimated following Landry et al. (2008). Briefly, by assuming exponential growth for phytoplankton, the net growth rate (k) in each bottle was calculated as $(1/t)\ln(P/dP_0)$, where P is the final biomass of the total phytoplankton and/or each of the three size classes of phytoplankton, which is represented by Chl *a* concentration or cell abundance of pico-phytoplankton, P_0 is the initial phytoplankton biomass/abundance, d is

233 the dilution factor (15% or 100%) of each bottle, and *t* is the duration of incubation time

234 (24 hours). The intrinsic growth rate (μ_n; d^{-1}) and mortality grazing rate (m; d^{-1}) of

235 phytoplankton were determined from the linear regression between net growth rate (k)

236 and dilution factor (d) by assuming an identical intrinsic phytoplankton growth rate in

237 each bottle. At *in situ* temperature, the instantaneous growth rate (μ_0; d^{-1}) was calculated

238 by adding the net growth rate of the bottles without adding nutrients to the mortality

239 grazing rate ($\mu_0 = m + k_{\text{100\%-without nutrient addition}}$).

240 *Estimation of temperature sensitivity and optimal temperature (T_{opt})*

241 According to the MTE, the Boltzmann-Arrhenius (BA) model of biochemical reaction

242 kinetics can be used to predict thermal responses of metabolism-linked rates within a

243 physiological temperature range (PTR; the temperature range below optimal temperature)

244 (Gillooly et al. 2001; Pawar et al. 2016). For the metabolism-linked rate (R), i.e., the

245 phytoplankton growth rate or microzooplankton grazing rate, the model can be described

246 as follows:

247 $$R = R_0 e^{\frac{-E_a}{k_b T}} \quad (1)$$

where R_0 is the normalization coefficient that includes the effect of body size, E_a is the activation energy (eV) that indicates temperature sensitivity, k_b is Boltzmann's constant (8.617×10^{-5} eV K^{-1}), and T is temperature in Kelvin (K). After logarithmically transforming the terms on both sides of *Eq.* (1), the activation energy (E_a) was estimated as the slope of linear regression of the log-transformed rate against the Boltzmann temperature $-1/k_bT$ (Brown et al. 2004; Kremer et al. 2017).

As the thermal responses of metabolism-linked rates are usually unimodal in a sufficiently wide temperature range, a unimodal extension of the BA model (Johnson and Lewin 1946; Dell et al. 2011; Chen and Laws 2017) is used to describe the relationship between metabolism-linked rates and temperature in a temperature range without restricting the data to the PTR:

$$r = r_0 \frac{e^{\frac{E_a}{k_b}(\frac{1}{T_0}-\frac{1}{T})}}{1+\frac{E_a}{E_h-E_a}e^{\frac{E_h}{k_b}(\frac{1}{T_{opt}}-\frac{1}{T})}} \quad (2)$$

where T_{opt} is the optimal temperature, at which the rate reaches the maximum value; E_h is added to describe the "steepness" of the decrease of the rate at higher temperature than T_{opt}, and E_a determines how fast the rate increases with temperature below T_{opt}, which shares

263 the same definition with the linear model mentioned above. r is the growth rate or grazing
264 rate at temperature T. r_0 is the normalization constant. Other items are the same as those in
265 *Eq.* (1).

266 In the majority of our experimental groups, the relationship between phytoplankton
267 growth rate (or microzooplankton grazing rate) and temperature within the 10-degree
268 thermal range was unimodal. *Eq.* (2) was used to fit the data in each set of experiments.
269 Nevertheless, sometimes the estimated values of E_a and E_h were extremely high with high
270 variance because the temperature range used in the model was relatively small and most of
271 the data located around the peak of the curve, which only allowed robust estimate of the
272 optimal temperature (T_{opt}). Insufficient sampling of temperature range was due to the
273 limitation of the short-term incubation experiments on natural communities. We had to
274 limit the experimental temperatures to a small range to avoid deteriorating the plankton
275 community and to be ecologically realistic. Practically, the number of temperature
276 treatments was restricted by resource and limited manpower. Therefore, the unimodal
277 function was only used in the estimate of T_{opt} but not of E_a.

The BA model was applied to estimate E_a of phytoplankton growth rate and microzooplankton grazing rate through fitting the rate vs. temperature data within the PTR. The data were restricted to the PTR by removing the rates that surpassed the optimal temperature from every temperature modulated experiment. Totally, 13 sets of experiments were conducted at each station, and 37% and 34% of the total data of phytoplankton community growth rate and microzooplankton grazing rate were excluded from the calculation. The E_a of each set of experiments was calculated through an OLS linear regression. As the E_a values at each station varied randomly without apparent seasonal pattern (Fig. S1), we used the linear mixed effects model treating months as random effects associated with E_a to estimate the average E_a at each station. This model allows random variations of both slope and intercept to account for hierarchical data structure, and has been applied in some studies on data analysis related to MTE (Van de Pol and Wright 2009; Bates et al. 2014; Kremer et al. 2017). The model for phytoplankton growth rate can be described as follows:

$$\ln v_{i,j} = (lnv_0 + \lambda D_i + \theta_{vi}) + \frac{E_{av} + \beta D_i + \theta_{Eavi}}{k_b}\left(\frac{1}{T_0} - \frac{1}{T_{i,j}}\right) + \varepsilon_{i,j} \quad (3)$$

where $\nu_{i,j}$ is the growth rate of total phytoplankton or each of the three size classes at the j^{th} temperature $T_{i,j}$ in the i^{th} experiment; ν_0 is the normalization coefficient at reference temperature T_0 (288.15K); $E_{a\nu}$ is the mean activation energy for phytoplankton growth rate; and $\theta_{\nu i}$ and $\theta_{Ea\nu i}$ represent the deviations of intercept ($ln\nu_0$) and slope ($E_{a\nu}$) in the i^{th} experiment from the mean, respectively. D_i is a dummy variable indicating the station information. D_i is set to 0 at Station EO, and to 1 at Station WE. Thus, λ and β are the differences in intercept and slope, respectively, between the two stations. Since we incubated all bottles at the same light condition and added the same concentration of nutrients (to ensure phytoplankton growth), we did not include the effects of light and nutrient in this model.

To calculate the temperature sensitivity of microzooplankton grazing rate, microzooplankton biomass was added to the model as follows:

$$\ln m_{i,j} = \alpha \ln(MZ_i) + (lnm_0 + \lambda D_i + \theta_{mi}) + \frac{E_{am}+\beta D_i+\theta_{Eami}}{k_b}\left(\frac{1}{T_0} - \frac{1}{T_{i,j}}\right) + \varepsilon_{i,j} \quad (4)$$

where $m_{i,j}$ is the microzooplankton grazing rate at the j^{th} temperature $T_{i,j}$ in i^{th} experiment, m_0 is the normalization coefficient at reference temperature T_0 (288.15K); E_{am} is the mean

activation energy for microzooplankton grazing rate; θ_{mi} and θ_{Eami} represent the random effects of intercept (lnm_0) and slope (E_{am}) in the i^{th} experiment, respectively; *MZ* is the microzooplankton biomass in carbon units (μg C L^{-1}) in the i^{th} experiment, which was estimated based on the microscopic enumeration and bio-volume measurement of the microzooplankton in each experiment (details are in Supplementary Information); and α is a constant describing the relationship between grazing rate (*m*) and biomass (*MZ*). Dummy variable (D_i) representing the station information is also included in this model as in *Eq.* (3).

