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Review

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Allometry and stoichiometry of unicellular, colonial and multicellular phytoplankton

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Summary

Phytoplankton life forms, including unicells, colonies, pseudocolonies, and multicellular organisms, span a huge size range. The smallest unicells are less than 1 μm^3 (e.g. cyanobacteria), while large unicellular diatoms may attain 10^9 μm^3, being visible to the naked eye. Phytoplankton includes chemo-organotrophic unicells, colonies and multicellular organisms that depend on symbionts or kleptoplastids for their capacity to photosynthesize. Analyses of physical (transport within cells, diffusion boundary layers, package effect, turgor, and vertical movements) and biotic (grazing, viruses and

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other parasitoids) factors indicate potential ecological constraints and opportunities that differ among the life forms. There are also variations among life forms in elemental stoichiometry and in allometric relations between biovolume and specific growth. While many of these factors probably have ecological and evolutionary significance, work is needed to establish those that are most important, warranting explicit description in models. Other factors setting limitations on growth rate (selecting slow-growing species) await elucidation.

I. Introduction

Photosynthetic planktonic organisms are extremely diverse with respect to taxonomy, growth form, and size. This polyphyletic group includes cyanobacteria, eukaryotes with chloroplasts derived from cyanobacterial endosymbionts (Raven *et al.*, 2005a,b), photosynthetic eukaryotes with diazotrophic (i.e. nitrogen-fixing) cyanobacterial endosymbionts (e.g. the diatom *Hemiaulus*), nonphotosynthetic ciliates that consume and maintain algae internally as kleptoplastids (e.g. *Myrionecta*), and nonphotosynthetic eukaryotes with photosynthetic symbionts (e.g. Radiolaria). In this review, we consider how the size, morphology, and elemental composition of phytoplankton relate to their functional biology, including traits such as growth rate, predation and resource requirements. Table 1 shows that, while unicellular representatives are found in all higher phytoplankton taxa, colonies are rather less common; very large unicells are restricted to three higher taxa, while multicellular organisms only occur in two higher taxa and pseudocolonies (apparently the result of genetically determined incomplete cell division) are restricted to the Dinophyceae. Understanding the constraints imposed by size, morphology and composition can help elucidate the relative advantages (and/or disadvantages) of being unicellular, colonial or multicellular. A major aspect of our analysis is the comparison of unicellular organisms, including those that are very large relative to the majority of phytoplankton organisms, with pseudocolonies, colonies and multicellular organisms. Ecological and evolutionary implications associated with the allometry and stoichiometry of these forms will also be considered. Our goal is to understand whether constraints imposed by size, morphology and composition can elucidate the relative advantages associated with being unicellular, colonial or multicellular. From a practical point of view, such information is also of value in determining the description of plankton function types and size structures within mathematical models of plankton ecology.

II. Unicellular, pseudocolonial, colonial and multicellular phytoplankton: definitions, taxonomy and morphology

1. Unicellular organisms

All phytoplankton groups have unicellular species (Table 1) whose size can vary by > 9 orders of magnitude in body volume (Table 2), from < 1 μm^3 (an equivalent spherical diameter (ESD) of < 1 μm) for the cyanobacterium *Prochlorococcus* and

Table 1 Occurrence of unicells, pseudocolonies, colonies and multicellular organisms in major taxa of phytoplankton organisms

Phylum	Class	Common name	Unicell	Very large unicell	Pseudo-colony	Colony	Multi-cellular
Cyanobacteria	Cyanobacteria	Cyanobacteria	+			+	+
Chlorophyta	Charophyceae		+			+	
Chlorophyta	Chlorophyceae	Green algae	+			+	+
Chlorophyta	Prasinophyceae	Green algae	+	+		+	
Chlorophyta	Trebouxiophyceae		+			+	
Dinophyta	Dinophyceae	Dinoflagellates	+	+	+		
Haptophyta	Pavlovophyceae		+				
Haptophyta	Prymnesiophyceae	Coccolithophores	+			+	
Heterokontophyta	Bacillariophyceae	Diatoms	+	+		+	
Heterokontophyta	Chrysophyceae		+			+	
Heterokontophyta	Eustigmatophyceae		+				
Heterokontophyta	Synurophyceae		+			+	

Information is from Kahn & Swift (1978), Van den Hoek *et al*. (1995), Kirk (1998), Villareal *et al*. (1999), Graham & Wilcox (2000), Kaiser (2001) and Hoppenrath & Leander (2007). Large unicells are defined as cells with a volume > 10^7 μm^3; equivalent spherical diameter (ESD) >267 μm.

