Supporting Information

Tansley review: Allometry and stoichiometry of unicellular, colonial and multicellular phytoplankton

Overview

Here we provide supplementary discussion of topics relating to Sections II, III, IV, V and VI of the main text:

Section II The motility of colonies and the differentiation of multicellular phytoplankton into specialized cell types are discussed in relation to morphology.

Section III Specific symbiotic relationships are described, including Kleptoplasty.

Section IV Cell shape is discussed in relation to the physical constraints mentioned in Section IV in the main text.

Section V The ecological stoichiometry of phytoplankton is expanded with consideration of the elemental content of giant unicells, the carbon content of mucilage and the possibility of storing other elements in it, the phosphorus content of ribosomes and the possible scaling effects on iron and manganese content.

Section VI Further discussion is provided on size scaling of growth rate in unicellular and colonial phytoplankton, emphasizing the need for the data used in such comparisons to be obtained under the same, preferably optimal conditions, and comparison of scaling in phytoplankton with that in other organisms.

Section II

Colonies

Most planktonic colonial forms are non-motile. This is always the case for filamentous colonies, invariably the case for the cyanobacteria (filamentous *Planktothrix*, which can show gliding motility on a solid surface but not when suspended in water ; spheroidal in *Microcystis*), and is the predominant situation for colonial eukaryotes. In marine environments, volumes can exceed 10^{11} μm^3 for spheroidal colonies of the haptophyte *Phaeocystis* and 10^{13} μm^3 for mats of the marine diatom *Rhizosolenia* (Shipe and Brzezinski 1999). In freshwater environments, colonies of the chlorococcoid green alga *Hydrodictyon reticulatum* (Chlorophyceae) can be 10 cm wide and 1 m long (van den Hoek *et al.,* 1995; Graham & Wilcox, 2000) (Table 2). The freshwater chlorococcoid green algae *Pediastrum* and *Hydrodictyon africanum* (Pocock, 1960) form mat-like colonies whereas spherical-cylindrical colonies are formed by *H. reticulatum*. The filaments, and *Phaeocystis*, do not have a fixed number of cells; *Pediastrum* and *Hydrodictyon africanum* (Pocock, 1960) have 2^n cells, while the cell number in *Hydrodictyon reticulatum* may be more variable. The extent to which *Hydrodictyon* can be regarded as planktonic is debatable; the large colonies are denser than water and they occur resting on sediment in shallow bodies of freshwater, but could be at least temporarily suspended by wind-induced mixing.

Multicellular Organisms

Heterocystous cyanobacteria are widespread but are not free-living organisms in tropical oceans (Stal *et al.,* 2003), although the heterocystous *Richelia* occurs as a diatom symbiont in tropical oceans (Burford *et al.,* 1995). Stal *et al.* (2003) propose that the use of a heterocyst to protect nitrogenase may become uncompetitive relative to the strategy employed by *Trichodesmium*, as warmer temperatures decrease O_2 concentrations at standard seawater salinities; the reverse argument applies to cooler and less saline seas. The high intracellular O_2 concentration in large photosynthesizing diatoms (Raven & Larkum, 2007) is proposed as the rationale for the occurrence of *Richelia* as a symbiont in the tropical ocean (Stal *et al.,* 2003).

The two flagella in *Volvox* cells protrude through holes in the cell walls, and the basal body of each flagellum also serves in nuclear division as a centriole; both flagella occur at the apical end of the cell. Since centrioles must occur at opposite ends of the cell for mitosis to proceed, the assumed rationale for cell differentiation of *Volvox* is that flagellar motility is incompatible with cell division in this organism. Thus, germ line cells give rise to new cells, which undergo cell division to produce new colonies within the parent colony, while somatic cells use their flagella to keep the parent colony in the euphotic zone. This evolutionary sequence has occurred at least four times in the polyphyletic genus *Volvox*, which is perhaps better described as an evolutionary

grade (Kirk, 2005). *Volvox* arose from colonial ancestors with fewer cells (4–16 in *Gonium*, 8–16 in *Pandorina* and *Stephanosphaera*) and very limited evidence of differentiation (van den Hoek *et al.,* 1995). However, some cells of the 16-64 found in *Eudorina* colonies have larger eyespots than do the others and could be thought of as representing an early stage of multicellularity in that a degree of division of labour and signalling among differently equipped cells may be involved (Van den Hoek *et al.,* 1995).