All statistical analyses were performed using the software R 3.1.2 (Team 2014). The nonlinear regression analysis was applied using the R function "*nls*". The linear mixed effects model was performed using "*lmer*" in R package "lme4". The conditional R^2 and marginal R^2 were calculated to assess the goodness of the fit of the model using "r.squaredGLMM" in the R package "MuMIn" (Nakagawa and Schielzeth 2013). To compare the activation energies among size classes at each station, Welch's ANOVA (R function "*oneway.test*") was used instead of the classic one-way ANOVA because the data

violated the assumption of homogeneity of variance. Finally, Welch's *t*-test was performed to identify the difference of activation energies between every group and every other group of rates. (Ruxton et al. 2006).

Results

Environmental condition and phytoplankton size structure

Pronounced seasonality was observed at both stations. Sea surface temperatures at the two stations varied similarly (Fig. 2), but the concentrations of inorganic nutrients differed dramatically between the two stations (Table S1). Both total dissolved inorganic nitrogen (TIN, the total concentration of nitrate, nitrite, and ammonia) and phosphate concentration at Station WE were remarkably higher (*ca.* 8-fold higher) than those at Station EO. Total Chl *a* concentration was also much higher at Station WE (7.58 ± 8.53 μg L^{-1}, range: 0.97-30.31 μg L^{-1}) than at Station EO (3.14 ± 1.89 μg L^{-1}, range: 1.13-7.06 μg L^{-1}) (Fig. 2). Micro-phytoplankton (> 20 μm) accounted for the major proportion of the phytoplankton biomass at both stations, and were more dominant at Station WE (WE: 44% ± 28%; EO: 38% ± 23%). Total Chl *a* concentration was positively correlated with

the concentration of micro-phytoplankton at Station WE, while it was correlated with the concentration of nano-phytoplankton at Station EO, where nano-phytoplankton accounted for 30% ± 16% of the total. Flow cytometric analysis demonstrated that *Synechococcus* was more abundant in summer at both stations, and their cell concentrations were positively correlated with temperatures (Spearman rank correlation test, for EO, $r = 0.91$, $p < 0.001$; for WE, $r = 0.95$, $p < 0.001$) (Fig. S2).

Temperature sensitivity of phytoplankton community growth rate and grazing mortality rate by microzooplankton

Monthly E_a varied randomly without any discernable trend at both stations (Fig. S1). Based on the linear mixed effects model (Eqs. 3, 4), the mean E_a of the whole phytoplankton growth rate was 0.35 eV (95% CI = 0.24 to 0.46 eV) at Station EO and was 0.37 eV (95% CI = 0.27 to 0.48 eV) at Station WE (Table 1, Fig. 3). No significant difference was found between these two stations (p value for β in *Eq.* 3: $p > 0.05$), which contrasted with our initial expectation. Both fixed and random effects in the mixed effects model explained about 90% variance of the whole phytoplankton community growth rate,

but only ~50% of the total variance can be interpreted by the fixed effects at each station, which suggested the importance of random effects (Table 1).

Surprisingly, microzooplankton biomass did not play a significant role in the model predicting grazing rate at Station EO ($\chi^2 = 0.45$, $p > 0.05$). Although it was found significant at Station WE ($\chi^2 = 6.23$, $p < 0.05$), the results were not much different from the ones without microzooplankton biomass (Welch's *t*-test, $p > 0.05$). Hence, the variable of microzooplankton biomass was removed from the models. The average E_a of microzooplankton grazing rate also showed no significant difference between the two stations (EO: 0.64 eV, 95% CI = 0.38 to 0.89 eV; WE: 0.57 eV, 95% CI = 0.36 to 0.78 eV; *p* value for β in *Eq.* 4: $p > 0.05$; Table 2, Fig. 4), which agreed with the canonic value (0.65 eV) of MTE at both stations.

At each station, E_a of bulk phytoplankton growth rate was significantly lower than that of microzooplankton grazing rate (Welch's *t*-test, EO: $p = 0.037 < 0.05$; WE: $p = 0.019 < 0.05$; Fig. 5), which again differed from our hypothesis. As such, the percentage of phytoplankton consumed by microzooplankton, which is represented by m: μ (Calbet

368 and Landry 2004), increased with rising temperature in each group. The result of the
369 linear mixed effects model using *Eq.* (3) on m: μ, exhibiting the distance in activation
370 energy between growth rate and grazing rate ($\Delta E_a = E_m - E_\mu$), was 0.30 eV (95% CI =
371 0.16 to 0.44 eV), which further supported that microzooplankton grazing rate was more
372 sensitive to temperature increase compared with phytoplankton growth rate.
373 *Temperature sensitivity of growth rate and grazing mortality rate by microzooplankton of*
374 *three size classes of phytoplankton*
375 The mean E_a values of phytoplankton growth rate at both stations differed for the
376 three size classes (Table 1, Fig. 5). At Station WE, only micro-phytoplankton growth rate
377 was less sensitive to temperature increase than the grazing rate (0.34 eV, 95% CI = 0.25
378 to 0.43 eV, Table 1, Fig. S3a). This was consistent with the whole phytoplankton
379 community growth rate (Welch's *t*-test, $p > 0.05$) due to the dominance of micro-
380 phytoplankton. The E_a values of nano- and pico-phytoplankton growth rates were slightly
381 higher than that of the whole phytoplankton community (Table 1; Welch's *t*-test, nano: p
382 = 0.009 < 0.01; pico: $p = 0.047 < 0.05$). While at Station EO, E_a of pico-phytoplankton

growth rate was the closest to that of the whole phytoplankton community (0.31 eV, 95% CI = 0.16 to 0.46 eV; Welch's *t*-test, $p > 0.05$), and the growth rate of micro-phytoplankton had a significantly higher value than that of the whole phytoplankton (0.52 eV, 95% CI = 0.33 to 0.7 eV, Welch's *t*-test, $p = 0.001 < 0.01$). However, E_a of micro-phytoplankton may be biased because of its data structure (Fig S3a). In the estimate of E_a, since data should be restricted to below optimal temperature to meet the requirement of the BA model, there were only two data points remained in some groups. Estimates based on restricted dataset in which at least three points were required were also carried out to examine the accuracy of our results (Table S2). No significant changes were observed in these estimates except for E_a of micro-phytoplankton growth rate with a value of 0.22 eV (95% CI = 0.06 to 0.38 eV). Therefore, more observations were required to confirm the E_a value of micro-phytoplankton growth rate at Station EO. The temperature sensitivity of *Synechococcus* was significantly greater than that of the whole phytoplankton community and that of pico-phytoplankton at both stations (Welch's *t*-test, $p < 0.001$; Fig. 5). These results were in concordance with previous studies (Kulk et al. 2012; Chen

et al. 2014; Stawiarski et al. 2016; Chen and Laws 2017), which revealed that cyanobacteria has a lower growth rate but higher temperature sensitivity than large eukaryotic phytoplankton such as diatoms.

