Errata and re-visitation of 'What is the limit for photoautotrophic plankton growth rates?' Flynn and Raven (2017)

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ABSTRACT

An error in our original work prompts a revisitation of factors constraining photoautotrophic plankton growth rates (μ_{max}). RuBisCO does not itself provide that constraint, but we identify other factors that result in our previously suggested value of ca. 2 doublings per day still likely being representative of the maximum for most photoautotrophs. μ_{max} likely evolves to balance the advantage of possessing a high competitive value while minimising the stresses incurred when the organism is incapable of routinely achieving a higher μ_{max} due to various limiting factors. Organisms with extreme high μ_{max} are thus expected to grow under conditions that provide the necessary environment (stable pH, non-limiting nutrients and light) for sufficient time that the evolution of higher μ_{max} becomes advantageous. Conditions in nature allowing the evolution of higher μ_{max} include the exploitation of an exceptional opportunity and then entering stasis (e.g., desert microalgae), or a situation where high grazing pressures match high phytoplankton growth thus maintaining non-limiting nutrient and light conditions. The latter, however, conflicts with the paradox of enrichment, as only under resource limitation would the necessary stability be attained in the predator-prey dynamic. Ultimately ecology, not biophysics, constrains phototroph μ_{max}.

Keywords: phytoplankton, mixoplankton, cyanobacteria, maximum growth-rate, photosynthesis, evolution

INTRODUCTION

In our previous paper (Flynn and Raven 2017), we argued that a major pinch point limiting the ultimate growth potential of phototrophic plankton (broadly equivalent to 'microalgae': Raven and Beardall, 2022) is the activity of RuBisCO, the primary enzyme involved in the fixation of CO_2. Ed Laws and Alex McClellan, working on factors limiting the growth of the cyanobacterium *Synechococcus* (Laws and McClellan, 2022), have brought to our attention an error in our analysis. Through neglecting to account for the fact that K_{cat} values for enzyme activity are reported per active site (of which RuBisCO has 8) and not per molecule, our calculations are too pessimistic, and RuBisCO is thus unlikely to ultimately constrain phototrophic growth rate. Several other developments and reconsiderations on this general topic of factors limiting photoautotrophic plankton growth have emerged since our 2017 paper that impact the consequence of this error for interpretations of both natural plankton and commercial microalgal growth.

Through a consideration of the maximum C-specific content of RuBisCO per cell, and the calculated activity of the enzyme, the sustained maximum specific rate of phototrophic growth (μ_{max}) suggested by Flynn and Raven (2017) is in the range of 1.3 d^{-1}. The recent work of Laws and McClellan (2022) established a maximum growth rate of 4.5 d^{-1} at 30-35°C for the cyanobacterium *Synechococcus*. Accounting for the high temperature used in that study by assuming Q_{10}=2, at 20°C this equates to a maximum growth rate of 1.6-2.25 d^{-1}, a value that is not too dissimilar to that we proposed in 2017. However, this rate is ca. 4-8× too low if it was to be limited by the potential RuBisCO activity, raising the question of what else other than the quantity of RuBisCO is constraining growth.

RuBisCO AND THE PHOTOTROPHIC LIMITATION OF GROWTH

Organism growth is ultimately limited by either the supply of resources, or the exploitation of those resources for biosynthesis and replication. In the bacterium *Escherichia coli*, it appears that ribosomal synthesis sets the ultimate limit (Belliveau et al., 2021). Microbial plankton, however, invariably live in resource-limiting environments, while biosynthesis commencing from inorganic substrates (as in photoautotrophy) inevitably also incurs additional costs compared to the osmotrophic growth of heterotrophs such as *E. coli*. The data in Weissman et al. (2021) suggest that there is no systematic difference in prokaryote versus eukaryote microbial growth rate potential in the upper ocean; we can thus ignore differences between these groups in our search. We are thus left with two ways to explain the excess amount of RuBisCO activity relative to μ_{max}. One is that the in vivo (i.e., effective) RuBisCO K_{cat} value is much lower than is the in vitro value; that could reflect a suboptimal substrate availability as CO_2 at the enzyme, and/or the inhibition of CO_2-fixation by rising concentrations of O_2 that may be expected to be increasingly problematic at a higher (non-limiting) irradiance. The other explanation is that RuBisCO activity is not the ultimate limiting factor and that any relationship between the cellular content of RuBisCO and μ_{max} is emergent; that is to say, the amount of RuBisCO is modulated via (de)repression to balance supply and demand for products of C-fixation. These two explanations are not mutually exclusive, and the latter may be expected to be functional in any case.

