



Reassessment of the cell surface area limitation to nutrient uptake in phytoplankton

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ABSTRACT: In many past theoretical and empirical studies of phytoplankton nutrient allometry, cell surface area has been considered the main limiting factor to nutrient uptake rates because of an assumption that the cell surface area is not large enough to accommodate enough uptake sites to meet the uptake demand. However, calculations in this paper show that the required uptake sites may only cover approximately 76% of the surface area of a typical phytoplankton cell. Cell volume could be the main factor controlling the maximum nutrient uptake rates of phytoplankton cells with a cell volume of $<40 \mu\text{m}^3$, while the cell surface becomes limiting for larger cells. The maximum nutrient uptake rate of phytoplankton cells generally increases almost linearly with cell volume, and that of smaller cells increases faster than that of larger cells.

KEY WORDS: Phytoplankton · Nutrient uptake · Growth rate · Limitation · Cell surface · Cell volume · Allometry

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INTRODUCTION

Cell surface area has been linked to rates of nutrient uptake in phytoplankton (Pasciak & Gavis 1974, Smith & Kalff 1982, Aksnes & Egge 1991, Litchman et al. 2007, Armstrong 2008, Edwards et al. 2012). Theoretical derivations based on a mechanistic model for inorganic nutrient uptake by Pasciak & Gavis (1974) and later by Aksnes & Egge (1991) led to a formula suggesting that the maximum nutrient uptake rate of a phytoplankton cell is linearly proportional to the number of nutrient ion uptake sites on the cell surface and inversely proportional to the ion handling time. The number of uptake sites was said to be dependent on the site density and the surface area. This led to the renowned hypothesis by Aksnes & Egge (1991) that if the site density is unchanged, the maximum nutrient uptake rate of a phytoplankton cell is linearly proportional to the area of the cell surface.

In later studies (Litchman et al. 2007, Ward et al. 2011, Edwards et al. 2012), the maximum nutrient uptake was considered to be limited by cell surface

area. This concept came from estimates that the total uptake area for nitrogen nutrient can comprise approximately 8.5% of the cell surface (Aksnes & Egge 1991) and that the cell needs to uptake at least 50 macro- and micronutrients (Litchman et al. 2007), leading to a perception that the cell surface must be too small to accommodate enough uptake sites for all nutrients (Ward et al. 2011). This concept was not only used for phototrophic phytoplankton but also for a general resource acquisition model for mixotrophic phytoplankton, where uptake sites for inorganic and organic nutrients have to share a limited area on the cell surface (Ward et al. 2011).

Empirical results, however, were not always consistent with theoretical results. For example, using power equations ($\rho_{\text{max}} = aV^b$, where ρ_{max} is the maximum nutrient uptake rate and V is the cell volume) to approximate an experimental dataset, Edwards et al. (2012) and Marañón et al. (2013) showed that the resulting scaling exponent, b , of the nitrogen and phosphorus maximum uptake rate–cell volume relationship was significantly higher than the theoretical

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value of $2/3$ and even exceeds 1. Litchman et al. (2007), when using a subset of the dataset of Edwards et al. (2012), derived a different result in which the scaling exponents were very close to $2/3$. Aksnes & Egge (1991) (and also Raven 1980) suggested that a cell can adopt one or more of the following strategies to increase its uptake rate, hence resulting in a larger-scale exponent: (1) increase the effective size of the uptake sites, (2) increase the number of uptake sites per individual, (3) reduce handling time and (4) reduce the effect of diffusion. Increase of the surface area-to-volume ratio (Edwards et al. 2012) could increase the number of uptake sites per individual. All these strategies, except for (4), assumed that the cell is not able to absorb all nutrients arriving at the cell surface because the surface area is limited for accommodating additional uptake sites.

Notably, there was a fundamental difference in the above theoretical and empirical studies. The theoretical work looked into the absorption capability of the cell surface without considering how the absorbed nutrients are processed within the cell. The empirical work was based on experimental data which reflect reality, in which a combination of the absorption capability of the cell surface and the processing capability of the cell internal machineries would define how much nutrient to take in.