Table 2 Maximum sizes of unicellular, pseudocolonial, colonial, and multicellular photosynthetic planktonic organisms

Structure	Organism	Volume (μm^3)	Reference
Unicellular	*Prochlorococcus*	10^0	Ting *et al*. (2007)
Unicellular	*Ostreococcus*	10^0	Courties *et al*. (1994)
Unicellular	Coccolithophore	10^4	Van den Hoek *et al*. (1995)
Unicellular	*Pyrocystis* (dinoflagellate)	10^7	Kahn & Swift (1978)
Unicellular	Prasinophyte phycoma	10^9	Van den Hoek *et al*. (1995)
Unicellular	*Ethmodiscus* (diatom)	10^9	Villareal *et al*. (1999)
Unicellular	Foraminiferan + photobiont	10^{10}	Caron *et al*. (1995)
Unicellular	Acanthophoran + photobiont	10^{11}	Caron *et al*. (1995)
Unicellular	Radiolarian + photobiont	10^{13}	Caron *et al*. (1995)
Pseudocolonial	Dinoflagellate *Polykrikos*	10^4	Hoppenrath & Leander (2007)
Colonial	Cyanobacterium *Microcystis*	10^7	Van den Hoek *et al*. (1995)
Colonial	Cyanobacterium *Trichodesmium*	10^{11}	Van den Hoek *et al*. (1995)
Colonial	Diatom *Rhizosolenia*	10^{13}	Shipe & Brzezinski (1999)
Colonial	Radiolarian + photobiont	10^{14}	Caron *et al*. (1995)
Colonial	Chlorophycean *Hydrodicyton*	10^{16}	Van den Hoek *et al*. (1995)
Multicellular	Cyanobacterium *Anabaena*	10^3	Van den Hoek *et al*. (1995)
Multicellular	Chlorophycean *Volvox*	10^9	Kirk (1998)

Values for *Prochlorococcus* and *Ostreococcus* represent the minimum cell sizes of cyanobacteria and eukaryotes, respectively.

the eukaryotic green alga *Ostreococcus* (Prasinophyceae), to over 10^9 μm^3 (ESD > 1 mm) for the diatom (Bacillariophyceae) *Ethmodiscus* (Courties *et al*., 1994; Moore & Villareal, 1996; Villareal *et al*., 1999). Among photosynthetic eukaryotes, the division Chlorophyta (green algae), class Prasinophyceae, includes both the smallest free-living eukaryote, *Ostreococcus* (ESD = 0.95 μm; Courties *et al*., 1994), and taxa with volumes of nearly 10^9 μm^3 in the nonmotile, phycoma stage (Van den Hoek *et al*., 1995; Dotzler *et al*., 2007). By contrast, coccolithophores, class Prymnesiophyceae, never exceed 10^4 μm^3 in volume (Van den Hoek *et al*., 1995). In the phylum Dinoflagellata, the largest dinoflagellates with flagella have volumes of 10^5 μm^3 (Menden-Deuer & Lessard, 2000). The cell size range can be extended another 4 orders of magnitude by including nonphotosynthetic eukaryotes with photosynthetic symbionts, such as Foraminifera (10^{10} μm^3), Acanthophora (10^{10} μm^3), and Radiolaria (10^{13} μm^3).

2. Pseudocolonial organisms

Typically dinoflagellates possess two flagella; one (the transverse flagellum) may be contained in a groove-like structure around the equator of the organism (the cingulum) and provides forward motion and spin to the dinoflagellate, and the other (the longitudinal flagellum) trails behind, acting as a rudder, and provides little propulsive force. Colonies of the marine dinoflagellate *Polykrikos*, however, are best regarded as 'pseudocolonies' because they result from a series of incomplete cell divisions (Table 1). One consequence of these incomplete divisions is that the total number of flagella does not match the number of nuclei in the coenocytes (Van den Hoek *et al*., 1995; Hoppenrath & Leander, 2007). The resulting multi-nucleated organisms can have volumes of up to 10^4 μm^3 (Table 2).