No pronounced difference was found in E_a of microzooplankton grazing rates of three size classes at Station EO (Welch's ANOVA, $p > 0.05$). The E_a value of grazing rate on nano-phytoplankton was slightly higher than that of nano-phytoplankton growth rate (Welch's *t*-test, $p = 0.038 < 0.05$; Fig. 5). At Station WE, the grazing rate on nano-phytoplankton had a high E_a value (0.97 eV, 95% CI = 0.57 to 1.37 eV, Fig. S4b). The E_a values for the other size classes and *Synechococcus* showed no obvious differences from that for the grazing rates on community and were close to the predicted values.

Optimal temperatures of phytoplankton growth rate and grazing mortality rate by microzooplankton

The nonlinear least-squares regression model was used to fit the data of each set of experiments when there was a unimodal relationship between growth rate (or grazing rate) and temperature (solid lines in Figs. 3, 4). The optimal temperatures for growth rate

and grazing rate were obtained from the nonlinear models. The overall mean optimal temperature of phytoplankton community growth rate was 23.7 ± 3.2ºC, significantly lower than that of microzooplankton grazing rate (25.9 ± 4ºC; paired t-test, df = 20, p = 0.013 < 0.05; Fig. 6). The optimal temperatures of both growth rate and grazing rate were positively correlated with the environmental temperature (growth rate: r = 0.88, df = 23, p < 0.001; grazing rate: r = 0.88, df = 20, p < 0.001; Fig. S5). For the three phytoplankton size classes, their growth rates showed slightly lower optimal temperatures compared with corresponding grazing rates, but the difference was not significant. For *Synechococcus* and pico-eukaryotes, no difference was observed in the optimal temperatures between growth rate and grazing rate.

Discussion

Implications of different thermal sensitivity of phytoplankton growth rate and microzooplankton grazing rate

Predicting how marine primary production, the efficiency of biological pump and food web stability respond to the projected warming entails reliably quantifying the

428 temperature sensitivity of various plankton rates, especially phytoplankton growth rate.
429 Accurately estimating the temperature sensitivity requires us to be aware of the potential
430 statistical problems in previous analyses (Chen and Laws 2017). In the current study, we
431 used a short-term temperature modulation approach to minimize the statistical problems
432 as much as possible, and to circumvent the issue of community structure shift under
433 warming conditions. Our results showed that at the community level, the average
434 temperature sensitivity of phytoplankton growth rate was 0.35 eV, lower than that of
435 microzooplankton grazing rate at the two contrasting stations in the subtropical coastal
436 waters. At the face value, this difference seems consistent with the classic conception of
437 lower temperature sensitivity of autotrophs (Allen et al. 2005; Rose and Caron 2007;
438 Yvon-Durocher et al. 2010; Chen et al. 2012; Regaudie-De-Gioux and Duarte 2012),
439 which has strong implications for the effect of warming on some critical ecosystem
440 processes, such as net community production (López-Urrutia et al. 2006; Regaudie-De-
441 Gioux and Duarte 2012) and the efficiency of the biological pump (Laws et al. 2000;
442 Cael and Follows 2016). Different thermal responses of phytoplankton and their

predators would also affect the dynamics and stability of marine food webs under global warming (Vasseur and McCann 2005; Rose and Caron 2007; Fussmann et al. 2014). Due to the lower temperature sensitivity, more phytoplankton biomass would subject to microzooplankton grazing as temperature increases, which would reduce possible occurrences of phytoplankton blooms under warming in the eutrophic coastal waters (Rose and Caron 2007; Cloern 2018). Nevertheless, the above speculations are based on projected transient responses. In the future, it is necessary to take into account thermal adaptive behaviors of phytoplankton for predicting warming effects on plankton ecosystems (García et al. 2018).

Why is phytoplankton temperature sensitivity lower? – Influence of optimal temperature on estimating activation energy

It is intriguing that our results still predict lower E_a of phytoplankton than that of microzooplankton even though we tried our best to minimize the statistical problems and took into account the influences of community composition (Chen and Laws 2017). We believe that the main reason is related to the effects of optimal temperature (T_{opt}) of

458 growth rate. Based on the nonlinear thermal response function that is extended from the

459 Arrhenius equation (Johnson and Lewin 1946; Dell et al. 2011; Chen and Laws 2017):

460 $$ln\mu = ln\mu_0 + \frac{E_a}{k_b}\left(\frac{1}{T_0} - \frac{1}{T}\right) - ln\left[1 + \frac{E_a}{E_h - E_a} e^{\frac{E_h}{k_b}\left(\frac{1}{T_{opt}} - \frac{1}{T}\right)}\right] \quad (5)$$

461 it is clear that the apparent slope of the linear regression of log-transformed growth rate

462 ($\ln\mu$) against Boltzmann temperature ($\frac{1}{k_b}\left(\frac{1}{T_0} - \frac{1}{T}\right)$) is affected not only by E_a but also by

463 $T_{opt.}$ When $T << T_{opt}$, the apparent linear slope is close to E_a. But when T approaches T_{opt},

464 the last term in Eq. (5) becomes significantly positive, leading to reduction of the

465 apparent linear slope. When $T > T_{opt}$, the slope eventually becomes negative. In addition,

466 the apparent linear slope also depends on the temperature range of the experiments,

467 which is necessarily small in our studies due to the concern of short-term thermal shock.

468 This phenomenon has been observed previously (Pawar et al. 2016). It has been

469 repeatedly reported that T_{opt} of many organisms including phytoplankton becomes closer

470 to environmental temperature in warm environments (Deutsch et al. 2008; Huey et al.

471 2009; Thomas et al. 2012; Chen 2015b). As a consequence, the reduced sensitivity of

phytoplankton growth rate to temperature should be a universal pattern in subtropical and tropical environments.