Laws and McClellan (2022) went to considerable lengths (using a high-dilution, continuous culture approach) to enable growth at maximum rates for *Synechococcus* over many generations while maintaining optimal nutrient, light and other conditions. This contrasts with typical laboratory batch experiments (starting with an inoculum of ca. 2-5% of the final abundance), in which exponential growth is only possible for a few generations; much of the time is spent with cells either recovering from, or entering into, periods of stress. If the supply of nutrients is not limiting, the extent of phytoplankton proliferation itself controls the growth rate through the light limitation caused by community self-shading. In addition, in the absence of high rates of aeration,

the pH of the water rapidly rises to deleterious levels coincident with the decline in availability of CO_2(aq) as the substrate for RuBisCO; there is then an increase in demand for (and potential limitation by) the activities of DIC uptake systems other than CO_2 diffusive entry (Clark and Flynn 2000; Huertas *et al*., 2000).

Factors other than resource availability also appear to control the potential for photosynthesis to limit phototrophic growth, as can be seen from studies of phytoplankton growth under different light-dark cycles and different daily photon doses. A simple interpretation of phototrophy would see a broadly linear relationship between the non-saturating daily photon-dose and growth rate. In reality, growth in a light:dark cycle versus that in continuous light at the same light-phase photon flux density does not show pro rata differences in daily growth rate, and the relationship between the light:dark cycle, the irradiance, and growth rate is complex (e.g., Eppley and Coatsworth, 1966; Paasche, 1968; Durbin, 1974; Iriate and Purdie, 1993; Sommer, 1994; Tang and Vincent, 2000). The data presented in Fig. 1, for example, show how the growth rate supported by a given daily photon dose varies depending on the acclimation of the organism. The potential for CO_2-fixation is modulated by factors related to other facets of physiology and cell cycle duration (Nelson and Brand, 1979); μ_{max} is not constrained simply by the maximum rate of photosynthesis.

OTHER LIMITATIONS AFFECTING SELECTION FOR HIGH PHOTOTROPHIC GROWTH RATES

Could diffusion-limitation of nutrient supply to the cell surface for transport constrain growth at very high rates? Using the approaches described in Flynn *et al*. (2018), in Fig. 2 we show the calculated external substrate concentrations required to supply 2× the half saturating nutrients for growth at different doubling times. It should be noted, that these calculations assume continuous light; in nature in a light:dark cycle the uptakes of DIC and to a large extent also of dissolved inorganic N would be confined to the light phase, requiring transport and assimilation rates for

growth in a 50:50 light:dark cycle of twice that calculated. Hence, for growth in a 50:50 light:dark cycle the values in Fig. 2 for a doubling time of 2 per day would require bulk water concentrations as indicated for 4 doublings per day. Half saturation values for growth limited by different resources are difficult to measure (Clark and Flynn 2000; Flynn *et al*., 2018), but it is clear that organism with cells below ca. 10 µm in diameter are far less likely to be subjected to significant nutrient limitation due to diffusion from plausible bulk water nutrient concentrations in natural eutrophic conditions. That is especially true for motile cells, but in culture non-motile cells are still subjected to high levels of turbulence in an aerated systems that would decrease the boundary layer thickness. The cost of motility is estimated at only a fraction of 1% of total cell energy expenditure (Raven and Lavoie, 2022); the nutritional gain far outweighs the cost for enhancing the growth rate. While for individual cells of size ca. <10 µm supply of macro nutrients is not likely to be limiting to phototrophic growth even to rates of 5.54 d^{-1} (8 divisions per day), the situation would be very different for colonies or clumps of cells, and especially if such aggregations are surrounded by mucus. For these aggregations, and for larger solitary cells, growth at doubling times above ca. 2 per day ($\mu > 1.4\ d^{-1}$), may require concentrations of DIN and DIP that are high relative to natural environmental levels. In consequence, there would be little selective pressure in evolving higher growth rate potentials as resource limitation would occur.