Section III

In the upper mixed layer of the surface ocean, members of the heterotrophic phylum Cercozoa (i.e. Acantharia, Foraminifera, Radiolaria) are often symbiotic with photosynthetic dinoflagellates, and occasionally symbiotic with Prymnesiophytes or Chrysophytes, *sensu lato* (Jørgensen *et al.,* 1985; Angel, 1991; Caron *et al.,* 1995; Gast & Caron, 1996). Unlike the other symbionts considered here, cercozoans are mineralised: acantharians have $SrSO_4$, foraminiferans have $CaCO_3$, and most radiolarians have SiO_2. In the oligotrophic ocean, almost all acantharians, and about half the species of Foraminifera and Radiolaria, have symbionts at some stage in their life cycle (Caron *et al.,* 1995). The volumetric-size ranges reported by Caron *et al.* (1995) for cercozoans are 10^6–10^{11} μm^3 for Acantharia, 10^8–10^{10} μm^3 for Foraminifera, and 10^9–10^{13} μm^3 for unicellularand 10^7–10^{14} μm^3 for colonial Radiolaria (Table 2). These estimates correspond to maximum ESDs of 2.2 mm for Acantharia, 1.5 mm for Foraminifera, 10.2 mm and 4.8 mm for unicellular Radiolaria and colonial Radiolaria. Caron *et al.* (1995) state in the same paper, however, that colonial Radiolaria can form gelatinous cylinders that are *centimeters* in diameter and over a metre in length.

Kleptoplasty is a means of introducing photosynthesis into a non-photosynthetic organism that does not involve the presence of complete photosynthetic cells as symbionts. The marine ciliate *Myrionecta rubra* (synonymous with *Mesodinium rubrum*) obtains photosynthate from cryptophyte kleptoplastids which it has obtained from cryptophyte food items. In some cases, these kleptoplastids can function over a relatively long period (weeks and more). In view of the very small fraction of the genes needed to maintain the full suite of plastid proteins it would be expected that the plastid activity would decay faster than is observed. It has recently been shown that the genes required for plastid function that are missing from the plastid genome are present in photosynthetic *Myrionecta* due to the retention of transcriptionally active cryptophyte nuclei (kleptonuclei), thus making the association closer to a symbiosis (Johnson *et al.,* 2007). The symbiotic and kleptoplastidic ciliates, foraminiferans, radiolarians and acoels are phagotrophic as well as photosynthetic.

This version of mixotrophy is also found in many non-symbiotic unicellular freshwater and marine photosynthetic flagellates (e.g. Bell & Laybourn-Parry, 2003; Laybourn-Parry & Marshall, 2003), as well as in the colonial freshwater flagellate *Dinobryon* (Raven, 1997). There are also ecologically important mixotrophic symbioses of planktonic ciliates with *Chlorella* (Laybourn-Parry *et al.,* 1997; Sand-Jensen *et al.,* 1997; Modenutti & Balseiro, 2002; Woelfl & Geller, 2002; Modenutti *et al.,* 2005). The best studied of these is probably the *Chlorella–Paramecium bursaria* association.

The only multicellular planktonic organisms with photosynthetic symbionts are acoel flatworms, which are turbellarians < 1 mm in length that proliferated prior to evolving symbioses with photosynthetic organisms. Symbiotic acoels are, in the words of Stoecker *et al.* (1989), "widespread, though sporadic". Like the littoral and benthic acoel flatworm *Convoluta*, they have prasinophytes and (less frequently) dinoflagellates as photosynthetic symbionts.

A case might be made for regarding the algae (and kleptoplastids) as colonies contained within the cells of an alveolate or cercozoan. However, there are many differences between symbioses and colonies of a single species. For example, in the great majority of cases, there is no vertical transmission of symbionts, so that fresh algae (or plastids) have to be obtained anew from the environment in each generation. This opens the possibility of a mixture of symbiont genotypes in a host, contrasting with the clonal nature of algal cells in "real" colonies.

Section IV

Lewis (1976) presented evidence that the axial ratio is greater for larger unicells both within genera and among higher taxa, although unicellular phytoplankton cells with the largest volumes (*Coscinodiscus* and *Ethmodiscus*) are centric diatoms that are compact and cylindrical in shape. Determinants of diatom shape, and the range of shapes that are possible, are not well understood, especially granted the constraints of the rigid frustules with their "semi-conservative" replication (van den Hoek *et al.,* 1995). For diatoms, the lower surface-to-volume quotients for the largest cells, which are just > 1 mm ESD, are partially offset by extensive vacuolation (Raven, 1987). The axial ratio , together with vacuolation, has impacts for all of the physical constraints and processes mention in Section IV, i.e. intracellular transport, diffusion boundary layers, package effect, vertical movement and turgor pressure and turgor-resisting walls.