The observed differences of temperature sensitivity (i.e., the apparent E_a estimated from the linear regression), hence, can be well explained by the discrepancy of T_{opt} between microzooplankton and phytoplankton. T_{opt} of microzooplankton grazing rate (17.3-32.2ºC) was higher than that of phytoplankton growth rate (18.2-29.6ºC) in our study (Fig. 6). Thus, higher T_{opt} of microzooplankton relative to the environment temperature T in *Eq.* (5) would result in a higher apparent linear slope compared with that of phytoplankton. Physiologically, the lower T_{opt} of phytoplankton might be related to the substantial requirement for RubisoCO enzyme at high temperature (Flynn and Raven 2016). In laboratory experiments, it is also true that T_{opt} of zooplankton was higher than that of phytoplankton or even cannot be observed in some designed experimental temperature range (Renaud et al. 2002; Chen and Laws 2017). Difference in the optimal temperatures between phytoplankton and microzooplankton holds the potential to influence the dynamics of microbial food web under climate warming. The

487 microzooplankton grazing rate would keep rising to reach its maximum when

488 temperature increases, while the phytoplankton growth is prone to deviate away from its

489 best fitness because it is more likely that the increased temperature can surpass the

490 optima. Thus, more phytoplankton biomass would be consumed by enhanced

491 microzooplankton grazing activities in a warming future. In addition, the ratio of m:μ,

492 which represents the grazing impact of microzooplankton on phytoplankton, also

493 increases with temperature and has a high optimal temperature (26.2 ± 3.6ºC), indicating

494 an increasing grazing pressure on phytoplankton under warming conditions. Interestingly,

495 Boersma et al. (2016) suggested that when going up the trophic level, fish also seem to

496 have much higher T_{opt} than phytoplankton under the same environmental temperature.

497 Further considering the evolution of endothermy, we suspect that as organisms evolve

498 from unicellular to more complicated forms that confer them maintain a higher body

499 temperature than the environment, their T_{opt} might also evolve to be higher to take

500 advantage of the greater fitness under higher temperature (Huey and Kingsolver 1989).

It is a challenging question why in our earlier investigation (Chen and Liu 2015), few incidences of T_{opt} were observed for both phytoplankton and microzooplankton (i.e., the rates kept increasing with temperature in most experiments). The most likely reason is that T_{opt} was much higher than the environmental temperature in the study region investigated in Chen & Liu (2015), leaving few opportunities to capture T_{opt} within the designed experimental temperature range. Some studies have suggested a considerably larger discrepancy between the environmental temperature and optimal temperature for the phytoplankton living in polar and temperate waters where the annual mean temperature is under ~25°C (see Fig. 2A in Thomas et al. 2012). Although the study region in Chen & Liu (2015) was in the subtropical waters, the annual mean temperature (20.2 ± 5.4°C) was lower than 25°C, and also lower compared with that of the current study region (25 ± 4.3°C). In addition, the study site in Chen & Liu (2015) was extremely eutrophic, with nitrate concentration always exceeding 15 μmol L^{-1}. It was also found that less nutrient availability can reduce T_{opt} for phytoplankton (Thomas et al. 2017).

Thus, the lower T_{opt} observed in the present study probably is related to the mesotrophic nature of the study region.

Different temperature sensitivities among three size classes of phytoplankton at the two stations

Community composition plays an important role in determining the temperature sensitivity of phytoplankton growth rate in natural environments (Chen and Laws 2017). We hypothesized that the phytoplankton community at Station EO dominated by small phytoplankton, mainly by cyanobacteria, would be more sensitive to temperature increase than that at Station WE, which was dominated by larger phytoplankton such as diatoms. However, the estimated E_a for phytoplankton growth rate of the whole community was not significantly different at the two stations, and was lower than that of microzooplankton grazing rate (Tables 1, 2, Fig. 5). This suggests that the influence of nonlinear temperature response (i.e., T_{opt}) is much greater than that of community structure.

529 The E_a value of phytoplankton growth rate varied among different size classes or

530 groups. Nano- and pico-phytoplankton growth rates showed higher E_a than micro-

531 phytoplankton growth rate at Station WE (Fig. 5b), which could drive an increase in the

532 contribution of small phytoplankton to the bulk biomass, and shift the community

533 structure under climate warming. It has also been shown that small phytoplankton

534 increased with increasing temperature in mesocosm experiments using either marine and

535 freshwater plankton communities (Sommer and Lengfellner 2008; Yvon-Durocher et al.

536 2011). The underlying mechanism could be a combination of faster response of small

537 phytoplankton to warmer temperature and enhanced grazing pressure on large

538 phytoplankton due to the difference in temperature sensitivities between micro-

539 phytoplankton and their predators (Fig. 5). Because small phytoplankton are less prone to

540 sinking and would accelerate nutrient regeneration by stimulating the growth of

541 microzooplankton, ocean warming, by shifting the phytoplankton community to smaller

542 sizes, would reduce the efficiency of carbon export (Laws et al. 2000).

543 *Cyanobacteria having high temperature sensitivity*

544 The estimated activation energy of *Synechococcus* growth rate was consistently high
545 at both stations (EO: 0.58 eV, 95% CI = 0.36 to 0.80 eV; WE: 0.54 eV, 95% CI = 0.41 to
546 0.67 eV; Fig. 5), close to the canonic value of 0.65 eV and in line with previous studies of
547 both freshwater and marine cyanobacteria (Joehnk et al. 2008; Chen et al. 2014; Chen
548 and Laws 2017). It has also been found that several eukaryotic strains isolated from the
549 oligotrophic ocean have lower temperature coefficient (Q_{10}) than pico-prokaryotes,
550 including *Synechococcus* and *Prochlorococcus* (Kulk et al. 2012; Stawiarski et al. 2016).
551 Similar results were observed at Station EO (Fig. 5a), which is closer to the open ocean.
552 Some studies pointed out that cyanobacteria have relatively higher T_{opt} than pico-
553 eukaryotes, which relieves them from high temperature inhibition in (sub)tropical
554 environments (Chen et al. 2014; Chen and Laws 2017). Thus, it is not surprising to find
555 its peak abundance in summer in coastal waters (Fig. S2; Agawin et al. 1998; Chen et al.
556 2009; Chen et al. 2014), and its abundance increases with rising annual mean temperature
557 under nutrient-sufficient conditions (Li 1998).

Potential problems in the estimate of E_a in such short-term temperature modulation experiments

Every methodology may have its own bias or drawback. For the incubation method we used, one problem might be the latent influence of the resource supply such as light and nutrients on the estimate of E_a. The effects of temperature on phytoplankton growth usually interact with light and nutrients. The interactive effects on growth are complicated as they are not simple combination of additive effects (Thomas et al. 2017). Practically, this study focused on the effect of temperature on phytoplankton growth under replete resource supply. Thus, additional nutrients were added to ensure sufficient nutrients for phytoplankton growth. Although the light intensity (about 100 μmol photons m^{-2} s^{-1}) used in the experiments may not be sufficient for some phytoplankton species during the incubation, especially when temperature increases (Collins and Boylen 1982), it was assumed to be saturating irradiance for the growth of phytoplankton community in some studies (Edwards et al. 2016). We also assumed that the effect of light should be negligible in the estimate of E_a in our study. Actually, as the light intensity was set to

573 imitate the *in situ* situation experienced by the phytoplankton, which were continuously
574 mixed within the mixed layer at a time scale less than 24 hours (Franks 2015; Chen and
575 Smith 2018), our estimates could be more relevant to the *in situ* conditions. Recent
576 studies found that nutrient-limitation or light-limitation would reduce the optimal
577 temperature of phytoplankton growth and diminish the temperature sensitivity (Edwards
578 et al. 2016; Thomas et al 2017). If so, the lower temperature sensitivity and optimal
579 temperature for phytoplankton growth found in the current study may be more critical
580 when involving the effects of resource supply in the real ocean.