In the theoretical context, the concept of surface area limitation and the Aksnes & Egge (1991) hypotheses would work well. However, in the real context, I would like to raise 2 questions: (1) Does surface area always constrain the cell's nutrient uptake? (2) Is the use of the Aksnes & Egge (1991) hypothesis to explain the empirical trend adequate? Investigations in this paper will provide possible answers for the above questions, reassessing the limitation of cell surface area to phytoplankton nutrient uptake. As carbon and nitrogen are the 2 most important elements that a phytoplankton cell needs to take in, experimental data on these 2 elements and growth rates of phytoplankton cells will be used for the investigations.

MATERIALS AND METHODS

Data collection and preparation

Laboratory experiment data on growth rates (μ , divisions d^{-1}), cellular carbon and nitrogen contents (C and N , $pg\ cell^{-1}$), cell volumes (V , μm^3) of phytoplankton and corresponding lighting durations of the experiments (I_d , $h\ d^{-1}$) were obtained from published

reports and articles (Sakshaug & Hansen 1977, Sakshaug et al. 1984, Montagnes et al. 1994, Maldonado & Price 1996, Bertilsson et al. 2003, Ho et al. 2003, Marchetti & Harrison 2007, Labry et al. 2008, Marañón et al. 2013). All considered phytoplankton were grown in nutrient-replete, light-saturated or near-saturated conditions. Batch cultures were harvested at the mid- or end-exponential growth phase. There are 86 sets of data containing growth rates, cellular carbon, cellular nitrogen and cell volumes.

Growth rates of phytoplankton in different light-dark cycles were adjusted to the same light conditions (here, all were adjusted to 24:0 light:dark) using the model of Flynn (2001) for non-fluctuating light. In Eq. (20) of Flynn (2001), the carbon-specific growth rate is modelled as a hyperbolic tangent function of light intensity and photoacclimation. Given the long and stable light cycle and saturated or near-saturated light intensity in the experiments, the photoacclimation could be neglected and the hyperbolic tangent function approaches 1. That gives the maximum carbon-specific growth independent of light, and a time-integral over a time period will give a linear function of time, making an acceptable correction for light.

Cell shapes and dimensions of the collected phytoplankton were obtained from databases of phytoplankton species, closest shapes and dimensions (Hillebrand et al. 1999, Sun & Liu 2003, Olenina et al. 2006). Cells of the same species were assumed to have the same shape so that cell dimensions could be normalized to a characteristic base length (L , μm) which was chosen as the diameter or the width of the cell's cross section. Using the base length, the cell volume and cell surface area (SA , μm^2) then can be computed as $V = V_0 L^3$ and $SA = SA_0 L^2$, where V_0 and SA_0 are the base volume and the base surface area, respectively, of a given cell shape having a unit base length.

Investigation method

Previous studies presumed that cell surface area is the limiting factor for the accommodation of uptake sites for all nutrients. The portion of surface area used for those uptake sites could be estimated as follows. The ratio of the total area for all uptake sites to the cell surface area, rSA , is estimated from the ratio of the area for nitrogen uptake sites to the cell surface area, rSA_N , and the Redfield ratios (Redfield 1934, Brzezinski 1985). The Redfield ratio N:Si = 16:15 indicates that the number of silicon atoms to be taken in is approximately equal to that of nitrogen. The number of atoms of phosphorus and other ele-

ments, in total, are approximately $a_2 = 30\%$ of the number of nitrogen atoms (Ho et al. 2003). Thus, the area ratio of total uptake sites for nutrients to the cell surface is

$$\begin{aligned} rSA &= rSA_N \times (C:N + 1 + 1 + a_2) \\ &= (C:N + 2.3) \times rSA_N \end{aligned} \quad (1)$$

