Cronfa - Swansea University Open Access Repository

_____

This is an author produced version of a paper published in :
Journal of Plankton Research

_____

Cronfa URL for this paper:

_____

Paper:

_____

What is the limit for photoautotrophic plankton growth rates?

Kevin J Flynn[1][*] and John A Raven[2,]

[1] Wallace Building; Swansea University, SA2 8PP, U.K.

[2] Division of Plant Science, University of Dundee at the James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK (permanent address), and Functional Plant Biology and Climate Change Cluster (C3), University of Technology Sydney, Ultimo, NSW 2007, Australia.

*author for correspondence k.j.flynn@swansea.ac.uk

Abstract

Knowing the potential maximum photoautotrophic growth rate for planktonic primary producers is fundamental to our understanding of trophic and biogeochemical processes, and of importance in applied phycology. When day-integrated C-specific growth is considered over natural light:dark cycles, plausible RuBisCO activity (K_{cat} coupled with cellular RuBisCO content) caps growth to less than a few doubling per day. Prolonged periods of C-specific growth rates above ca. 1.3 d^{-1} thus appear increasingly implausible. Discrepancies between RuBisCO-capped rates and reported microalgal specific growth rates, including temperature-growth rate relationships, may be explained by transformational errors in growth rate determinations made by reference to cell counts or most often chlorophyll, or by extrapolations from short term measurements. Coupled studies of enzyme activity and day-on-day C-specific growth rates are required to provide definitive evidence of high growth rates. It seems likely, however, that selective pressure to evolve a RuBisCO with a high K_{cat} (with a likely concomitant increase in K_m for CO_2) would be low, as other factors such as light limitation (developing during biomass growth due to self-shading), nutrient limitations, CO_2 depletion and pH elevation, would all rapidly depress realised specific growth rates.

Introduction

Factors that limit primary production have various applied and fundamental implications. In ecology, such information affects our understanding of the input rates of energy and materials into the base of most open-water food chains, and in models for setting the maximum specific growth rate of primary producers. The more recent enhanced interest in applied aspects of microalgae and planktonic production (e.g., Smith and McBride, 2015) has also highlighted the importance of a robust understanding of the limits of primary production (Kenny and Flynn, 2014; Raven and Ralph, 2015).

Oxygenic photoautotrophic primary production on Earth is universally dependent on the activity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, RuBisCO. RuBisCO is thus arguably the most important enzyme for life on Earth. Another accolade is that it is the most abundant enzyme on Earth by mass (Ellis, 1979; Raven, 2013), though this is tempered by the allied accolade of having one of the lowest substrate saturated specific reaction rate (K_{cat}) among enzymes (Tcherkez et al. 2006). It is because of these features that the cellular content of RuBisCO, which may attain over 10% of total microalgal protein (Raven, 2013; Raven et al., 2013), has the potential to ultimately limit the specific growth rate of these photoautotrophs. From hereon we refer mainly to microalgae, rather than to phytoplankton, noting that microalgae are not necessarily planktonic.

Enzyme activity as described by the specific reaction rate, K_{cat}, is the maximum number of moles of substrate processed (or mole of product produced) per mole of enzyme per second. This biochemical definition is itself not of great consequence for a cost-benefit analysis of organism physiology as it takes no account of the size (and hence effective cost) of the enzyme molecule itself nor of the cellular demand for the product. It is important to account for such cost-benefit considerations using an appropriate currency; here that currency is C. The important issue for a consideration of factors limiting growth is thus not the mole-specific K_{cat}, but the C-specific transform of K_{cat}, here with units of g CO_2 fixed per g RuBisCO-C per time.

By considering the values of K_{cat} and enzyme molecular weights (http://www.brenda-enzymes.org,) together with the Redfield C:N:P mass ratio it is possible to compare the structural costs for different major enzymes in microalgae. For RuBisCO that activity is ca. 1g substrate C handled per g enzyme-C per hr (we consider the range of values further, below). For major enzymes of N-assimilation and metabolism (GS, GDH, NR and NiR) values are ca. 50-400 g substrate-N per g enzyme-C per hr, and for P-acquisition (alkPase) values are of ca. 50-250 g substrate-P per g enzyme-C per hr. By taking into account a

Redfield mass ratio for C:N:P of 41:7.2:1, and assuming that this ratio aligns with substrate demand for these elements, then the structural cost demand of a microalgal cell for RuBisCO can be seen to be 100 – 1000 times greater than that for these other enzymes. These are all very simplistic calculations, with many assumptions. There are many other factors, other costs, of importance to the synthesis and operation of enzymes and synthesis of structural proteins, most notably the need for metal cofactors and the concentration of other substrates. There is also the consequence of enzyme turnover time to consider; for protein D1 (a component of the photochemical reaction centre of Photosystem II), for example, that is of particular importance (Table 3b of Raven, 2015).

Nonetheless, even assuming a robust enzyme with minimal turnover, the cellular cost for RuBisCO in microalgae is highly significant. And there is one other issue that is often not taken into account – the diel variation of phototrophic activities. While K_{cat} is typically reported with units of s^{-1}, growth in terms of cell doublings occurs over periods of many hours if not days and expressed enzyme activity is not necessarily continuous. For RuBisCO the useful enzyme activity is restricted to the light phase of growth when its operation is supported by the generation of photoreductant and ATP. This does also apply, of course, to other photosynthesis-specific proteins and their cofactors. It applies to major enzymes associated with primary production (notably, for nitrite reductase, NiR, which may also be driven by photoreductant), and to intrinsically light-independent catalysts such as ribosomes when the bulk of net protein synthesis occurs in the photophase, leaving only protein synthesis following breakdown as a ribosome function in the darkness of the scotophase, as is the case for some algae (Raven 2013b). However, while some level of periodicity may be expected in the operation of these other enzymes and processes, for the operation of RuBisCO this situation is critical. It means that this enzyme (RuBisCO), which may account for over 10% of cell-protein, is in natural populations essentially lying dormant for ca. half the day. The dark-phase dormancy of RuBisCO means that twice the cellular rate of activity is required during the light phase (assuming for simplicity a 50:50 L:D cycle) to provide a given specific rate of daily microalgal growth.

