Comment: Unimodal relationship between phytoplankton-mass–specific growth rate and size: A reply to the comment by Sal and López-Urrutia (2011)

Bingzhang Chen,[a],* and Hongbin Liu[b]

[a] State Key Laboratory of Marine Environmental Science, College of Oceanography and Environmental Science, Xiamen University, Xiamen, China
[b] Division of Life Science, Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong

Sal and López-Urrutia (2011) had two criticisms of our analysis on the relationship between community-based, temperature-corrected, mass-specific growth rates of natural phytoplankton assemblages under nutrient-enriched conditions and average size based on data from dilution experiments in surface waters of the global ocean (Chen and Liu 2010). Two individual data sources (Chen et al. 2009; Sherr et al. 2009) used photoacclimation-corrected growth rates, while other data were not corrected for photoacclimation. If uncorrected data in Chen et al. (2009) were used in the analysis, the quadratic term in the unimodal fit became insignificant, but the slope of the linear fit was still significantly positive, suggesting that larger phytoplankton grow faster. However, if a lower temperature coefficient (activation energy E = 0.32 eV instead of 0.41 eV) were used to normalize the temperature effect, there was no relationship between phytoplankton specific growth rate and average size, which is consistent with the prediction of the Metabolic Theory of Ecology (MTE) that cell-specific production rate should scale isometrically with cell size expressed in terms of carbon (López-Urrutia et al. 2006).

We argue that, for the first point, photoacclimation-corrected data should be used for calculation of phytoplankton-mass–specific growth rates. In dilution experiments, chlorophyll a (Chl a) concentrations are usually used as a proxy for total phytoplankton biomass. However, phytoplankton assemblages can undergo changes in chlorophyll : carbon ratios during incubation due to the photoacclimation effect induced by changes of the external light environment. To estimate the carbon-based, phytoplankton-mass–specific growth rate, the chlorophyll-based growth rates must be corrected for photoacclimation. Flow cytometry provides a useful tool for corrections because the side-scattering and red fluorescence of phytoplankton cells excited by a 488-nm laser can be related to cell size and chlorophyll content, respectively (Li 1995). Owing to the inferior ability of flow cytometry to analyze large and rare cells, this kind of correction is most reliable in oligotrophic waters where picophytoplankton cells dominate. Therefore, we argue that, for estimation of phytoplankton growth rate using dilution experiments, the correction for the photoacclimation effect should be conducted as long as flow cytometry (FCM) data are available. When we assembled the data set in Chen and Liu (2010), however, the correction using FCM data was not conducted in most

studies. We assume that the problem of changed chlorophyll : carbon ratios of phytoplankton before and after incubation was not serious or counteracted each other (Calbet and Landry 2004). However, in our own study (Chen et al. 2009) in which the photoacclimation effect was corrected, we observed significant increases in fluorescence of picophytoplankton cells (especially for *Synechococcus*) relative to size increases in some experiments, probably owing to some problems in creating a perfect match of the in situ light environment. We believe that the photoacclimation effect should be corrected in these experiments to get the right values of phytoplankton-mass–specific growth rates. If the corrected data are used, the unimodal relationship between log phytoplankton-mass–specific growth rates (μ_n) and log-average phytoplankton size (M) still holds even if a smaller temperature coefficient (activation energy = 0.32 eV) is used (Fig. 1A).

We admit that the data from Chen et al. (2009) affect the final shape of the fitting curve (linear vs. quadratic). If these data are removed or uncorrected data are used as in Sal and López-Urrutia (2011), the quadratic term is not significant. Inclusion of more data would probably help reveal the underlying shape of the curve, but is beyond the scope of this reply (but *see* below).

A critical point underpinning Sal and López-Urrutia's (2011) argument, which is that phytoplankton-cell–specific production rate should scale isometrically with cell size (i.e., mass-specific growth rate should not depend on cell size), results from López-Urrutia et al.'s (2006) analysis based on a data set composed of lab measurements (table 3 in the supplemental materials of López-Urrutia et al. [2006]). Inspired by Sal and López-Urrutia's (2011) argument that, "Theory and experiments should have a major say in elucidating whether phytoplankton growth rates scale according to models of resource distribution networks as proposed by MTE or are constrained by surface diffusion," we converted the cell-specific phytoplankton production rates (unit: mmol O_2 cell^{-1} d^{-1}) in the data set given by López-Urrutia et al. (2006) into mass-specific growth rates (unit: d^{-1}) using a photosynthetic quotient of 1.25 provided in the supplemental materials of López-Urrutia et al. (2006). After normalization for temperature and photosynthetically active radiation (PAR) using the parameters given in table 1 of López-Urrutia et al. (2006), $\log_{10}$ mass-specific growth rates were better described by a quadratic curve than a straight line (Fig. 1B). The quadratic term was highly significant (t-test, $t = -17.2$, $p \ll 0.001$, df = 1057). The fitted quadratic