Section V

Work on the nitrogen and phosphorus subsistence quotas of cells of unicellular phytoplankton organisms, and of cells from colonies, as a function of cell volume shows a decrease in nitrogen and phosphorus per unit volume as cell volume increases (Shuter, 1978). However, the cell volume range covered in Shuter's analysis is only 10^3-fold (4–4.10^3 μm^3), so that the largest cells considered are still 10^{-6} the volume of *Ethmodiscus.*

Villareal *et al.* (1999) examined the C, N and Si contents of the vertically migrating giant-celled diatom *Ethmodiscus* from the Central North Pacific gyre, and found C:N ratios higher than the Redfield ratio; they pointed out that this is typical of vertically migrating phytoplankton cells that take up nitrate (and other nutrients) at depth and move them to nearer the surface where there is more PAR available (see also the arguments in Clark *et al.,* 2002). The Si content of *Ethmodiscus* on a cell volume basis was close to what is predicted from extrapolation to larger volume of the Si:cell volume relationship for a number of diatoms with a range of cell volumes that are much smaller than that of *Ethmodiscus* (Villareal *et al.,* 1999), i.e. $biovolume^{-0.87}$ (Brzezinski, 1985) – $biovolume^{-0.91}$ (Conley *et al.,* 1989). Further metabolic implications of cyclic vertical migrations in diatoms and in flagellates are discussed by Clark *et al.* (2002), Flynn (2002), Flynn *et al.* (2002) and Needoba & Harrison (2004).

Villareal *et al.* (2007) examined the N, P and Fe composition of *Ethmodiscus* along a 3000 km transect in the Central North Pacific gyre. The N:P ratio was close to the Redfield ratio, and there was little alkaline phosphatase activity, suggesting no P-limitation; Fe content expressed on a cytoplasmic volume basis was as expected for a Fe-sufficient organism. Villareal *et al.* (2007; cf. Woods & Villareal, 2008) suggest that there is a high vacuolar organic C content, based on the need for low-density organic osmolytes to generate the low vacuolar density of these (at least when ascending) buoyant cells (Boyd & Gradmann, 2002). However, analysis of inorganic ions in the vacuolar sap of the positively buoyant giant-celled diatom *Ethmodiscus rex* and the positively buoyant photosynthetic large-celled dinoflagellate *Pyrocystis noctiluca*, as well as the giant-celled non-photosynthetic dinoflagellate *Noctiluca scintillans* shows that buoyancy can be explained in terms of the composition of the vacuolar sap (Beklemishov *et al.,* 1961; Kahn & Swift, 1978). Here there is no need to invoke organic osmolytes in the generation of buoyancy and, where examined, the ionic components account for the observed osmolarity of the sap, so there is no room for organic solutes either (Kahn & Swift, 1978).

Cells of colonies of cyanobacteria such as *Microcystis* are embedded in mucilage; if this consists only of polysaccharide the mucilage would add to the C relative to mucilage-free unicell (e.g. Wu & Song, 2008) but would not increase the N or P content relative to corresponding mucilage-free unicells unless these nutrients are stored in the mucilage. Thus the elemental stoichiometry (C:N:P) of *Microcystis* colonies would be affected relative to its unicellular equivalent. *Phaeocystis* colonies, by contrast, are contained within a pellicle whose mechanically tough layer may be < 1 μm thick (Hamm *et al.,* 1999) though the overall thickness may be 7 μm (van Rijssel *et al.,* 1997), and has pores with an effective radius for solute diffusion of <4.4 nm (Hamm *et al.,* 1999). Building the *Phaeocystis* colony requires relatively little extracellular polysaccharide (van Rijssel *et al.,* 1997), so that the colonies are more like "bags of water"' than "balls of jelly" (Hamm *et al.,* 1999).

For *Phaeocystis*, colony size of field populations is related to cell numbers and carbon and nitrogen content (Verity *et al.,* 2007). Plots of log cell number against colony volume had a slope of 0.54, so there is a significant decrease in number of cells per unit volume as the colony size increases. Corresponding slope values for carbon and nitrogen were 0.92 and 1.22, so that carbon per unit volume decreased slightly with colony volume, while nitrogen per unit volume increased with colony volume (Verity *et al.,* 2007). Since the carbon and nitrogen content per cell increased as colony size increased, data are consistent with the occurrence of very significant extracellular carbon and nitrogen accumulations (Verity *et al.,* 2007), contrasting with the suggestion of a "bag of water" proposed by van Rijssel *et al.* (1997) and Hamm *et al.* (1999) for the structure of *Phaeocystis* colonies.