581 Another problem is the possible damage to plankton community caused by the
582 temperature manipulation especially temperature extremes, which could impose "thermal
583 shock" to the plankton. To alleviate this problem, the experimental temperature gradients
584 were prudently designed to be confined to small deviations from the *in situ* temperature.
585 The experimentally elevated temperatures would not cause damage to the majority of
586 plankton in our experiments because significant increase of phytoplankton biomass can
587 be observed after incubation. Our estimates of E_a were consistent with many previous

588 studies, such as Laws et al. (2000), Allen et al. (2005), Lopez-Urrutia et al. (2006), Rose
589 and Caron (2007), Cael and Follows (2016), and Kremer et al. (2017), although for
590 different reasons, which suggested that the problem of temperature manipulation did not
591 bias the estimate of E_a substantially. However, the restricted temperature range might
592 lead to high variation (uncertainty) of the estimates of E_a. Moreover, as ocean warming is
593 very slow relative to warming used in the experimental design, the results of such short-
594 term experiments should be applied cautiously in predicting the effects of warming on the
595 marine plankton on a long-term scale. Despite the above-mentioned issues of short-term
596 experiments, our estimates provide some useful information about the temperature
597 sensitivity, which is an important trait of the plankton community to predict how the
598 marine ecosystem responds to ocean warming.

599 **Conclusion**

600 Our results suggest that the heterotrophs are more sensitive to the increase of
601 temperature than the autotrophs in the subtropical regions even when the statistical
602 problems were minimized and the community composition was taken into consideration

603 in our experiments. This difference arises due to a new problem that is the different
604 discrepancies of the optimal temperature of phytoplankton and microzooplankton to the
605 environmental temperature. It does not suggest the above-mentioned statistical problems
606 do not occur in previous studies.
607 Our study highlights the importance of considering nonlinear temperature responses
608 when estimating the temperature sensitivity of plankton in the subtropical and tropical
609 regions, where environmental temperatures are often close to the optimal temperature of
610 phytoplankton, but probably less so for zooplankton. This has significant implications for
611 the impact of global warming on ocean ecosystems. We expect that warming will
612 continue to shift phytoplankton to small size (cyanobacteria) and microzooplankton will
613 probably flourish, leading to a more active microbial loop. How these repercussions will
614 affect key ocean ecosystem functioning, such as the marine biological pump or fishery,
615 remains important tasks for oceanographers and ecologists.
616

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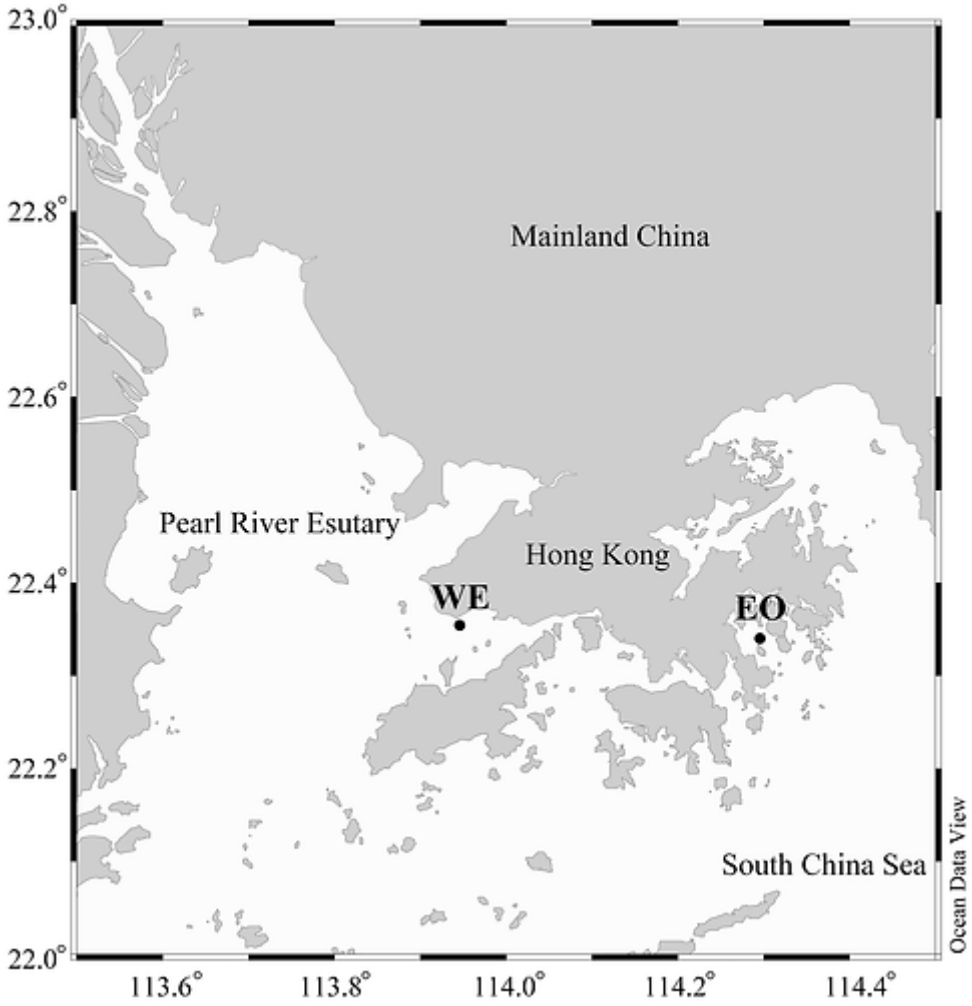
861