* Corresponding author: bzchen2011@xmu.edu.cn

A

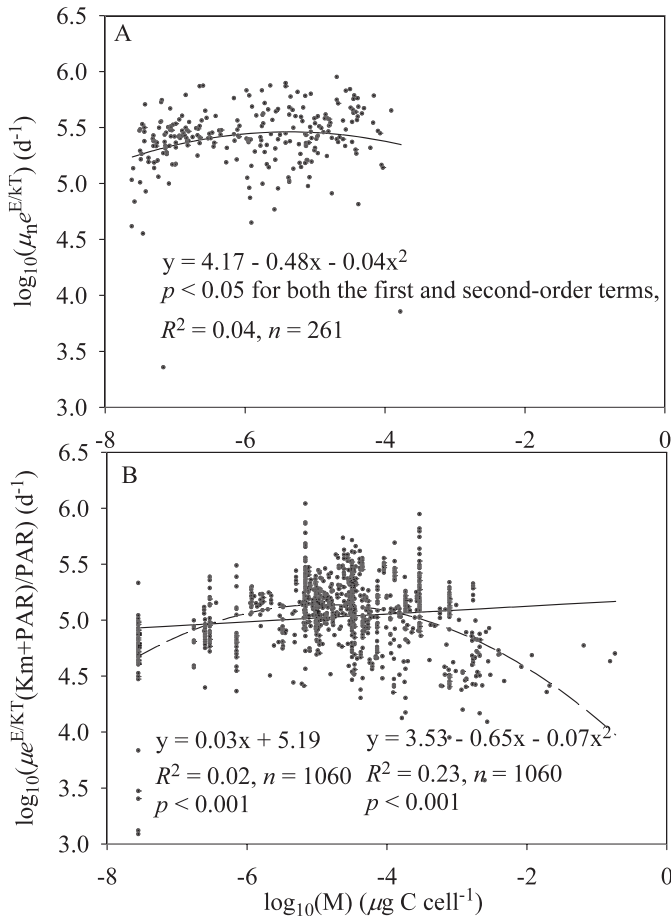$$\log_{10}(\mu_n e^{E/kT}) \ (d^{-1})$$

$y = 4.17 - 0.48x - 0.04x^2$

$p < 0.05$ for both the first and second-order terms,

$R^2 = 0.04, n = 261$

B

$$\log_{10}(\mu e^{E/KT}(K_m + PAR)/PAR) \ (d^{-1})$$

$y = 0.03x + 5.19$
$R^2 = 0.02, n = 1060$
$p < 0.001$

$y = 3.53 - 0.65x - 0.07x^2$
$R^2 = 0.23, n = 1060$
$p < 0.001$

$$\log_{10}(M) \ (\mu g \ C \ cell^{-1})$$

Fig. 1. (A) Logarithmic temperature (T, in Kelvin) normalized nutrient-enriched phytoplankton-mass–specific growth rates (d^{-1}) against phytoplankton average size (M) based on the dilution data set in Chen and Liu (2010). (B) Logarithmic phytoplankton-mass–specific growth rates after correction for temperature and photosynthetically active radiation (PAR, mol photons $m^{-2} \ d^{-1}$) against log cell size based on the lab data set from López-Urrutia et al. (2006). Both linear and quadratic regression are shown. The left equation represents the linear fit, while the right equation represents the quadratic fit. Activation energy $E = 0.32$ and 0.29 eV in A and B, respectively. $K_m = 1.51$ mol photons $m^{-2} \ d^{-1}$.

Table 1. Quadratic regression equations of $\log_{10}$ phytoplankton-mass–specific growth rates (y, d^{-1}) against $\log_{10}$ cell size (x, $\mu g \ C \ cell^{-1}$) with activation energies ranging from 0 eV to 0.8 eV based on the data set in López-Urrutia et al. (2006). Photosynthetically active radiation (PAR, mol photons $m^{-2} \ d^{-1}$) is corrected according to the parameters given in table 1 of López-Urrutia et al. (2006). All p-values for the second-order term are highly significant ($< 10^{-15}$).

Activation energy (E, eV)	Regression equation	R^2
0	$y = -1.30 - 0.55x - 0.05x^2$	0.14
0.2	$y = 2.03 - 0.62x - 0.06x^2$	0.21
0.3	$y = 3.70 - 0.66x - 0.07x^2$	0.24
0.4	$y = 5.36 - 0.69x - 0.07x^2$	0.26
0.6	$y = 8.69 - 0.76x - 0.08x^2$	0.29
0.8	$y = 12.02 - 0.83x - 0.09x^2$	0.31

unimodal pattern is clearer in the larger data set of lab experiments, which also circumvents the problem of confounding correlation between size and nutrient supply (i.e., phytoplankton average size being larger in more eutrophic waters). The unimodal relationship between phytoplankton-mass–specific growth rate and size is not a result of nutrient limitation, but may reflect evolutionary adaptation of picophytoplankton to the oligotrophic environment where nutrient requirements of phytoplankton must be kept low, which limits the proportion of scalable components devoted to cell growth (Raven 1998).