There seem to be very few data on the elemental stoichiometry of planktonic photosynthetic symbioses, although there are data on the organic and inorganic carbon content of two benthic photosynthetic foraminifera (Ter Kuile & Erez, 1991). For nitrogen and phosphorus, Uhle *et al.* (1999) resorted to the Redfield ratio in analysing nitrogen isotope data, and computing nitrogen and phosphorus uptake, in the planktonic symbiotic foraminiferan *Orbulina universa.*

In addition, there have been suggestions of a more general relationship between cell metabolism and the need for particular elements as a function of growth rate and organism size. Sterner & Elser (2002) summarise the evidence as to the correlation of specific growth rate with biomass-based rRNA content of the organism or, as, a surrogate, the biomass-based phosphorus content; this is the basis of the so-called "Growth Rate Hypothesis". The argument here is that (maximum) growth rate is ultimately limited by the rate of protein synthesis via the content of ribosomes. While this applies to some non-photosynthetic micro-organisms and metazoan, little of the available evidence on phytoplankton is immediately consistent with it (Raven *et al.,* 2005). There is little explicit mention of organism size and specific growth rate relationships in Sterner & Elser (2006): more work is needed on this, not least for phytoplankton. There are predictions of the content of iron and maganese content of the photosynthetic apparatus as a function of organism size and the package effect (Raven, 1990); more testing of these is also needed.

Section VI

Sarthou *et al.* (2005) analysed the size scaling of the specific growth rate of diatoms and found that the rate scales with the -0.13 power of the cell volume. This analysis considered volumes from 13 to $7x10^5$ μm^3, and did not include the very largest diatoms, but the estimates of Villareal *et al.* (1999) for *Ethmodiscus* agree with an extrapolation of this relationship. Maranon (2008) found scaling factors for growth rate as a function of cell volume that are never as low as -0.25 for growth rate and sometimes not significantly different from zero for natural assemblages of a number of higher phytoplankton taxa. However, the use of natural populations means that there are variations in the extent to which organisms are resource-saturated or resource limited, greatly complicating interpretation of the data (Finkel & Irwin, 2000; Finkel, 2001; Finkel *et al.,* 2004). There may also be decreases in the specific growth rate at the very smallest sizes (Bec *et al.,* 2008). In addition there is the matter of top-down size-dependent relationships, which may also interact with phytoplankton physiological status (Mitra & Flynn, 2006), which have potential to overturn size-related autecological arguments.

Nielsen (2006) examined the size-dependence of specific growth rate of unicells and filamentous cyanobacterial colonies, and of unicells, colonies and a multicellular organism that span 4 classes of green algae (Charophyceae, Chlorophyceae, Prasinophyceae and Trebouxiophyceae). Together these data encompass a wide range of structures and taxa, cells that divide by binary and multiple fission, flagellate and non-flagellate forms, and even *Prototheca*, a non-photosynthetic relative of *Chlorella*. For unicells, Nielson (2006) found that specific growth rate scales as about the -0.25 power of the minimum external cell dimension. For colonies, specific growth rate appeared to be independent of size, although there was significant scatter. It would be interesting to know the light regimes of the treatments because, if any of the data came from cultures not growing at light saturation, this could affect the scaling exponent (Finkel & Irwin, 2000; Finkel, 2001; Finkel *et al.,* 2004).Indeed, all such comparisons need to be conducted under similar or

optimal conditions of temperature, light and nutrient regime. There are no comprehensive data sets that permit such comparisons, and in consequence it is difficult to judge just how sound our understanding is.

It is also of interest to investigate how specific growth rate scales in unicells and cells in colonies of the same species. For instance, Wilson *et al.* (2006) compared 32 isolates of the colonial cyanobacterium *Microcystis aeruginosa*, and found a positive correlation of specific population growth and the mean colony surface area; this is, of course, the opposite of the 'normal' inverse relationship between specific growth rate and organism/colony size described by Nielsen (2006). It is also contrary to expectations based on boundary layer effects, as discussed above. Shorter-term investigations of unicells and colonies from a number of strains of *Microcystis aeruginosa* have been carried out by Shen & Song (2007) and Wu & Song (2008). Colonies had significantly higher resource-saturated photosynthetic rates on a chlorophyll *a* basis, though there were no significant differences in the values of the initial slope of increase in photosynthetic rate per unit increase in incident photon flux density at low irradiance (α), of light compensation (E_c) or of the light saturation parameter (E_k). Although the chlorophyll *a* per unit volume of medium was greater for unicells than for colonies over 18 days of culture, the data provided do not permit calculation of chlorophyll *a* per cell or per dry matter, or the growth rate on anything but a chlorophyll *a* basis. The ETR_{max} values cited were not corrected for the absorptance of unicells relative to colonies, so the larger difference between unicells and colonies than for resource-saturated photosynthetic rates measured as O_2 evolution could be a function of a higher absorptance of the colonies. The other datum obtained by Wu & Song (2008) was that of external inorganic C dependence of photosynthetic O_2 evolution, showing that the inorganic C affinity was greater in colonies than in unicells. This work shows that, at least for the inorganic C dependence of photosynthesis, colonies are much closer to the limits imposed by diffusion boundary layers than are unicells, observations that are consistent with the possible increased diffusion limitation of CO_2 supply in *Trichodesmium* as colony size increases (Tchernov & Lipschiltz, 2008). It is not clear how significant this effect is on the fitness of the organisms concerned.