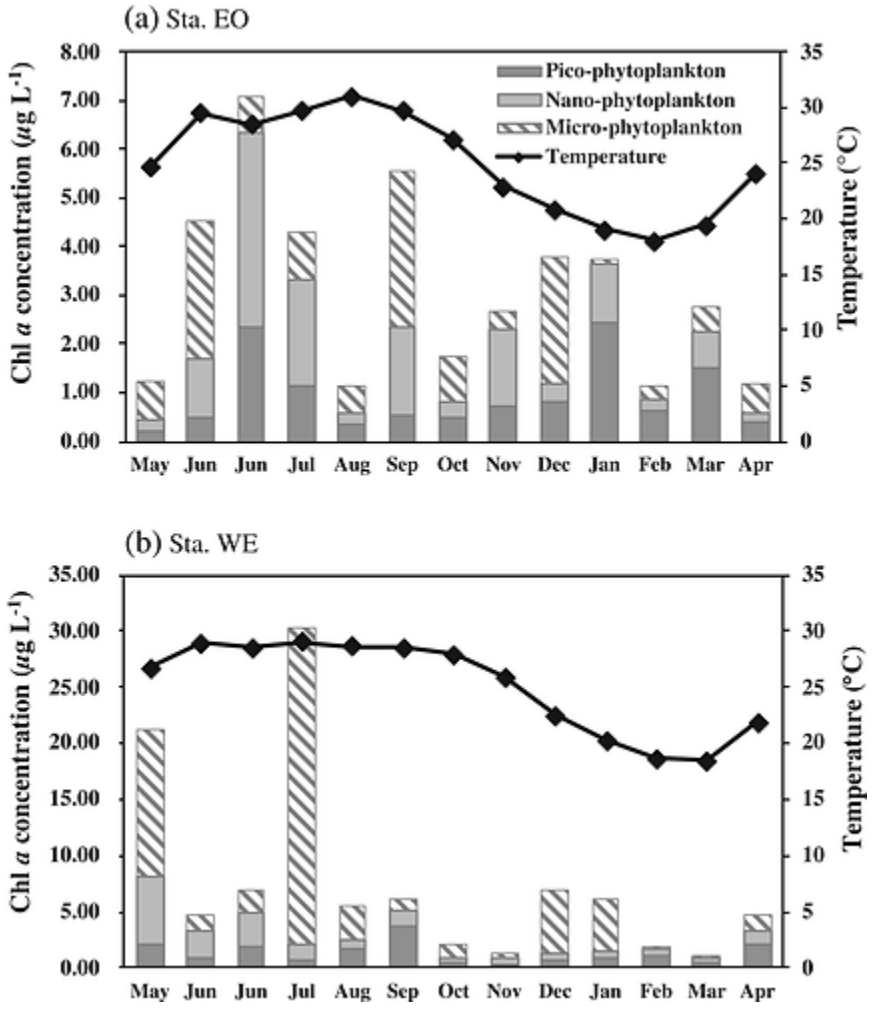
862 **Figures**



863

864 Fig. 1 Locations of study stations in Hong Kong waters. WE: Western Estuarine Station

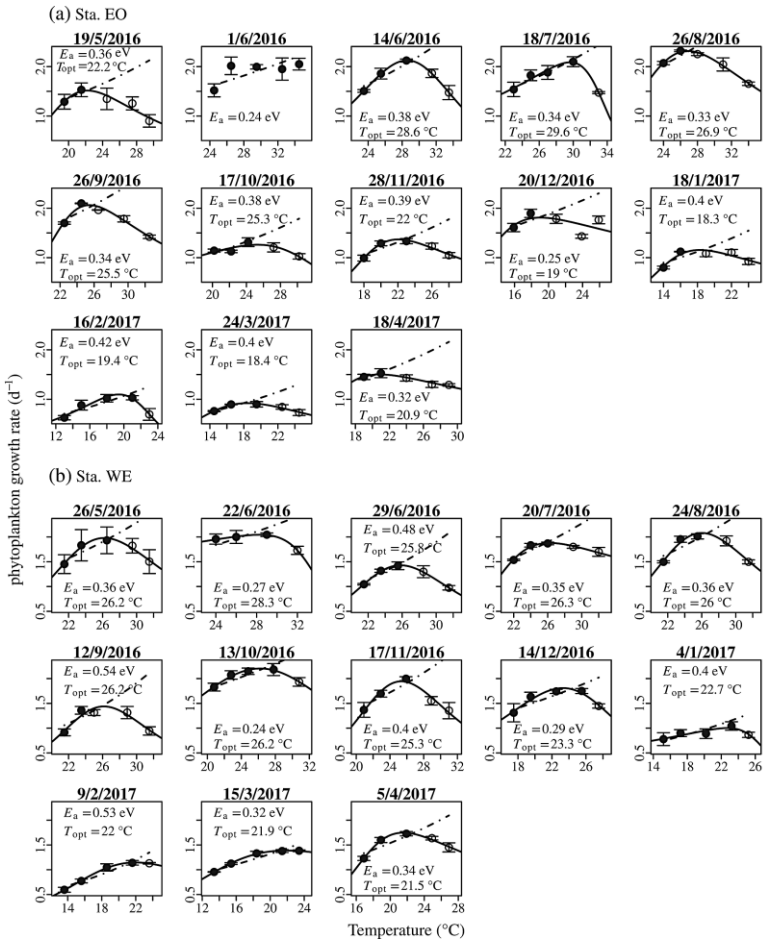
865 (22°21.32'N, 113°56.78'E); EO: Eastern Oceanic Station (22°20.45'N, 114°17.70'E).



866

867 Fig. 2 Monthly variations of temperature and chlorophyll *a* (Chl *a*) concentration of three

868 phytoplankton size classes at Station EO (a) and Station WE (b).



869

870 Fig. 3 Linear mixed effects model and nonlinear regression fits of phytoplankton

871 community growth rates and experimental temperature for individual experiments at

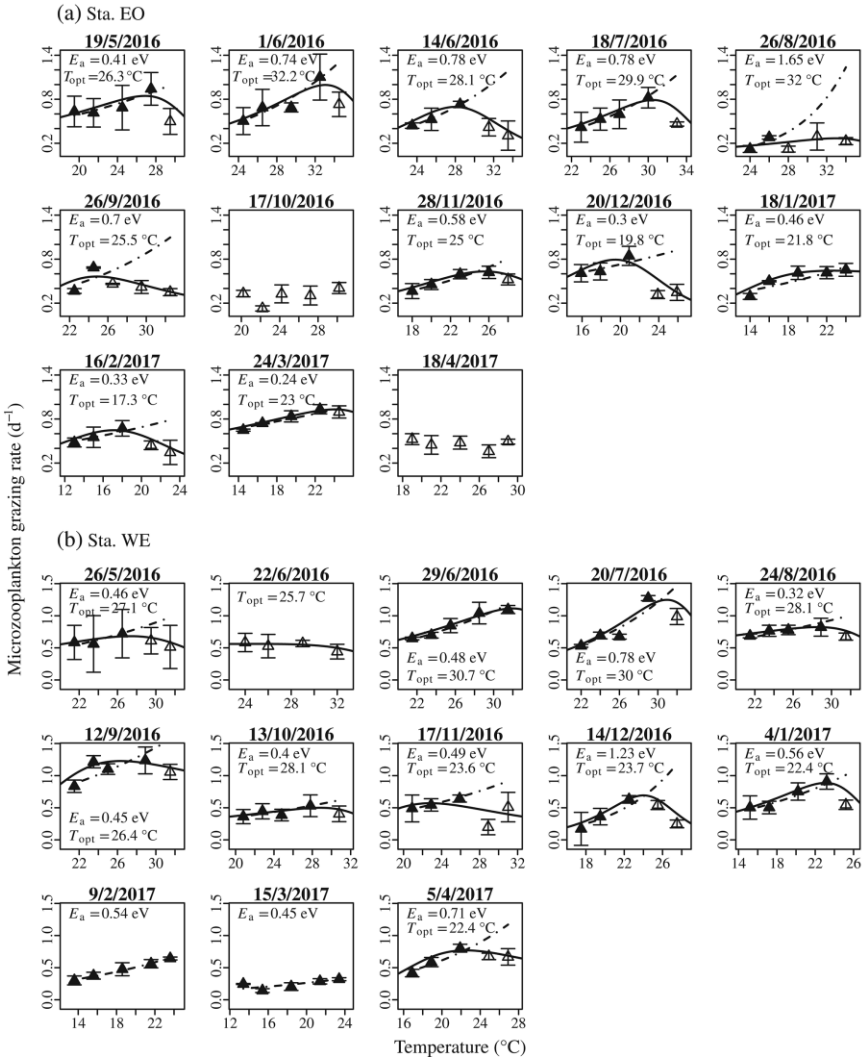
872 Station EO (a) and Station WE (b). The dash-dotted line is the fitted line of the

873 linear mixed effects model results, and the solid line is the fitted line of the nonlinear

874 regression. Open circles: Data not used in the linear mixed effects model. E_a:

875 Activation energy obtained from the linear mixed effects model; T_{opt}: Optimal

876 temperature of growth rate obtained from the nonlinear regression.