Here we are concerned with what limits the maximum possible primary production rate, so while we recognise that there are a host of non-linearities linking the photon dose to photosynthesis and ultimately to day-integrated growth, we will assume that the activity of RuBisCO is indeed the process limiting growth (e.g., for *Prochlorococcus* with its effective CO_2 concentrating mechanism; Hopkinson et al., 2014; Zorz et al., 2015), and explore the resultant potential cellular C-specific growth rate under different L:D cycles, and nitrate vs

ammonium supply. In doing so we also note that growth using nitrate differs both over the L:D cycle and with respect to the drain on photoreductant (Flynn et al. 2002). We then compare these specific growth rate potentials with those reported for microalgae, concluding that these two rates do not align, and thus that something appears to be amiss.

The limit for primary production according to RuBisCO

For all of what follows we assume that *in vivo* concentrations of substrates and inhibitors are such that net RuBisCO activity (i.e., for fixation of CO_2) can indeed realise a rate equivalent to the value of K_{cat}. We assume also that RuBisCO oxygenase activity is not significant as a result of sufficiently high concentrations of CO_2 aided by diffusive CO_2 entry from a high external CO_2 concentration or an effective CO_2 concentrating mechanism. We also assume a negligible loss of dissolved organic-C from the cell. In short, what follows tends to the optimistic, to enable higher rather than lower C-fixation rates.

We have used data for enzyme molecular weights and activities documented in the BRENDA data base (http://www.brenda-enzymes.org). We assume the following: a molecular weight for RuBisCO of 490000, a mole-specific K_{cat} over the range of 2 -16 s^{-1} (noting that K_{cat} values around 3 s^{-1} are not uncommon, e.g., for diatoms, Young et al., 2016), and a standard mass transform for protein:N of 6.25. From these data, and assuming as an example at the temperature of interest a RuBisCO mole-specific K_{cat} of 10 s^{-1}, we derive a C-specific rate of CO_2 fixation of 1.69 gC (g-RuBisCO-C)$^{-1}$ hr^{-1}.

We then consider the contribution of RuBisCO-protein to total protein over the range 2-16% (Raven 2013a; Raven et al. 2013), a contribution of protein-N to cell-N of 0.75 (Geider and LaRoche 2002), and a mass cellular C:N for nutrient-replete microalgae of 6 (Geider and LaRoche 2002; noting that C:N in ammonium-growing cells may be lower than nitrate-growing cells). This enables us to estimate a rate of gross C-fixation. With the above example, of a RuBisCO mole-specific K_{cat} of 10 s^{-1} applied with a contribution of 10% of protein as RuBisCO in a cell growing under continuous illumination, we thus obtain a gross C-fixation rate of 1.653 gC (gC)$^{-1}$ d^{-1}.

In the light, certain energy costs may be met directly from photochemical reactions. Most notably in this regard is the cost of reducing nitrate through to ammonium. However, during growth in nutrient-saturated conditions in a light-dark cycle a significant proportion of nitrate reduction may occur during darkness (Clark et al., 2002); such a process may consume fixed-C at a rate equivalent to 1.71 gC (g nitrate-N)$^{-1}$ (Flynn and Hipkin, 1999). There is also an additional respiratory cost of 1.5 gC (gN)$^{-1}$ for assimilation of ammonium-N into organic-

N. Under continuous illumination the above demands a respiratory cost of 0.25 gC (g assimilated-C)$^{-1}$; this increases to 0.39 gC (gC)$^{-1}$ under a 12:12 L:D cycle when growing on nitrate, assuming half the nitrate assimilation occurs at the expense of non-photo-generated reductant (Clark et al., 2002). So, under continuous illumination, with the afore mentioned (rather high) K_{cat} and RuBisCO contribution to cell protein, we obtain a net C-fixation rate of 1.322 gC (gC)$^{-1}$ d^{-1}; this value of a C-specific growth rate equates to approaching 2 doublings per day. It should be noted that the above respiratory demands are optimistic, with equivalent gross:net photosynthesis ratios of ca. 1.25 – 1.4. The gross:net photosynthesis ratios for microalgae reported in the literature can be very much higher than these (Halsey and Jones, 2015), and would thus require significantly higher RuBisCO levels to support a given maximum specific growth rate.

Extending the wide range of the input values in the above calculations, in Fig.1 we present across a matrix of L:D periodicity and K_{cat} values, the proportion of cellular protein required as RuBisCO in order to support a net C-specific growth rate of 0.693 d^{-1} (i.e., 1 doubling per day). This is shown both when growth is supported by nitrate (f-ratio 1) or by ammonium (f-ratio 0); there is only a ca. 10% difference between them (ammonium-supported growth being energetically cheaper) which is not readily discernible against the log-axis used for the RuBisCO protein contribution. At typical values of K_{cat} (i.e., <10 s^{-1}) and in a 12:12 hr (i.e. 0.5) L:D cycle, to attain a specific growth rate of a doubling per day requires RuBisCO to account for > 20% of cell protein which is a value far exceeding those typically reported (Raven, 2013a; Raven et al., 2013).

In Fig.2 we have assumed a K_{cat} of 5 s^{-1} (in line with those reported for diatoms, which are amongst the fastest growing microalgae; Young et al., 2016) and show the resultant specific growth rate for given L:D ratios and RuBisCO:protein contributions. From this it appears that the maximum specific growth rate, under continuous illumination, appears to approach 2 doublings per day (C-specific growth rate 1.1 d^{-1}). Differences between potential specific growth rates supported by ammonium vs nitrate (f-ratio 0 vs 1) appear of consequence only at low L:D.

If we now consider a doubling of K_{cat}, to 10 s^{-1}, which is at the upper end of the documented range (Tcherkez et al., 2006; Raven et al., 2013), or a value of K_{cat} consistent with performance with a reference K_{cat} of 5 s^{-1} (as used for Fig.2) but at a temperature elevated by 10°C with Q_{10}=2, and we assume a very high 20% contribution of RuBisCO to total cell protein, then a maximum potential C-specific growth rate under continuous illumination is achieved of 2.645 d^{-1}; this equates to 3.8 doublings per day. These values are

halved under a 12:12 L:D growing on ammonium, and fall further, to 1.187 d^{-1} (i.e., 1.71 doublings d^{-1}), when using nitrate.