Cell surface area is theoretically the limiting factor if rSA reaches 100%. The area ratio of nitrogen uptake sites to the cell surface, correspondingly, will also reach a maximum, rSA_{Nmax} . The corresponding theoretical cell-surface-limited growth rate (μ_s ; divisions d^{-1}) is derived from theoretical formulae given in Table 2 of Aksnes & Egge (1991). The derived formula is

$$\mu_s = \frac{rSA_{Nmax} \times SA \times K_{sN} \times D}{N \times r} \quad (2)$$

where $D (= 1.5 \times 10^{-9} \text{ m}^2 \text{ s}^{-1})$ is the molecular diffusion coefficient for nutrient ions in water, and r (μm) is the spherical equivalent radius of the cell. The half-saturation constant for nitrogen uptake, K_{sN} , was unfortunately unavailable in the collected dataset. Therefore, the linear function of cell radius $K_{sN} = K_{sN0} \times r$ provided in Aksnes & Egge (1991) was used. The function is also fitted to the values given in Table 2 of the same paper, i.e. $K_{sN} = 0.5 \mu\text{M}$ for an $r = 3 \mu\text{m}$ algal cell. This gives $K_{sN0} = 0.167 \text{ M m}^{-1}$.

Eq. (2) was applied to every phytoplankton individual in the collected dataset with available cellular nitrogen content and cell surface area. The theoretical surface-limited growth rates was compared with the actual growth rates at different cell volumes. The comparison was expected to identify a cell size threshold where surface area becomes limiting, i.e. the actual growth rate approaches the theoretical growth rate.

To verify the empirical findings of the past nutrient allometry studies and to provide additional information for the investigation in this paper, variations of carbon production rates against cell volumes and cell surface areas were investigated. As carbon is the most important element in a cell and the chloroplast (considered the processing machinery for carbon) takes up a large portion of the cell volume, carbon uptake will be the most suitable to verify the hypothesis of surface and volume limitation. The carbon production rates (C_{prd} , $\text{pg cell}^{-1} \text{ d}^{-1}$) were computed using the collected growth rates and

the cellular carbon contents and were corrected to the same light duration as

$$C_{prd} = (24/I_d) \times \mu \times C \quad (3)$$

C_{prd} was plotted against cell volume and cell surface area. The plotted data were fitted by power functions, $y = a x^b$, using a least squares regression method. The curve fitting was also applied to 2 sub-datasets of cell size classes, i.e. small and large phytoplankton, separated by the cell size threshold defined above.

RESULTS

We can estimate from Eq. (1) that the maximum area ratio of uptake sites for nitrogen to the cell surface of a typical phytoplankton (C:N = 106:16) is $rSA_{Nmax} = 11.15\%$. The theoretical surface-limited growth rates of phytoplankton species listed in the dataset are computed by plugging the actual C:N into Eq. (1) to obtain rSA_{Nmax} (silicon uptake sites are excluded for non-diatom species), then plugging rSA_{Nmax} into Eq. (2). Obtained results are plotted in Fig. 1 together with the actual growth rates. It can be seen from Fig. 1 that at small cell volumes, the actual growth rates are well below the theoretical surface-limited growth rates. The 2 values approach each other as the cell volume increases. At cell volumes of above approximately $40 \mu\text{m}^3$, the actual and the theoretical surface-limited growth rates are in the same range. It is also observed from the experimental data shown in Fig. 1 that the growth rate of phytoplankton reaches its maximum at a cell volume of 40 to $100 \mu\text{m}^3$.

The carbon production rates, cell volumes, cell surface areas and curve fittings are shown in Fig. 2. The

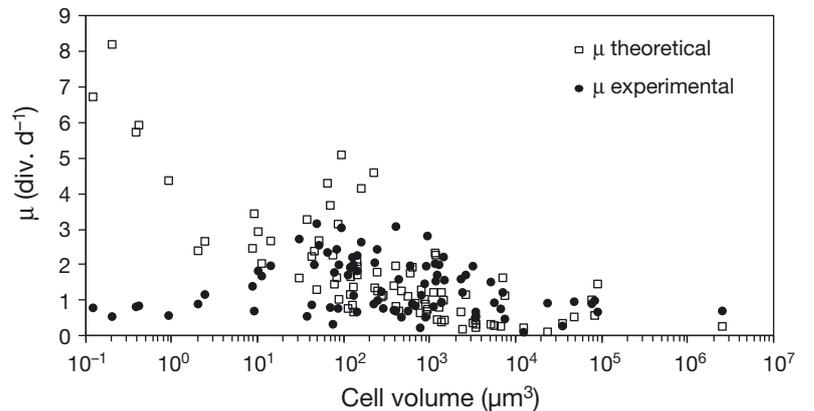


Fig. 1. Theoretical cell surface-limited and experimental measured growth rates (μ) of phytoplankton of different cell volumes