One reason that such a unimodal relationship was often overlooked is probably because a simple power-law function was taken for granted relating cell-specific production rate and size without looking at the pattern of mass-specific grow rate against size. The intrinsic second-order effect was inappropriately treated as random error in such a log–log linear regression. The slope of the linear curve of log-cell–specific production rate or mass-specific growth rate against log size depends on the size range considered (Fig. 2), which could explain why different exponents were sometimes obtained (Tang 1995; López-Urrutia et al. 2006; Litchman et al. 2007).

Curvature in metabolic scaling is not uncommon (Dodds et al. 2001; Kolokotrones et al. 2010). There has been no satisfactory theory explaining the empirically observed 3/4-law, although significant progress has been made (West et al. 1997; Banavar et al. 2010). Even the universality of the 3/4 exponent has been questioned (Dodds et al. 2001; Glazier 2005; also *see* Fig. 2, which strongly discourages the use of a single allometric exponent). Mechanistic models are still needed to explain the unimodal pattern between phytoplankton growth rate and size.

We need to add that, although 0.32 eV might be an appropriate, approximate activation energy for the temperature dependence of the C_3 photosynthetic rate (limited by Rubisco carboxylation) of one chloroplast (Allen et al. 2005), phytoplankton cells can adjust biomass partitioning to temperature-sensitive vs. temperature-insensitive compartments at different temperatures to achieve optimal growth at a given temperature (Raven and Geider 1988). On the whole-cell level, the activation energy of phytoplankton growth may deviate from the 'canonical' 0.32 eV,
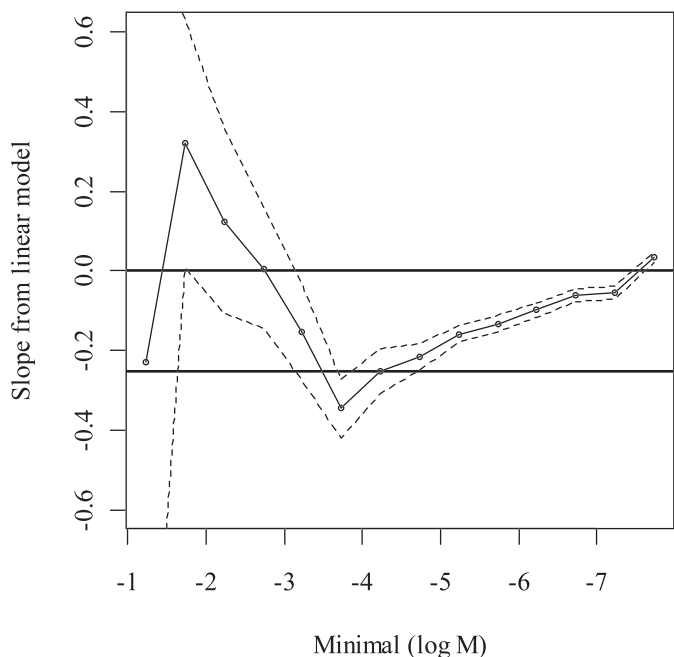
equation and the corresponding modal size ($10^{-4.64} = 2.29 \times 10^{-5} \ \mu g \ C \ cell^{-1}$) are nearly identical with those given in fig. 2A of Chen and Liu (2010). Hence, the unimodal relationship between phytoplankton-mass–specific growth rate and size is also apparent in lab measurements. The close match between lab and field data suggests that the growth rates of individual species obtained in lab cultures can be well-scaled up to natural communities. We also find that the unimodal relationship in this lab data set is robust to the specific temperature coefficients used (Table 1). Even if the temperature effect is not corrected (activation energy = 0 eV), the unimodal relationship still holds.

The number of data points matters in revealing such a relationship. The number (1060) of data points in the data set of López-Urrutia et al. (2006) is four times of that (261) in fig. 2C in Chen and Liu (2010) and, therefore, the

Fig. 2. Slopes of linear regressions of log-transformed, phytoplankton-mass–specific growth rates and cell size (M) as in Fig. 1 vs. the minimal cell sizes in the subsets of the data set of López-Urrutia et al. (2006), for which the maximal cell size was not changed. The dashed lines represent the 95% confidential intervals. Horizontal lines of 0 and −0.25 are also shown for reference.

and it is still argued that there is no preference for choosing the Arrehnius equation over the simpler exponential Q_{10} equation (Clarke and Johnston 1999). More intriguingly, Montagnes and Franklin (2001) have shown that, for a number of diatom species, growth rates increase linearly with temperature at certain temperature ranges, suggesting that the growth rate–temperature relationship within species could be different from that among species and the underlying patterns and mechanisms could be more complex than is currently understood.