Finally, we consider the required K_{cat} and contribution of RuBisCO to cellular protein required to support the extremes of the temperature-growth rate envelope as given by Eppley (1972). The upper plot in Fig.3 shows the form of the Eppley equation relating temperature to microalgal specific growth rate, while the contour plots show the RuBisCO:protein contributions required to realise those growth rates under continuous illumination or in a 50:50 light:dark cycle. Much of the data space in the contour plots at elevated temperature requires implausible RuBisCO contributions to total cell protein (the maximum likely value being around 20%) or extreme values of K_{cat}. This mismatch is significant for continuous light scenarios (the conditions used for most studies reporting high growth rates), and is of course greater again for 50:50 light:dark scenarios.

Matching of potential RuBisCO-limited specific growth rates with other evidence

The fact that the primary metabolic processes of C-fixation are largely (cf. Mortain-Bertrand et al. 1988) restricted to the light phase of the diel cycle is often (surprisingly) overlooked. Some (especially older) models of phytoplankton primary production reference the daily photon dose, rather than an explicit light-dark cycle (Flynn and Fasham, 2003). In reality, growth in a L:D cycle has various obvious consequences for microalgal physiology (e.g., Rost et al., 2006) and also less obvious consequences, such as upon the potential value of these organisms as prey (Cuhel et al., 1984). Many specific growth rate estimates for microalgae living within L:D cycles are around, if not less than 0.693 d^{-1} (1 doubling per day) (Flynn et al., 2010). This is consistent with cell cycle synchronisation to the L:D cycle, with cell growth during light and division in darkness (Nelson and Brand, 1979). Such specific growth rates also appear consistent with typical cellular activities of RuBisCO (Fig.1).

There are, however, also many reports of growth rates far in excess of 0.693 d^{-1}, and some extreme values of over 5 divisions per day (Ichimi et al., 2012). The Eppley (1972) temperature-growth rate equation describes microalgal growth rates above this value of 5 divisions a day at temperatures > 28°C, and over 2 doublings per day at >14°C. (See Behrenfield and Falkowski (1997) for further discussion on temperature-production relationships.) However, from Fig.2, and considering that natural L:D periodicity at high latitudes (usually coupled with low temperature) may permit L:D photoperiods exceeding 0.5, plausible maximum C-specific growth rates would appear to peak around 1 d^{-1} (cf. Westbury et al., 2008, noting that their Fig. 4 plots division rates and not specific growth

rates). It thus seems that highly implausible configurations of RuBisCO K_{cat} and of this enzyme's contributions to cellular protein are required to enable specific growth rates that are consistent with the Eppley temperature-growth rate equation (Fig.3); certainly that must be so for growth in natural light:dark cycles.

Thus, in total, it appears from our analysis to become increasingly implausible to justify estimates of microalgal C-specific growth rates above 2 doublings per day (1.386 d^{-1}). However, there is a wide spread conviction, supported by citations to classic works such as Eppley (1972), that maximum microalgal growth rates far in excess of this are indeed possible. We thus have a conundrum; how can so many specific growth rate estimates appear to be at odds with the biochemical and stoichiometric data?

Challenges when measuring specific growth rates

Growth rates are variously reported in the literature with respect to division rates (for cells), doubling times (for population size or biomass) and specific growth rates (for biomass or components thereof). Here we are specifically concerned with C-specific growth rates (i.e., gC $(gC)^{-1}$ d^{-1}), because this defines primary production.

Most microalgal specific growth rates are reported from measurements of changes in numeric cell abundance, or of changes in bulk chlorophyll content (e.g., µg Chl L^{-1}). Eppley (1972) describes the types of measurements used to derive growth rates in the data sets he considered; they variously involve cell counts, Chl measurements, changes in biovolume, ^{15}N or ^{14}C incorporations, and he also emphasises the value of short term estimates (which are typically of necessity for work with field samples). Subsequent studies used similar methods, for example - Holt and Smayda (1974) used cell counts; Gilstad and Sakshaug (1990) use *in vivo* Chl fluorescence to study impacts of L:D periodicity on growth rates and Thompson et al. (1989) used the same approach in comparing ammonium vs. nitrate supported growth rates; Nicklisch et al. (2008) used biovolume measurements in semi-continuous turbidostats. Measurements of actual changes in C-biomass are rare. To derive primary production rates, an assumption must then made that cell-specific and/or Chl-specific growth rates are the same as C-specific growth rates. Such assumptions are often implicit; most reports do not refer to the specificity of "specific growth rate" (lacking mention of the base units, as for example cell $(cell)^{-1}$ d^{-1}, C $(C)^{-1}$ d^{-1} or Chl $(Chl)^{-1}$ d^{-1}).

It is quite possible for different approaches to the measurement of "specific growth rates" to yield different results; indeed one would expect them to do so during all but steady-state growth in continuous light. Values of cell:C (which halve and double over the cell cycle

and may also halve with light and N-limitation but double with P-limitation) and Chl:C (which varies over ca. 5 fold with nutrient status and photoacclimation) are not constants. Thus calculations of specific growth rate using these values cannot be assumed to be the same as C-specific values. Chlorophyll-specific growth rates present a particular challenge as the rates of Chl synthesis respond to increasing self-shading as population growth develops (thus Chl-specific growth rates would be higher than C-specific rate). Chl-specific growth rates may also decrease (perhaps even becoming negative) on exhaustion of nutrients, when Chl:C content can decline rapidly, giving a false impression of cell death (negative growth rates). Additional concerns can be levied against *in vivo* vs *in vitro* Chl measurements during growth into nutrient limitation (Kruskoff and Flynn, 2006). In most batch experiments, and in nature, specific growth rates (measured by what every criteria) can therefore change rapidly, and different specific rate determinations go out of synchronisation (Kruskopf and Flynn, 2006). It may be expected that both cell- and Chl- specific growth rates will exceed C-specific rates on occasion; rapid sequences of cell division with little pro rata change in biomass are possible (multiple forking in DNA replication in prokaryotes is an extreme example; the topic is discussed for eukaryote microalgae further below). In diatoms, which are often highly vacuolated and may show a decline in cell size over vegetative generations, there is additional scope for cell- and C-specific growth to become decoupled (Flynn and Martin-Jézéquel, 2000). In long-running steady-state systems, of course, physiological processes synchronise, but such systems are invariably light or nutrient-limited while we are concerned here with measuring maximum specific growth rates.