Further evidence that μ_{max} is not controlled simply by the potential for phototrophy is that different strains of the same phytoplankton species have different μ_{max}, and that there is a great spread in μ_{max} observed in different phytoplankton of a given cell size (e.g., Finkel *et al*., 2010; Lynch *et al*. 2022). There is also a very wide range of μ_{max} values for microbial plankton (heterotrophs, mixotrophs, phototrophs, prokaryote and eukaryote) with temperature and geographic spread (Rose and Caron, 2007; Weissman et al., 2021). This indicates that possessing a high μ_{max} comes at a cost, else why are otherwise comparable phytoplankton (same functional group, genus, or even members of the same species) incapable of expressing high growth rates under what are assumed to be optimal conditions?

Evidence for a general trait-trade-off between the competitive advantage of an organism possessing a high growth rate potential versus the physiological stress incurred in being unable to realise that potential, comes from various sources (e.g., Droop, 1974; Arendt, 1997; Monaghan *et al*., 2009; Dmitriew, 2011). Though log-log regressions through growth rate data may provide insights to explain ecological functionalities, from the wide spread of data values there are clearly factors that confound simple interpretation. Using a plankton model to explore a trade-off between the benefits and costs of processing a high μ_{max} (Flynn and Skibinski, 2020), after many iterations of interaction with the zooplankton that ate them and also regenerated the nutrients required for the continued growth of individual phytoplankton cells, and also decreased self-shading of the phytoplankton community, the value of phytoplankton μ_{max} evolved to an optimum that reflected resource supply and demand. Starting with a μ_{max} for phytoplankton >5 d^{-1} in these simulations, μ_{max} evolved down to around a value of 2 d^{-1}. Evolution at a higher temperature (within bounds of lethality) eventually led to an evolved lower expressed μ_{max} at the reference temperature for both phytoplankton and zooplankton, largely cancelling out Q_{10} effects (Flynn and Skibinski, 2020).

If the evolution of the growth rate potential (which would modulate the maximum rate of photosynthesis) is indeed involved in constraining phototrophic μ_{max}, as we expect from culture work (Droop, 1974; Zhang *et al*., 2021), then under what conditions could natural populations of phytoplankton grow over a sufficiently long period such that μ_{max} would evolve to a high value? To provide a suitable stable 'fast' environment would require that the high phytoplankton growth rates are countered by stable high rates of zooplankton grazing and growth, and thence phytoplankton growth would be supported of fast nutrient recycling activities. These conditions are required to prevent self-shading and/or nutrient limitation of the phytoplankton growth, conditions that are akin to those provided by a high-dilution culture regime in the laboratory as used by Laws and McClellan (2022). However, the stability in predator-prey interactions required to keep a thin phytoplankton suspension is most apparent in low resource systems (Rosenzweig, 1971); high nutrient loads promote oscillations in predator-prey dynamics. Low resource systems are by

definition nutrient-limiting conditions that would inevitably constrain phytoplankton growth. It is also not plausible for a stable high-growth-rate predator-prey system to operate in nature over many months to support evolution of an extreme high μ_{max}, especially when set against the vagaries of the weather (notably affecting light), and the emergent differences in growth rate potential for phototrophs and their predators (Rose and Caron, 2007; Flynn and Skibinski, 2020; Pulsifer and Laws, 2021). The greater, and variable, mixed layer depths of natural bodies of water, even with a low Chl content, would also inevitably restrict light availability.

There is an alternative evolutionary mechanism – emerge and grow very rapidly when conditions are good, and then enter stasis as soon as conditions deteriorate. Thus, the extreme growth rate potential ($> 12\ d^{-1}$) seen in the desert microalga *Chlorella ohadii* (Ananyev *et al*., 2017) is only sustained for a few hours; it exploits the temporary availability of moisture enabled by the unique functioning of this organisms' photosystems and a disconnect between the potential for photosynthesis and the potential for cell growth. Such high growth rates would doubtless exhaust nutrients very quickly, and growth is in any case restricted by a co-occurring and competing cyanobacterium that aids the *Chlorella*'s rehydration cycle (Kedem et al., 2021).

Ultimately then, the selective pressure for the evolution of high phototrophic growth rates in natural populations of phytoplankton, and microalgae in general, may be expected to be restrained by combinations of abiotic and biotic (ecological) factors associated with light and/or nutrient limitations. These factors include interactions with zooplankton. The evolution of a higher growth rate potential is of no advantage as it cannot be expressed for long enough in nature to compensate for the intervening periods of stress. In nature a plankton phototrophic growth rate exceeding our previously suggested maximum (Flynn and Raven, 2017) is unlikely; this rate converted into a depth-integrated rate is also consistent with that observed at upwellings (Sarmiento and Gruber, 2006).