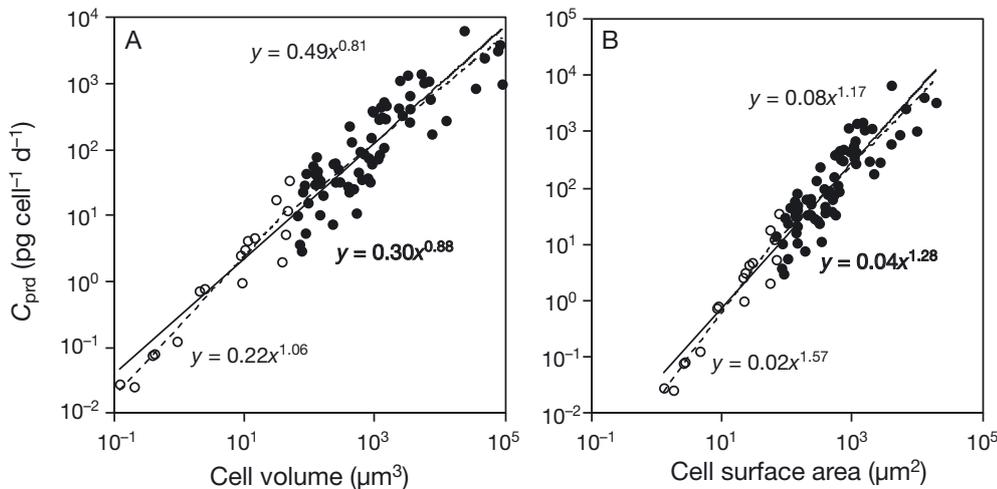


Fig. 2. Variation in carbon production rates (C_{prd}) with (A) cell volume and (B) cell surface area. Dashed lines and solid lines show curve fitting to sub-datasets and the full dataset, respectively. (○) Smaller size classes (below threshold where surface area becomes limiting to growth); (●) larger size classes

scaling exponent of the $C_{\text{prd}}-V$ function is slightly lower than 1 ($b = 0.88$; $R^2 = 0.89$; Fig. 2A), while that of the $C_{\text{prd}}-SA$ function is significantly higher than 1 ($b = 1.28$; $R^2 = 0.89$; Fig. 2B). In the 2 size classes below the cell volume threshold, the scaling exponents of both the $C_{\text{prd}}-V$ and $C_{\text{prd}}-SA$ functions of the small size class are significantly higher than those of the large size class (1.06 and 1.57 compared to 0.81 and 1.17). Greater fluctuations in carbon production rates are also observed in the large size class. The ratio of the scaling exponents of the $C_{\text{prd}}-SA$ and $C_{\text{prd}}-V$ functions is 1.48 for the small size class and 1.44 for the large size class.

DISCUSSION

According to Eq. (1), if uptake sites for nitrogen occupy 8.5% of the cell surface, then the total area occupied by all uptake sites is 76% of the cell surface. The cell surface area is entirely occupied by uptake sites if the area ratio of uptake sites for nitrogen to the cell surface is 11.15%, which is approximately 30% higher. Moreover, Eq. (1) assumed that silicon is taken in and all nutrients are simultaneously absorbed through the cell surface, which may be a stressful scenario. Nutrients like nitrogen, phosphorus and other minerals may be taken in by the cells during the dark period, while carbon may be given the first priority during the light period. This strategy could increase the usage efficiency of the uptake sites on the cell surface. Hence, we could say that surface area is not really limiting, at least for the typical phytoplankton used in the study of Aksnes & Egge (1991).