Photosynthesis rates (by C-fixation or O_2 evolution, or derived from ^{15}N assimilation), are usually estimated using short-term measurements and/or are not referenced against cell C-biomass (more often they are referenced against cell count or Chl). There is an important distinction to be made between day-average net growth rates and short term, or perhaps even instantaneous estimates of specific growth rate (values of which Eppley aspired to obtaining back in 1972, and are now arguably realisable using fluorescence techniques, though these do not measure C-fixation, only potential for that fixation – Suggett et al. 2009). For most trophic modelling and commercial interests, it is the day-average rate that is important, not short burst rates. RuBisCO activity caps even those short burst-rates of CO_2-fixation, but it is important to note that such short-term rates may not be sustainable over the longer term, especially at elevated temperature. Thus RuBisCO-defined rates may indeed match the bounds of the Eppley (1972) temperature relationship for short periods. It may perhaps be constructive for researchers to thus differentiate between "net photosynthesis rates" measured

over the short term, and reserve the term "specific growth rate" to describe estimates of C-specific biomass increase over multi-generational and/or 24hr periods.

Estimates of specific growth rate from rates of ^{14}C-inorganic C fixation over 24 h (Reynolds et al., 1985) under-estimate the requirement for RuBisCO catalysis since they do not take into account ^{14}C-inorganic C that has been assimilated by RuBisCO and that has been lost as dissolved ^{14}C-organic or respiratory ^{14}CO$_2$. What are actually needed are measurements (ideally taken over several consecutive days) of net, day-integrated, C-specific growth rate. The time-course development of C-biomass needs to be made either directly by elemental analysis of cell-C, or via estimates of biovolume (i.e., cell volume x cell abundance). The former method is subject to levels of sensitivity of typically around 10 µgC per sample; even assuming a reproducible sensitivity level for standard elemental analysis as low as 2 µgC, the initial biomass densities reported by Ichimi et al. (2012; 1.44 µgC L^{-1}) in a report of the highest documented rate of microalgal growth, would require filtration of almost 1.4 L of culture onto one filter. At levels of C sufficient for ready elemental analysis, biomass densities become increasingly likely to contribute to self-shading, thus lowering the specific growth rate potential.

In field sampling there are facets of all the above challenges to contend with, together with additional complexities of advection, vertical migration, mixed communities, and measuring net growth against predation, viral lysis, etc. Further, Chl is most often used as a surrogate for biomass in field studies, which (as we have seen above) is in itself highly problematic. There is an additional potential explanation for high specific growth rates of natural populations of "phytoplankton", and that is mixotrophy. It is now apparent that mixotrophy amongst protists that would typically be identified as "phytoplankton" on account of them containing Chl (which includes ca. 1/3rd of photic-zone micro-zooplankton) is far more common that previously considered (Flynn et al., 2013; Mitra et al., 2016). To what extent the heterotrophic input of organic C could augment the growth in nature of these Chl-containing organisms to rates above those expected from solely C-fixation is unknown.

Methods of estimating organism specific growth rates are thus complicated by many factors, but most are likely to err on the side of over estimating those rates. There are, however, data that irrefutably indicate values of 4 or so divisions per day in synchronous cultures of green microalgae (Molendijk et al., 1992; Bišová and Zachleder, 2014). These organisms undergo multiple fission events, so that a single cell generates not 2, but 4, 8 16, or even 32 daughters. Bišová and Zachleder (2014) discuss the complexity of the triggers for cell and DNA division in these organisms, including phasing of light and photosynthesis and

the need for sufficient previously accumulated readily-mobilisable C (as starch) to fuel the cell divisions. Although there is good evidence in these studies of a parallel increase in biovolume indicative of a commensurate growth in terms of biomass, actual measurements of C-biomass are absent, and the experiments are of only a few days at most. Ultimately, we do not know what the C-specific rates of growth are from such organisms. Doubt can then be cast on whether such rates of growth are meaningful in a sustained ecological, or indeed in a commercial production, context.

Challenges with interpreting the biochemical and stoichiometric data

Our calculations of the contributions of RuBisCO-C to total cell-C assume a series of well-studied stoichiometric relationships; there is likely little scope for significant error here. Of the biochemical data, the most problematic is the estimate of K_{cat} itself. This is determined under conditions of pH, temperature, substrate supply, and exclusion of inhibitory factors (notably here, the concentration of the alternative substrate for RuBisCO, namely O_2) that are chosen to be optimal for enzyme operation. Whether an *in vitro* enzyme assay reports kinetic values that are lower (suboptimal conditions *in vitro*) or higher (suboptimal *in vivo*) always presents a conundrum in biochemistry. However, recent work with bacteria (Davidi et al., 2016) suggests that in general K_{cat} values correlate well with potential *in vivo* rates.

A factor of importance in enzyme kinetics is the trade-off between enzyme K_{cat} and half saturation constant (K_m) for the substrates. Such a trade-off may be of especial importance for microalgae growing under conditions in which the supply of CO_2 for RuBisCO is limiting (Read and Tabita, 1994). Allied to this is the potential effect of temperature, if one assumes scope for a significant Q_{10} enhancement of RuBisCO activity. Whether the rest of the cellular physiology can support (for light reactions), or make best use of, such enhanced C-fixation over periods exceeding the few hours typical of enzyme assay durations is unclear. The availability of nutrients (including CO_2) may likely become limiting over the longer term.