COMMERCIAL GROWTH OF MICROALGAE AND MIXOTROPHY

The work of Kenny and Flynn (2017) on microalgal biofuels production assumed maximum growth rates, informed by Flynn and Raven (2017), as high as 3 d^{-1}. Kenny and Flynn (2017) concluded that, from an economic standpoint, microalgal biofuels would only become viable if productivity increased ca.10 fold. Ostensibly the above noted ×8 error in RuBisCO reaction-rate calculations provides that potential for increased productivity. This view would be strengthened if the performance of RuBisCO could be enhanced from its assumed low efficiency (Tcherkez *et al*., 2006). However, a wide ranging analysis of different enzymes (Bar-Even *et al*., 2011) provides evidence that RuBisCO is actually a rather average enzyme, with kinetic characteristics in line with expectations and hence that there is less scope for artificial enhancement than may have seemed possible. The real physiological limitation in commercial phototrophic microalgal production is related to light harvesting for photosynthesis during growth in the extremely high density (and thus self-shading) cell suspensions necessary to provide high areal production rates (i.e. 10's gC m^{-2} d^{-1}) to make culturing and harvesting cost effective. This challenge can be mitigated by using modified microalgal strains with a restricted maximum Chl:C (Beckmann *et al*., 2009; Kenny and Flynn, 2017); the catch is that the inevitable appearance of an elevated Chl:C in mutants will enhance the competitive advantage of those mutated individual cells, and eventually lead to an increased self-shading of the collective.

Running the microalgal production decision support tool of Flynn (2021), we see that increasing values of μ_{max} give diminishing returns on productivity, topping out at around a value for μ_{max} ca. 4 d^{-1} (depending on the operational conditions used to culture and harvest the biomass). However, biofuels production is usually optimised by growth under nutrient limitation (Kenny and Flynn, 2017); we must expect cultured microalgae forced to grow slowly in such a regime to gradually evolve such that their μ_{max} will decline (Droop, 1974). To maintain maximum productivity will require a periodic complete restart of the culture systems with the optimised seed.

We have specifically referenced features limiting 'photoautotrophy' in the discussion above to discriminate against the growth of microalgae (co-)supported through heterotrophy. Uptake of sugars, amino acids and other low molecular weight dissolved organics, is likely ubiquitous across prokaryote and eukaryote microbes (e.g., Muñoz-Marín *et al*., 2020; Godrijian *et al*., 2021; Meyer *et al*., 2022), although to what extent in microalgae this just provides a recovery mechanism (Flynn and Berry, 1999) against the well documented leakage of metabolites (Biddanda and Benner, 1997; Wetz and Wheeler, 2007) is unclear. The leakage of DOC can be extreme; Larsson *et al*., (2022) report that 50-70% of CO_2-fixation is released as mucus and other DOC by the mixoplanktonic dinoflagellate *Prorocentrum* cf. *balticum* – the slow growth rate of this species (ca. doubling every 2 days) totally belies the specific rate of photosynthesis. In commercial culture, heterotrophy (via osmotrophy) can be used to compensate for self-shading (even to the extent of allowing high growth rates in darkness; Gladue and Maxey, 1994; Zaslavskaia *et al*., 2001; Harel and Place, 2004), but of course production is no longer totally autotrophic, being partly mixotrophic, and thus conflicts with the desire to maximise primary production of microalgal biomass. Likewise, phagotrophy in mixoplankton (phototrophic protists that can eat – Flynn *et al*., 2019) provides an additional nutritional route. Mixoplankton are typically not fast growing organisms, dominating as they do in mature ecosystems where growth rates are typically slower (Mitra *et al*., 2014), with their growth rates likely reflecting also the selective advantage of lower values of μ_{max}.

CONCLUSION

Taken together with our (corrected) calculations, a revised view develops that sees factors other than RuBisCO activity as the pinch point in plankton phototrophic potential. Factors related to the matching of resource supply and physiological demand, with consequential evolution balancing competitive advantage against stress of possessing a high μ_{max}, appear most likely to limit primary production and phototroph growth in nature. In microalgal cultivation, the 'selfish genes' of

228 photoacclimation, that enhances growth of the individual but self-shades the collective, appears as
229 the critical factor.