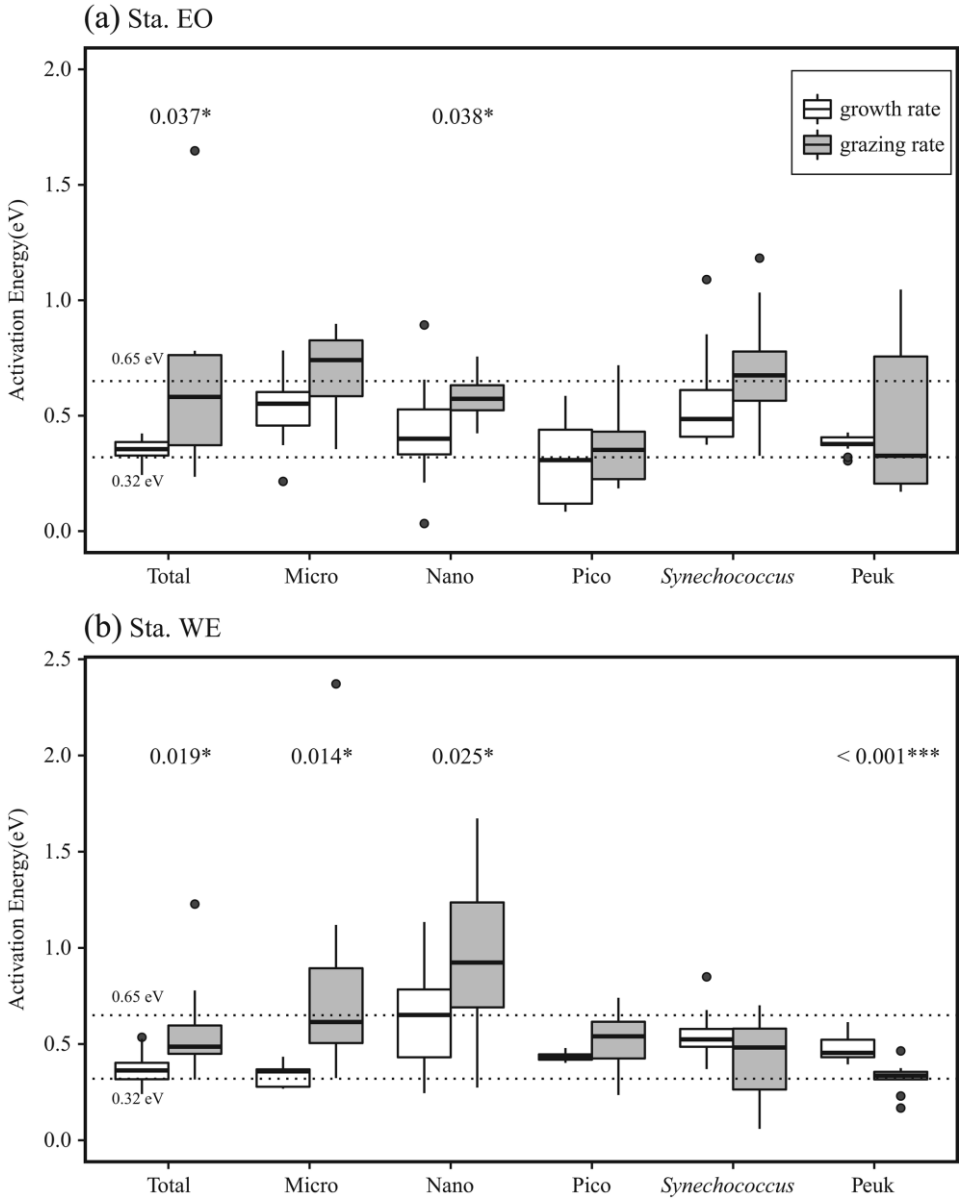


877

878 Fig. 4 Linear mixed effects model and nonlinear regression fits of microzooplankton

879 grazing rates on the total phytoplankton at experimental temperatures at station EO

880 (a) and station WE (b). Same as Fig. 3.



881

882 Fig. 5 Activation energies of growth rates of total phytoplankton, three size classes of

883 phytoplankton and two pico-phytoplankton and corresponding grazing mortality

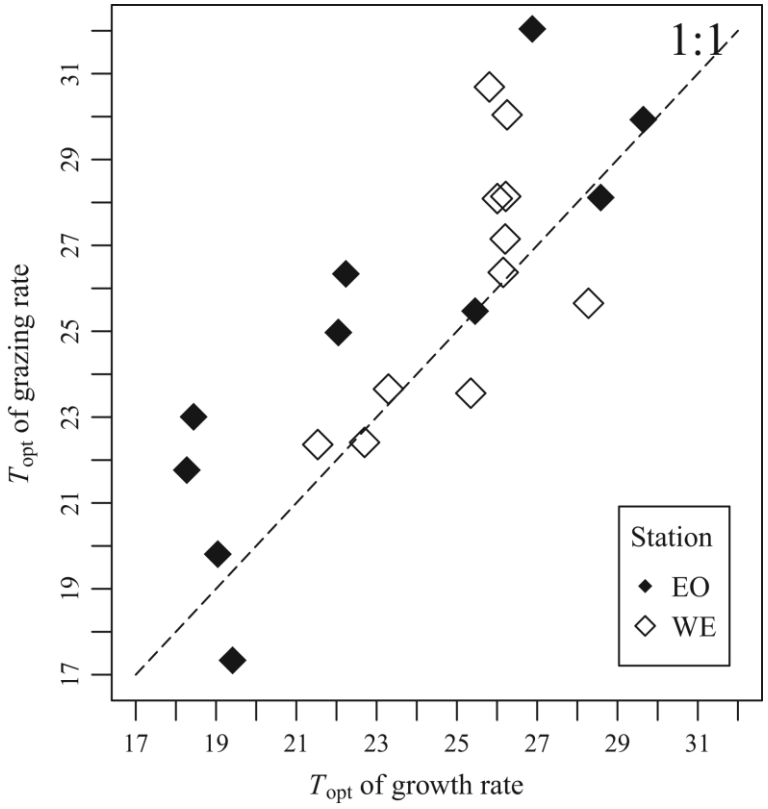
884 rates by microzooplankton at Station EO (a) and Station WE (b). The two dashed

885 lines represent the theoretical activation energies of autotrophic processes (0.32 eV)

886 and heterotrophic processes (0.65eV). Significant levels between activation energies

887 of growth rate and grazing rate are given by the *p*-values with the asterisks (* : $p <$

888 0.5; ** : $p < 0.1$; *** : $p < 0.001$).