All that said, and despite the impression given that the structural functionality of RuBisCO is essentially "frozen" (Shi et al., 2005), there are clear kinetic differences among the RuBisCO's sourced from various organisms, including among species of diatom (Young et al., 2016) and evidence of positive selection for these kinetics within microalgae (Young et al., 2012). Given that typically there is a trade-off in enzyme functionality such that K_{cat} and K_m cannot be simultaneously optimal, it is worth noting that a microalga with RuBisCO of a high K_{cat} (hence with potential to support a high specific growth rate), will exhaust nutrients

rapidly, and that includes the substrate for RuBisCO itself, as CO_2(aq); the expressed cellular activity of a high K_{cat} RuBisCO would then likely become restrained by its high K_m for CO_2. At high temperature, the decline in CO_2 solubility, even set against the activity of CO_2 concentrating mechanisms, may be expected to also counter a Q_{10}-inspired elevation of K_{cat}.

There are in addition several other factors that operate to cap specific growth rates in microalgal suspensions. Under conditions where nutrients (nitrate, ammonium, phosphate) do not limit growth directly, high growth rates will rapidly lead to biomass levels causing self-shading and light limitation, to CO_2 depletion, and also to increases in pH (basification) that also limits growth (Flynn et al., 2015). Under low-biomass conditions in nature, where self-shading and CO_2 depletion are not issues, nutrient limitation will rapidly constrain specific growth rate potentials. The structure of the enzyme, and thence the balance of K_{cat} and K_m, has itself been suggested to actually be rather well configured for the task at hand (Tcherkez et al., 2006). The implication is that while cellular RuBisCO activity may indeed closely match phototrophic growth, it may do so as a reflection of the time-integrated scope for growth set against resource limitations. Critically, resource limitation includes the implications of the self-shading that so rapidly limits the potential of cellular growth and multiplication in most microalgal suspensions. In consequence, except in biotechnology scenarios where CO_2 may be maintained at elevated levels (and inhibitory O_2 removed), and the effective optical depth minimised, any advantage in possessing a high RuBisCO K_{cat} may be expected to be short lived and hence not a strong selective characteristic.

Conclusion

Not with standing all of the discussion above, even when we combine the highest values of K_{cat} and of RuBisCO:protein, and assume continuous illumination, the maximum C-specific growth rate potential for microalgae seems to fall significantly below the higher rates of microalgal growth claimed in the literature. Mismatch between extrapolating (or integrating) short-term growth rates into net day-averaged growth rates provides an additional challenge in comparing estimates, but ultimately all that CO_2 *de facto* passes through RuBisCO.

If we assume a maximum plausible "typical" K_{cat} 10 s^{-1} and 16% RuBisCO:protein, then we obtain a maximum gross photosynthetic rate (P_{max}) of 2.645 d^{-1}; this equates to a 12:12 L:D maximum specific growth rate using ammonium-N of 1.06 d^{-1} (1.53 doubling per day). Although rates above this value may be expected in the short term at elevated temperature, one may expect K_{cat} (and with it, K_m) to become adapted in the longer term. The

implication for the long-term growth of microalgae is that maximum specific growth rates under natural illumination are likely held to significantly less than 2 doubling a day, and may be expected to adapt to much lower levels in consequence of enforced slow growth in chemostat-style growth reactors (Droop, 1974). In this context it is noteworthy that the review by Laws (Laws, 2013) suggests typical phytoplankton doubling times in tropical and temperate waters to be around 1 day. It is also interesting to return to Eppley's (1972) introductory paragraphs, where he questions whether there is a strong temperature linkage to specific growth rates; the implication is that other factors limit the maximum phytoplankton growth rate, a notion that could be argued as being borne out by the data scatter in his Fig.1 seen below the headline, and much cited, temperature-growth rate curve (Eppley, 1972).

Assuming that our analysis is correct, we suggest that estimates of C-specific growth rates in excess of those given above, derived from considerations of RuBisCO activity, may often reflect combinations of error in interpretation or misrepresentation of what is measured vs. real medium-to-long term C-specific growth rates. We do accept that these assumptions requires a belief in the extrapolation of RuBisCO activities, but we suggest that the biochemical and stoichiometric data are well founded. Either way, it is important to resolve the issue because the indiscriminate use of values of maximum specific-growth rates for microalgae in models, for example referenced to temperature via the Eppley (1972) equation, has important ramifications that need to be questioned.

Without care, the use of artificially assumed high primary producer specific growth rates in models require, or drive, a matched artificially elevated rate of secondary production and a resultant misrepresentation of events such as trophic dynamics and biological carbon pump activity. In a commercial microalgal setting, the assumption of implausible specific growth rates leads to implausible business projections. RuBisCO activity is literally and metaphorically at the centre of these issues; additional data linking RuBisCO K_{cat}, K_m, and contribution to cellular protein together with day-integrated C-specific growth rates would be of great benefit in resolving the conundrum.

References

Behrenfield M.J., Falkowski PG (1997) A consumer's guide to phytoplankton primary productivity models. *Limnol. Oceanogr*. **42**, 1479-1491.

Bišová, K., Zachleder, V. (2014) Cell-cycle regulation in green algae dividing by multiple fission. *J. Exp. Bot*. **65**, 2585–2602, doi:10.1093/jxb/ert466

Clark, D. R., Flynn, K. J., Owens, N. J. P. (2002) The large capacity for dark nitrate-assimilation in diatoms may overcome nitrate limitation of growth. *New Phytol.* **155**, 101-108.

Cuhel, R. L., Ortner, P. B., Lean, D. R. S. (1984) Night synthesis of protein by algae. *Limnol. Oceanogr.* **29**, 701-744

Davidi D., Noor, E., Liebermeister. W., Bar-Even, A., Flamholz, A., Tummler, K., Goldenfeld, M., Shlomi, T., Milo, R. (2016) Global characterization of in vivo enzyme catalytic rates and their correspondence to in vitro k_{cat} measurements. *Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.1514240113

Droop, M. R. (1974) The nutrient status of algal cells in continuous culture. *J. Mar. Biol. Assoc. U. K.* **54**, 825–855.

Ellis, R. J. (1979) Most abundant protein in the world. *Trends Biochem. Sci.* **4**, 241-244.

Eppley, R. W. (1972) Temperature and phytoplankton growth in the sea. *Fish. Bull.* **70**, 1063–1085.

Flynn, K. J., Fasham, M. J. R. (2003) Operation of light-dark cycles within simple ecosystem models of primary production and the consequences of using phytoplankton models with different abilities to assimilate N in darkness. *J. Plankt. Res.* **25**, 83-92.