In addition, data in Fig. 1 show that the actual growth rates of cells with volumes smaller than a

threshold of approximately $40 \mu\text{m}^3$ are well below the theoretical surface-limited growth rates. This interesting result could imply that those cells are not surface-limited because if all their surface areas were used up for accommodating uptake sites, their growth rates would have been significantly higher. Aksnes & Cao (2011) also showed that a *Vibrio splendidus* cell having a cell volume of $0.52 \mu\text{m}^3$ could theoretically increase its growth rate by almost 2 orders by increasing its uptake site density by 100 times. But this is not true for the actual growth rates. A prominent possibility is that the processing capability of the cellular machineries of those cells is lower than the absorbing capability of the cell surfaces, resulting in a lower actual growth rate. When the cell volume increases, the cellular machinery as well as its processing capability would increase accordingly and eventually catch up with the absorbing capability of the cell surface. As a result, the actual growth rate would approach the theoretical value as observed in the figure. Hence, the cell's growth rate is constrained by its volume. Once the area density of uptake sites reaches the maximum, the cell becomes surface-limited.

Cell volume constraining the cell growth is consistent with empirical results of nutrient allometry studies. The scaling exponent of 0.88 for the $C_{\text{prd}}-V$ function obtained in this study (Fig. 2A) agrees with that obtained by Marañón (2008), Edwards et al. (2012) and Marañón et al. (2013) for carbon, nitrogen and phosphorus. These results indicate that the cell's nutrient uptake rate is almost linearly proportional to the cell volume. Furthermore, the increase of carbon production rate due to increasing cell volume is more prominent in the small cell class and indeed almost linear (Fig. 2A). The significant reduction in the scaling exponent from 1.06 for the small cell class to 0.81

for the large cell class is probably because other limitations (surface area, diffusion flux) start influencing the absorption rate as the cell size becomes larger.

The ratios of the scaling exponents of the $C_{\text{prd}} - SA$ curve to the $C_{\text{prd}} - V$ curve also support the hypothesis that nutrient uptake rates of the small size class are not constrained by the cell surface area. As discussed above, nutrient uptake rates of the small size class are constrained by the cell volume; hence, the cells should maximize their volume-to-surface ratio, i.e. tend to have spherical shape, to achieve maximum uptake rates. The ratio of the scaling exponents of 1.48 for the small size class is close to that of a spherical cell (1.5). The large size class, whose nutrient uptake rates are constrained by the cell surface area or diffusion flux, should maximize their surface area to volume. Hence, the ratio of the scaling exponents is reduced (to 1.44). Various strategies of the cells to reduce the effect of the cell surface area or diffusion flux limitation could be attributed to the fluctuations of the growth rates and carbon production rates as observed in Figs. 1 & 2.

In summary, maximum nutrient uptake rates of an actual phytoplankton cell having a cell volume less than a threshold of approximately $40 \mu\text{m}^3$ could be constrained by the cell volume. While the maximum uptake rate of phytoplankton cells generally increases almost linearly with the cell volume, that of smaller cells increases faster than that of larger cells. The hypothesis of Aksnes & Egge (1991) (maximum uptake rates are linearly proportional to the cell surface area) probably explained the absorption capability of the cell surface but not the cell as a whole. This hypothesis is correct if the cell is truly surface-limited, i.e. the absorption capability of the cell surface is lower than the processing capability of the cell and lower than the diffusion flux of nutrients towards the cell. Although in most experiments phytoplankton cells would have cell volumes larger than the threshold, using the hypothesis of Aksnes & Egge (1991) to explain experiment results should be done with care and may require appropriate modifications.

Acknowledgements. The research described in this publication was funded in whole or in part by the Singapore National Research Foundation (NRF) through the Singapore-MIT Alliance for Research and Technology (SMART), Center for Environmental Sensing and Modeling (CEN-SAM).

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*Editorial responsibility: Antonio Bode,
A Coruña, Spain*

*Submitted: January 30, 2013; Accepted: June 4, 2013
Proofs received from author(s): August 19, 2013*