Gao & Ai (2004) and Li & Gao (2004) examined the effect of colony size on growth, photosynthetic and respiratory parameters of the heterocystous filamentous cyanobacterium *Nostoc* spp. According to our definition, this species forms colonies comprised of many multicellular organisms. When this terrestrial species was grown in culture, there was an inverse relationship between colony size and specific growth rate, light-saturated photosynthetic and respiratory rates on a dry matter basis, and the light saturation parameter and the light compensation point, but no significant effect of colony size on the chlorophyll content per dry matter (Gao & Ai, 2004; Li & Gao, 2004).

Veldhuis *et al.* (2005) examined the specific growth rate of cells of the haptophyte *Phaeocystis*, which has free-living single cells and colony-forming cells as components of the life cycle, with cells 4–7 μm diameter and colonies up to 10 mm diameter. They showed that the specific growth rate (on a cell basis) of cells in colonies was 1.5–3.8 times that of single cells in *Phaeocystis globosa* and *Phaeocystis pouchetti*: it is not clear how this relates to the possible preference of unicells for NH_4^+, an N-source likely to be present at much lower concentrations than the NO_3^- used by colonies (Riegman & van Boeckjel, 1996). Thus, the various physico-chemical reasons for lower growth rates of cells in colonies, as opposed to unicells, as have been suggested for growth under resource-limiting conditions (i.e. package effect, boundary layer effect), clearly did not apply in *Phaeocystis* any more than in *Microcystis.* Indeed, for these organisms the specific growth rate is faster for larger rather than smaller colonies, and also for colonies rather than unicells. However, for *Nostoc,* small colonies grew faster than large colonies; it is not clear if this difference from *Microcystis* and *Phaeocystis* is a function of phylogeny, or of the terrestrial habitat of the *Nostoc* strain used. Specific growth rates can also be expressed on a cell basis (whether in a colony or as a unicell, see Veldhuis *et al.,* 2005), and in these terms the "penalty" in maximum specific growth rate of living in a colony seems to be small relative to the expected effect on growth rate of enlarging individual cells to the size of the colony.

Overall, the rather limited literature seems to be consistent in showing that there is a smaller dependence of resource-saturated specific growth rate on an organism biomass basis for colonial and multicellular organisms than for 'comparable' unicells.

There appear to be no relevant data on the specific growth rate as a function of organism size for symbiotically photosynthetic planktonic organisms (Raven 2004).

Of relevance to the question of scaling growth rates with colony size is work on colonies of sessile benthic marine metazoans; the examples used here lack photosynthetic symbionts, though these are common in (especially) tropical cnidarian corals and ascidian urochordates. Hughes & Hughes (1986) showed no effect of colony size on the growth and growth rates of the constituent zooids of the ectoproct *Electra pilosa*, whereas Nakaya *et al.* (2003) showed that the colony respiration rate of the ascidian *Botrylloides simodensis* scales as -0.25 with colony size. Nakaya *et al.* (2002) suggested that the difference between the two colonies is that those of the zooids of the ectoproct are in a rigid skeletal box, with relatively little communication between them, while the zooids of the ascidians are joined by a common vascular network. Edmonds (2006) also draws attention to the occurrence of both isometry and allometry in colonial modular invertebrates, and adds phenotypic effects of environmental variations to the determinants of the rate: colony size relationship. This is illustrated by his work over a decade on the growth of juvenile scleractinian corals *in situ*, with isometric growth in warm years and positive allometry in cool years (Edmonds, 2006).

For unitary multicellular zooplankton the dependence of specific growth rate on body size often fits reasonably well with a -0.25 scaling exponent, e.g. for copepods (Hirst & Sheader, 1997).

A final comment on allometry is the basis on which the growth rates are compared. For modeling purposes growth on a C basis is generally more appropriate. However, a per cell (genome) basis may be more relevant for studies of the determinants of the mechanism(s) underlying differences in growth rate.

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