Flynn, K. J., Martin-Jézéquel, V. (2000) Modelling Si-N limited growth of diatoms. *J. Plankt. Res.* **22**, 447-472.

Flynn, K. J., Clark, D. R., Owens, N. J. P. (2002) Modelling suggests that optimization of dark nitrogen-assimilation need not be a critical selective feature in phytoplankton. *New Phytol.* **155**; 109-119.

Flynn, K. J., Raven, J. A., Rees, T. A. V., Finkel, Z., Quigg, A., Beardall, J. (2010) Is the growth rate hypothesis applicable to microalgae? *J. Phycol.* **46**, 1-12 DOI: 10.1111/j.1529-8817.2009.00756.x

Flynn, K. J., Stoecker, D. K., Mitra, A., Raven, J. A., Glibert, P. M., Hansen, P. J., Granéli, E., Burkholder, J. M. (2013) Misuse of the phytoplankton-zooplankton dichotomy:

the need to assign organisms as mixotrophs within plankton functional types. *J. Plankt. Res.* **35**, 3-11 *doi:10.1093/plankt/fbs062*.

Flynn, K. J., Clark, D. R., Mitra, A., Fabian, H., Hansen, P. J., Glibert, P. M., Wheeler, G. L., Stoecker, D. K., Blackford, J. C., Brownlee, C. (2015) Ocean acidification with (de)eutrophication will alter future phytoplankton growth and succession. *Proc. Roy. Soc. B* **282**, 20142604 http://dx.doi.org/10.1098/rspb.2014.2604

Gilstad, M., Sakshaug, E. (1990). Growth rates of ten diatom species from the Barents Sea at different irradiances and day lengths. *Mar. Ecol. Prog. Ser.* **64**, 169-173.

Halsey, K. H., Jones, B. M. (2015) Phytoplankton strategies for photosynthetic energy allocation. *Annu. Rev. Mar. Sci.* **7**, 265–297 doi: 10.1146/annurev-marine-010814-015813

Holt, M. G., Smayda, T. J. (1974) The effect of daylength and light intensity on the growth rate of the marine diatom *Detonula confervacea* (Cleve) Gran. *J. Phycol.* **10**, 231-237.

Hopkinson, B. M., Young, J. N., Tansik, A. L., Binder, B. J. (2014) The minimal CO_2-concentrating mechanism of *Prochlorococcus* spp. MED4 is effective and efficient. *Plant Physiol.* **166**, 2205-2217

Ichimi, K., Kawamura, T., Yamamoto, A., Tada, K., Harrison, P. J. (2012) Extremely high growth rate of the small diatom *Chaetoceros salsugineum* isolated from an estuary in the eastern Seto inland sea, Japan. *J. Phycol.* **48**, 1284–1288 DOI: 10.1111/j.1529-8817.2012.01185.x

Kenny, P., Flynn, K. J. (2014) In silico optimization for production of biomass and biofuel feedstocks from microalgae. *J. Appl. Phycol.* **27**, 33-48 doi 10.1007/s10811-014-0342-2

Kruskopf, M., Flynn, K. J. (2006) Chlorophyll content and fluorescence responses cannot be used to gauge reliably phytoplankton biomass, nutrient status or growth rate. *New Phytol.* **169**, 525-536.

Laws, E. A. (2013) Evaluation of in situ phytoplankton growth rates: a synthesis of data from varied approaches. *Ann. Rev. Mar. Sci.* **5**, 247-268. DOI: 10.1146/annurev-marine-121211-172258

Mitra, A., Flynn, K. J., Tillmann, U., Raven, J. A., Caron, D., Stoecker, D. K., Not, F., Hansen, P. J., Hallegraeff, G., Sanders, R., Wilken, S., McManus, G., Johnson, M., Pitta, P., Vågen, S., Berge, T., Calbet, A., Thingstad, F., Jeong, H. J., Burkholder, J-A., Glibert, P. M., Granéli, E., Lundgren, V. (2016) Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition; incorporation of

diverse mixotrophic strategies. *Protist* **167**, 106–120.
http://dx.doi.org/10.1016/j.protis.2016.01.003

Mortain-Bertrand, A., Descolas-Gros, C., Jupin, H. (1988) Patterns of dark inorganic carbon fixation in two species of diatoms: influence of light regime and regulator factors on diel variations. *J. Plankt. Res*. **10**, 199-217.

Molendijk, A. J., Van Egmond, P., Haringf, M. A., Klis, F. M., van den Ende, H. (1992) Characterization of the cell cycle in synchronous cultures of *Chlamydomonas eugametos* in relation to gametogenesis. *J. Gen. Microbiol.* **138**, 1941 - 1947.

Nelson, D. M., Brand, L. E. (1979) Cel1 division periodicity in 13 species of marine phytoplankton on a 1ight:dark cycle. *J. Phycol.* **15**, 67-75

Nicklisch, A., Shatwell, T., Köhler, J. (2008) Analysis and modelling of the interactive effects of temperature and light on phytoplankton growth and relevance for the spring bloom. *J. Plankt. Res.* **30**, 75-91.

Raven, J. A. (2013a) Rubisco: still the most abundant protein in Earth? *New Phytol*. **198**, 1-3.

Raven, J. A. (2013b) RNA function and phosphorus use by photosynthetic organisms. *Frontiers Plant Sci*. **4**: Article 536.

Raven, J.A. (2015) Implications of mutation of organelle genomes for organelle function and evolution. *J. Exp. Bot.* **66,** 5639-5650.

Raven, J. A., Ralph, P. J. (2015) Enhanced biofuel production using optimality, pathway modification and waste minimization. *J. Appl. Phycol.* **27**, 1-31. doi: 10.1007/s10811-0140323-

Raven, J. A., Beardall, J., Larkum, A. W. D., Sánchez-Baracaldo, P. (2013) Interactions of photosynthesis with genome size and function. *Phil. Trans. Roy. Soc. B* **368**, 20120264,

Read, B. A., Tabita, F. R. (1994) High substrate specificity factor ribulose bisphosphate carboxylase/oxygenase from eukaryotic marine algae and properties of recombinant cyanobacterial RuBisCO containing "algal" residue modifications. *Arch. Biochem. Biophys*. **312,** 210 - 218.