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FIGURE LEGENDS

Fig.1 Growth of *Emiliania huxleyii* at different irradiance (photon flux density, PFD, of 10, 26, 60 or 102 µmol photons m^{-2} s^{-1}) delivered in different light:dark periods (L:D; where 1 is continuous light). Panel (a) shows growth rates, while panel (b) shows efficiency as growth rate per daily photon dose (d^{-1}×(mol m^{-2} d^{-1})$^{-1}$). Original data sourced from Paasche (1967). The initial elevation in efficiency as L:D increases in low PFD cultures most likely reflects the changing relative importance of basal respiration rate upon growth. Otherwise, though, efficiency falls as the contribution of the light period in the L:D cycle increases, and decreases in cells acclimated to growth in higher PFD, demonstrating that growth is not simply related to photosynthesis even at low (non-saturating) PFD.

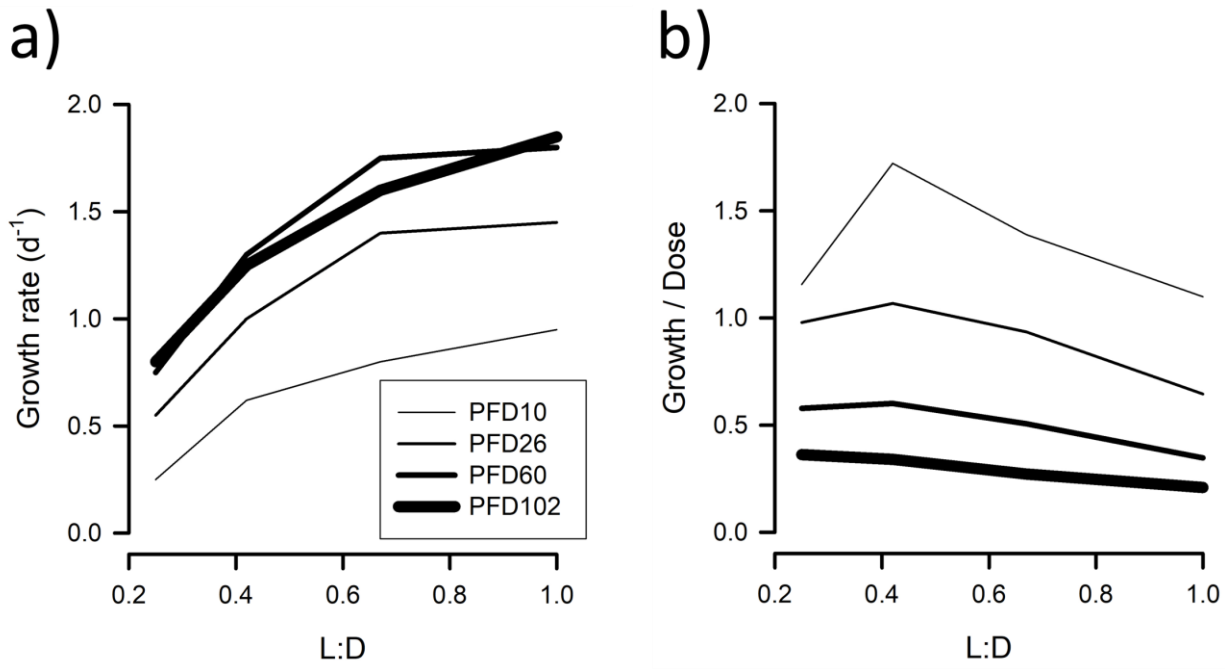

Fig. 2 Interaction between doublings per day and bulk water substrate concentration required to supply near-cell substrate values of 2× the half saturation constant for growth (K_G) for different sized cells (ESD, µm). Assumed values of K_G were 100, 1 and 0.1 µM for DIC, DIN and DIP respectively; the greatest relative range is seen for DIN (panel b). For each panel, the plot is shown for non-motile and motile cells. Growth was modelled assuming continuous irradiance. Motility was computed according to Flynn and Mitra (2016), as v (µm s^{-1}) = $38.542 \times ESD^{0.5424}$; this gives a swimming speed relative to cell size (v/ESD) ranging from ca. 23.3 at ESD 3 and ca. 4.7 at ESD 100µm.