References

ALLEN, A. P., J. F. GILLOOLY, AND J. H. BROWN. 2005. Linking the global carbon cycle to individual metabolism. Funct. Ecol. **19:** 202–213, doi:10.1111/j.1365-2435.2005.00952.x

BANAVAR, J. R., M. E. MOSES, J. H. BROWN, J. DAMUTH, A. RINALDO, R. M. SIBLY, AND A. MARITAN. 2010. A general basis for quarter-power scaling in animals. Proc. Natl. Acad. Sci. U. S. A. **107:** 15816–15820, doi:10.1073/pnas.1009974107

CALBET, A., AND M. R. LANDRY. 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnol. Oceanogr. **49:** 51–57, doi:10.4319/lo.2004.49.1.0051

CHEN, B. Z., AND H. B. LIU. 2010. Relationships between phytoplankton growth and cell size in surface oceans: Interactive effects of temperature, nutrients, and grazing. Limnol. Oceanogr. **55:** 965–972, doi:10.4319/lo.2010.55.3.0965

————, ————, M. R. LANDRY, M. H. DAI, B. Q. HUANG, AND J. SUN. 2009. Close coupling between phytoplankton growth and microzooplankton grazing in the western South China Sea. Limnol. Oceanogr. **54:** 1084–1097, doi:10.4319/lo.2009.54.4.1084

CLARKE, A., AND N. M. JOHNSTON. 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol. **68:** 893–905, doi:10.1046/j.1365-2656.1999.00337.x

DODDS, P. S., D. H. ROTHMAN, AND J. S. WEITZ. 2001. Re-examination of the '3/4-law' of metabolism. J. Theor. Biol. **209:** 9–27, doi:10.1006/jtbi.2000.2238

GLAZIER, D. S. 2005. Beyond the '3/4-power law': Variation in the intra- and interspecific scaling of metabolic rate in animals. Biol. Rev. **80:** 611–662, doi:10.1017/S1464793105006834

KOLOKOTRONES, T., V. SAVAGE, E. J. DEEDS, AND W. FONTANA. 2010. Curvature in metabolic scaling. Nature **464:** 753–756, doi:10.1038/nature08920

LI, W. K. W. 1995. Composition of ultraphytoplankton in the central North-Atlantic. Mar. Ecol. Prog. Ser. **122:** 1–8, doi:10.3354/meps122001

LITCHMAN, E., C. A. KLAUSMEIER, O. M. SCHOFIELD, AND P. G. FALKOWSKI. 2007. The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. Ecol. Lett. **10:** 1170–1181, doi:10.1111/j.1461-0248.2007.01117.x

LÓPEZ-URRUTIA, A., E. SAN MARTIN, R. P. HARRIS, AND X. IRIGOIEN. 2006. Scaling the metabolic balance of the oceans. Proc. Natl. Acad. Sci. U. S. A. **103:** 8739–8744, doi:10.1073/pnas.0601137103

MONTAGNES, D. J. S., AND D. J. FRANKLIN. 2001. Effect of temperature on diatom volume, growth rate, and carbon and nitrogen content: Reconsidering some paradigms. Limnol. Oceanogr. **46:** 2008–2018, doi:10.4319/lo.2001.46.8.2008

RAVEN, J. A. 1998. The twelfth Tansley Lecture. Small is beautiful: The picophytoplankton. Funct. Ecol. **12:** 503–513, doi:10.1046/j.1365-2435.1998.00233.x

————, AND R. J. GEIDER. 1988. Temperature and algal growth. New Phytol. **110:** 441–461, doi:10.1111/j.1469-8137.1988.tb00282.x

SAL, S., AND Á. LÓPEZ-URRUTIA. 2011. Temperature, nutrients, and the size-scaling of phytoplankton growth in the sea. Limnol. Oceanogr, **56:** 1952–1955, doi:10.4319/lo.2011.56.5.1952

SHERR, E. B., B. F. SHERR, AND A. J. HARTZ. 2009. Micro-zooplankton grazing impact in the western Arctic Ocean. Deep-Sea Res. Part II **56:** 1264–1273.

TANG, E. P. Y. 1995. The allometry of algal growth-rates. J. Plankton Res. **17:** 1325–1335, doi:10.1093/plankt/17.6.1325

WEST, G. B., J. H. BROWN, AND B. J. ENQUIST. 1997. A general model for the origin of allometric scaling laws in biology. Science **276:** 122–126, doi:10.1126/science.276.5309.122