Reynolds, C. S., Harris, G. P., Gouldney, D. N. (1985) Comparison of carbon-specific growth rates and rates of cellular increase of phytoplankton in large limnetic enclosures. *J. Plankt. Res.* **7**, 791-820.

Rost, B., Riebesell, U., Sültemeyer, D. (2006) Carbon acquisition of marine phytoplankton: Effect of photoperiod length. *Limnol. Oceanogr*. **51**, 12–20.

Shi, T., Bibby, T. S., Jiang, L., Irwin, A. J., Falkowski, P. G. (2005) Protein interactions limit the rate of evolution of photosynthetic genes in cyanobacterial. *Mol. Biol. Evol.* **22**, 2179-2189.

Smith, V. H., McBride, R. C. (2015) Key ecological challenges in sustainable algal biofuels production. *J. Plankt. Res.* **37**, 671-682. doi: 10.1093/plankt/fbv053

Suggett, D. J, MacIntyre, H. L., Kana, T. M., Geider, R. J. (2009) Comparing electron transport with gas exchange: parameterising exchange rates between alternative photosynthetic currencies for eukaryotic phytoplankton. *Aquat. Microbial Ecol.* **56**, 147-162.

Tcherkez, G. G., Farquhar, G. D., Andrews, T. J. (2006) Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *Proc. Natl. Acad. Sci. USA* **103**, 7246-51.

Thompson, P. A., Levasseur, M. E., Harrison, P. J. (1989) Light-limited growth on ammonium vs. nitrate: What is the advantage for marine phytoplankton? *Limnol. Oceanogr.* **34**, 1014-1024.

Westberry, T., Behrenfeld, M. J., Siegel, D. A., Boss, E. (2008) Carbon-based primary productivity modelling with vertically resolved photoacclimation. *Glob. Biogeochem, Cycles* **22**, GB2024, doi:10.1029/2007GB003078

Young, J. N., Rickaby, R. E. M., Kapralov, M. V., Filatov, D. A. (2012) Adaptive signals in algal Rubisco reveal a history of ancient carbon dioxide. *Phil. Trans. Roy. Soc. B* **367**: 483-492.

Young, J. N, Heureux, A. M. C., Sharwood, S. E., Rickaby, R. E. M., Morel, F. M. M., Whitney, S. M. (2016) Large variation in the Rubisco kinetics of diatoms reveals diversity among their carbon-concentrating mechanisms. *J. Exp. Bot.* doi:10.1093/jxb/erw1633

Zorz, J. K., Allanach, J. R., Murphy, C. D., Roodvoets, M. S., Campbell, D. A., Cockshutt, A. M. (2015) The RUBISCO to photosystem II ratio limits the maximum photosynthetic rate in picocyanobacteria. *Life* **5**, 403-417; doi:10.3390/life5010403

Figure Legends

Fig. 1. Matrix plot across a range of light:dark (L:D) values and RuBisCO K_{cat} values, showing the proportion of cellular protein required as RuBisCO protein in order to support a microalgal net C-specific growth rate of 0.693 d^{-1} (1 doubling per day). The relationship is shown both for growth supported by nitrate-N (f-ratio 1) and ammonium-N (f-ratio 0); there is only a ca. 10% difference between them which is not readily discernible against the log-axis used for the RuBisCO protein contribution.

Fig. 2. Matrix plot across a range of light:dark (L:D) values and RuBisCO:protein contributions, assuming a RuBisCO K_{cat} of 5 s^{-1}, showing the resultant microalgal net C-specific growth rate. The relationship is shown both for growth supported by nitrate-N (f-ratio 1) and ammonium-N (f-ratio 0); the differences between these is only of consequence at low L:D.