889

890 Fig.6 Optimal temperatures of phytoplankton community growth rate and grazing

891 morality rate by microzooplankton grazing. Dashed line is the 1:1 line.

892

Table 1. Estimated activation energies (E_a, eV) of total, three size classes, and taxa-specific phytoplankton growth rates derived from the linear mixed effects model and OLS regression. The parameters of the linear mixed effects model include the average energy (E_{av}, with 95% CI in brackets), the normalization constant (ln $v_0 \pm$ standard error), standard deviations of the random effects of ln v_0 and E_{av} (θ_v and θ_{Eav}), percentage of variance explained by the random and fixed effect (V_{fr}), percentage of variance explained by the fixed effect only (V_f), number of observations used in the model (N_o), and number of groups used in the model (N_g). E_a (OLS) (eV) is the mean activation energy derived from the OLS regression, 95% CIs are in brackets.

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		Linea mixed effects model								Linear model (OLS)
Station	**Growth rate**	**ln ν_0**	**$E_{a\nu}$**	**θ_ν**	**$\theta_{Ea\nu}$**	**V_{fr}**	**V_f**	**N_o**	**N_g**	**$E_{a(OLS)}$**
EO station	Bulk phytoplankton	0.05 ± 0.11	0.35 (0.24, 0.46)	0.23	0.09	0.93	0.56	37	13	0.48 (0.32, 0.63)
	Micro-phytoplankton	−0.03 ± 0.11	0.52 (0.33, 0.70)	0.35	0.22	0.98	0.50	31	13	0.54 (0.39, 0.69)
	Nano-phytoplankton	−0.00 ± 0.15	0.43 (0.29, 0.57)	0.54	0.22	0.96	0.35	39	13	0.45 (0.34, 0.55)
	Pico-phytoplankton	−0.02 ± 0.12	0.31 (0.16, 0.46)	0.38	0.2	0.81	0.37	39	13	0.40 (0.23, 0.57)
	Synechococcus (FCM)	−0.83 ± 0.27	0.58 (0.36, 0.80)	0.9	0.23	0.97	0.19	40	13	0.88 (0.57, 1.19)
	Picoeukaryotes (FCM)	−0.15 ± 0.10	0.38 (0.29, 0.47)	0.31	0.04	0.95	0.29	42	13	0.44 (0.36, 0.52)
WE station	Bulk phytoplankton	0.02 ± 0.09	0.37 (0.27, 0.48)	0.29	0.12	0.92	0.41	44	13	0.47 (0.27, 0.66)
	Micro-phytoplankton	0.19 ± 0.08	0.34 (0.25, 0.43)	0.2	0.09	0.91	0.39	45	13	0.38 (0.29, 0.46)
	Nano-phytoplankton	−0.36 ± 0.11	0.63 (0.41, 0.84)	0.34	0.33	0.95	0.44	46	13	0.64 (0.43, 0.85)
	Pico-phytoplankton	−0.15 ± 0.14	0.43 (0.25, 0.61)	0.38	0.02	0.80	0.21	43	13	0.65 (0.34, 0.97)
	Synechococcus (FCM)	−0.15 ± 0.10	0.54 (0.41, 0.67)	0.3	0.14	0.88	0.65	42	13	0.69 (0.47, 0.9)
	Picoeukaryotes (FCM)	−0.06 ± 0.13	0.48 (0.35, 0.60)	0.38	0.07	0.94	0.36	35	12	0.65 (0.43, 0.87)

Table 2. Estimated activation energies (E_a, eV) of total, three size classes, and taxa-specific phytoplankton grazing mortality rates by microzooplankton derived from the linear mixed effects model and OLS regression. The parameters of linear mixed effects model include the average energy (E_{am}, with 95% CI in brackets), the normalization constants (ln $m_0 \pm$ standard error), standard deviations of the random effects of ln m_0 and E_{am} (θ_m and θ_{Eam}), percentage of variance explained by the random and fixed effect (V_{fr}); percentage of variance explained by the fixed effect only (V_f). Other parameters are the same with Table 1.

		Linear mixed effects model								Linear model (OLS)
Station	**Grazing rate**	**ln m_0**	**E_{am}**	**θ_m**	**θ_{Eam}**	**V_{fr}**	**V_f**	**N_o**	**N_g**	**$E_{a(OLS)}$**
EO station	Bulk phytoplankton	−1.36 ± 0.31	0.64 (0.38, 0.89)	0.99	0.4	0.97	0.27	38	11	0.70 (0.39, 1.00)
	Micro-phytoplankton	−1.79 ± 0.52	0.45 (−0.34, 1.24)	1.03	0.75	0.58	0.09	25	8	1.78 (0.71, 2.85)
	Nano-phytoplankton	−1.31 ± 0.23	0.58 (0.34, 0.81)	0.57	0.09	0.78	0.29	39	10	0.86 (0.45, 1.27)
	Pico-phytoplankton	−0.70 ± 0.22	0.37 (0.16, 0.59)	0.62	0.19	0.78	0.22	39	11	0.59 (0.35, 0.82)
	Synechococcus (FCM)	−1.17 ± 0.27	0.69 (0.36, 1.03)	0.78	0.38	0.92	0.28	37	12	0.74 (0.45, 1.03)
	Picoeukaryotes (FCM)	−1.00 ± 0.30	0.49 (0.19, 0.80)	0.85	0.35	0.82	0.19	40	11	0.66 (0.18, 1.14)
WE station	Bulk phytoplankton	−1.11 ± 0.14	0.57 (0.36, 0.78)	0.44	0.3	0.91	0.40	47	12	0.61 (0.35, 0.87)
	Micro-phytoplankton	−1.83 ± 0.31	0.77 (0.40, 1.14)	1.07	0.6	0.94	0.28	50	13	0.66 (0.41, 0.91)
	Nano-phytoplankton	−1.44 ± 0.26	0.97 (0.57, 1.37)	0.76	0.54	0.87	0.44	45	12	1.12 (0.64, 1.60)
	Pico-phytoplankton	−0.43 ± 0.21	0.53 (0.32, 0.72)	0.62	0.17	0.90	0.22	39	12	0.72 (0.41, 1.03)
	Synechococcus (FCM)	−0.3 ± 0.16	0.43 (0.24, 0.63)	0.52	0.27	0.97	0.21	33	12	0.44 (0.27, 0.61)
	Picoeukaryotes (FCM)	−0.15 ± 0.18	0.33 (0.20, 0.46)	0.57	0.13	0.98	0.10	33	11	0.35 (0.23, 0.48)

906