Fig. 3. Values of the RuBisCO contribution to total cell protein required to enable the microalgal specific growth rates projected by the Eppley (1972) temperature-growth rate relationship for a given expressed value of RuBisCO K_{cat}. These contributions are computed assuming ammonium to be the N-source, and under either continuous illumination (L:D 1) or with light supplied in a 50:50 light:dark cycle (L:D 0.5). The Eppley relationship is shown in the upper plot. The contour plots show red where the RuBisCO contribution to total cell protein exceeds 0.5; values in the literature are suggestive of a maximum of ca. 0.16.
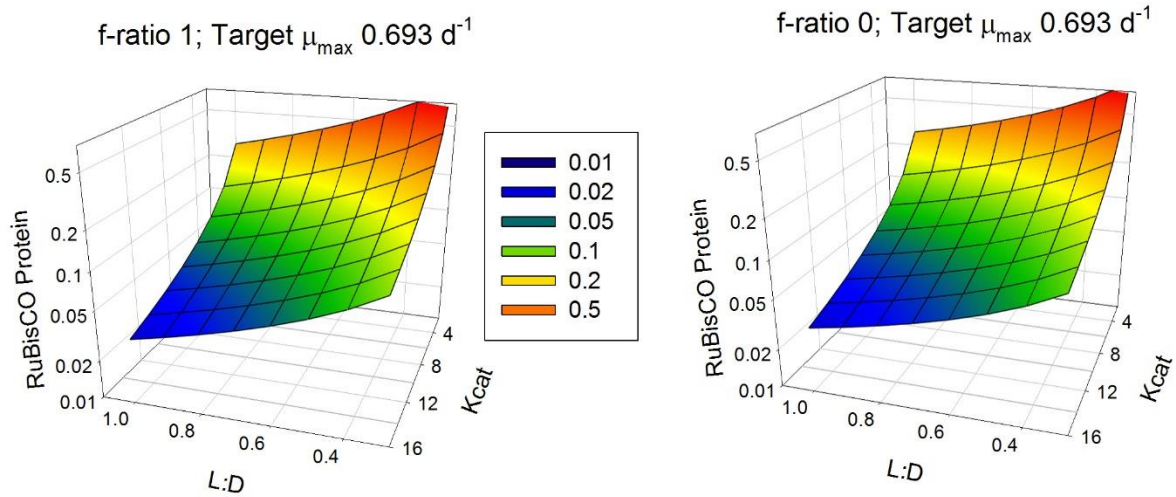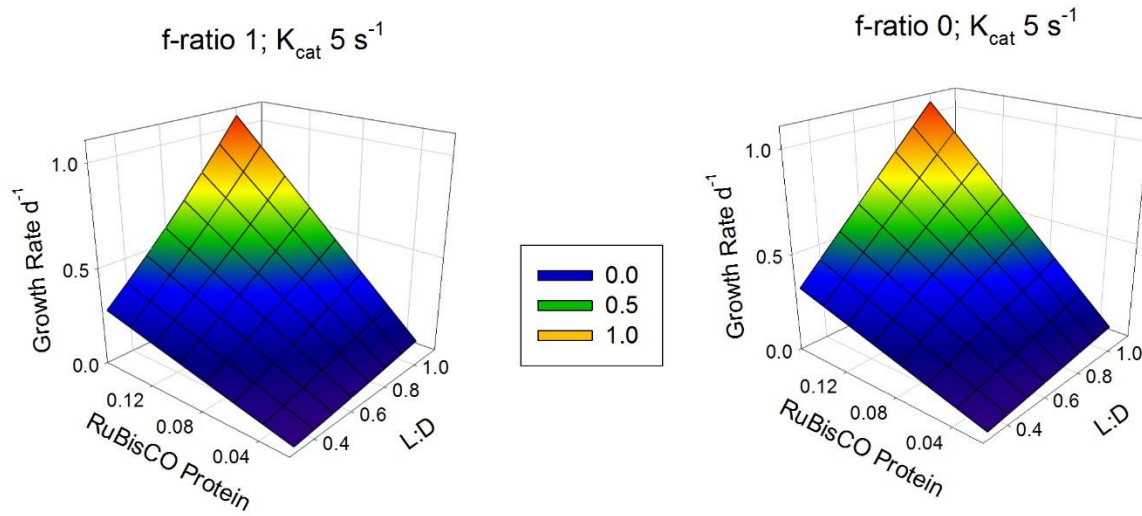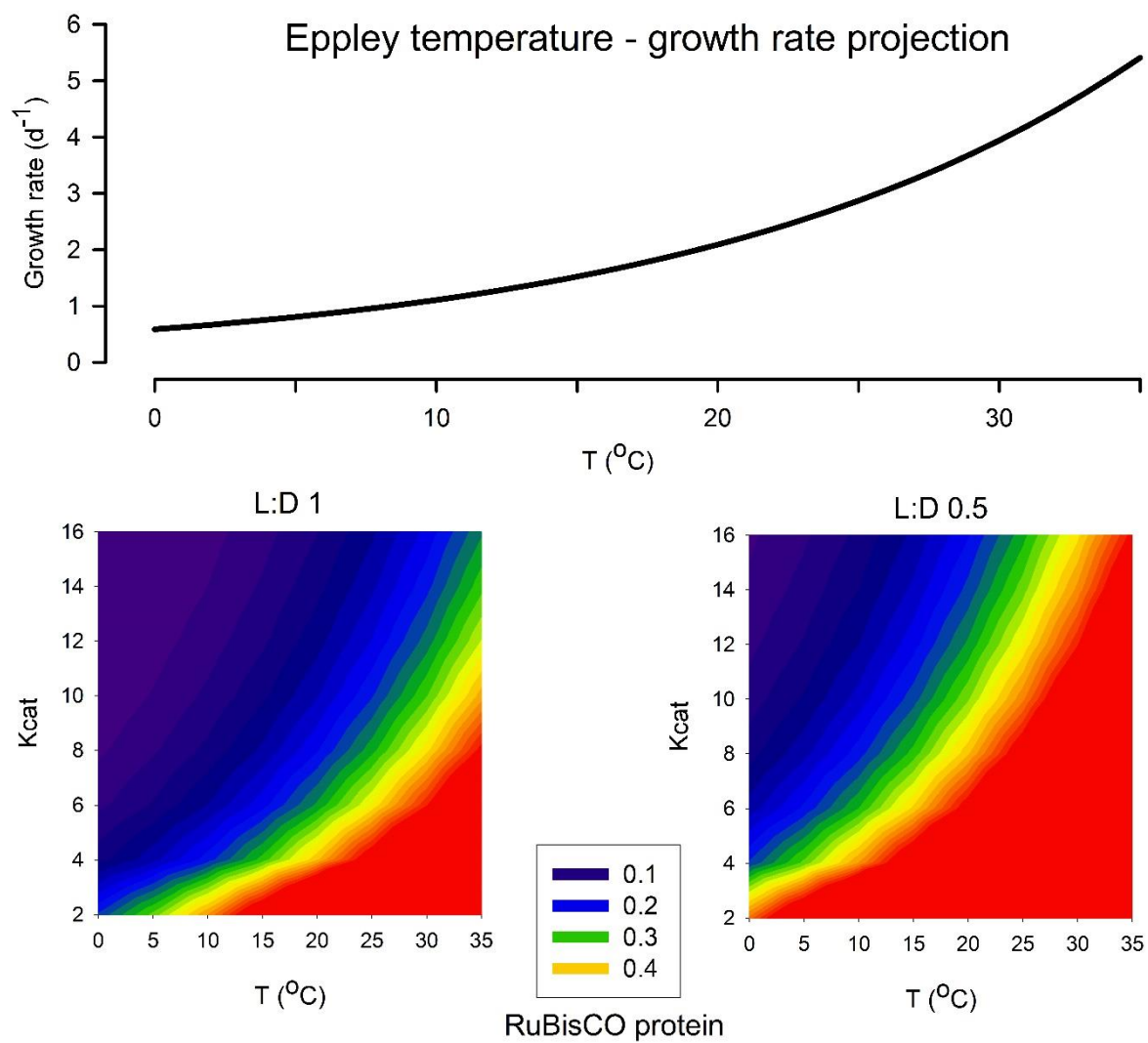
f-ratio 1; Target μ_{max} 0.693 d^{-1}

f-ratio 0; Target μ_{max} 0.693 d^{-1}

Fig.1

f-ratio 1; K_{cat} 5 s^{-1}

f-ratio 0; K_{cat} 5 s^{-1}

Fig.2

Fig.3