3. Colonial organisms

Colonial organisms occur in the cyanobacteria, Bacillariophyceae, Charophyceae, Chlorophyceae, Chrysophyceae, Pavlovophyceae and Prymnesiophyceae (Table 1). These colonies consist of aggregates of well-differentiated, morphologically identical cells of the same genotype that form one-dimensional filaments (also referred to as chains), two-dimensional mats (also referred to as plates), or three-dimensional cylinders, spheres, or amorphous structures (Van den Hoek *et al*., 1995; Graham & Wilcox, 2000). Colonial organisms can be as small as a few μm in ESD, or many orders of magnitude larger. Aspects of nonmotile and motile colonies are considered further in the Supporting Information (Text S1).

Motile colonies comprise freshwater representatives from three algal classes – Chlorophyceae, Chrysophyceae and Synurophyceae – and freshwater ciliates with photosynthetic symbionts (Sand-Jensen *et al*., 1997). Chrysophyte and synurophyte colonies are spherical, with a highly variable number of cells. Chlorophytes can be in mats (*Gonium*) or spheroidal (e.g. *Pandorina*), and have a fixed (2^n) number of cells. Some nonmotile colonies (e.g. *Hydrodictyon* and *Phaeocystis*) also have motile unicells as part of their life cycle (Van den Hoek *et al*., 1995; Graham & Wilcox, 2000).

4. Multicellular organisms

Here we define multicellular organisms as having many cells of the same genotype, in which there is some level of

morphological differentiation and division of labour among cell types (Kirk, 1998, 2005; Kaiser, 2001). There are few examples of multicellular photosynthetic plankton defined in this way. We consider two here: the Nostocalean cyanobacteria, and *Volvox* in the Chlorophyceae (Table 1).

Nostocalean cyanobacteria comprise ~5-μm ESD cells, which form filaments that can be hundreds of μm long (Table 2). In *Nostoc* (which is typically free-living in freshwater benthos or on land, and is also symbiotic), aggregations of the muliticellular organisms form attached or unattached 'balls' on land and in water (Dodds *et al.*, 1995). Filaments consist of vegetative cells responsible for photosynthesis, heterocysts (cells responsible for nitrogen fixation), and (in some cases) resting-stage cells referred to as akinetes. Heterocysts maintain low-oxygen environments internally, allowing nitrogen fixation by oxygen-sensitive nitrogenase reductase–nitrogenase complexes to proceed even in the light when adjacent vegetative cells are photosynthesizing. By contrast, filamentous colonies of the nonheterocystous marine diazotroph *Trichodesmium* appear to maintain the low oxygen concentrations necessary for nitrogen fixation without cell differentiation in the strict sense (Berman-Frank *et al.*, 2003). However, it should be noted that even single filaments of *Trichodesmium* are capable of fixing nitrogen in the light (Milligan *et al.*, 2007) without recourse to a colonial form, possibly through the formation of micro-anaeobic zones between and within tightly packed cells. We consider factors limiting the distribution of heterocystous cyanobacteria in the Supporting Information (Text S1).

Volvox is a flagellate genus of green algae that forms hollow spherical colonies up to 1 mm in diameter, made up of (approximately) 2^n cells (*c.* 500 to several thousand) (Table 2). Cell differentiation in *Volvox* is related to colony size; multicellularity is only achieved by large colonies, which comprise somatic and germline cells. Large colonies are denser than their freshwater environments, and would sink below the euphotic zone in the absence of flagella (Kirk, 1998, 2005, 2006; Raven, 1998b; Solari *et al.*, 2006a,b), which allow diel vertical excursions of over 20 m. The evolution of migration in *Volvox* is considered in the Supporting Information (Text S1).

III. Symbioses

Unicellular and colonial forms of photosynthetic plankton sometimes maintain symbioses. For example, the marine diatoms *Rhizosolenia* (unicellular or colonial) and *Hemiaulus* (colonial) can obtain at least a fraction of their nitrogen requirements through the nitrogen-fixing activities of cells of the diazotrophic cyanobacterium *Richelia* that they have incorporated as (probably) vertically transmitted endosymbionts (Janson *et al.*, 1999). Other planktonic organisms are photosynthetic because they have photosynthetic symbionts. Among ciliates, mixotrophs of unicellular (e.g. *Stentor* spp.: Laybourn-Parry *et al.*, 1997; Woelfl & Geller, 2002; Modenutti *et al.*, 2005) and colonial (*Ophrydium versatile*: Sand-Jensen *et al.*, 1997; *Ophrydium naumanni*: Modenutti & Balseiro, 2002) forms are sometimes symbiotic with *Chlorella*, and can be the dominant primary producers in some inland water bodies. In the ocean there are photosynthetic symbioses of some planktonic acantharia, acoels, foraminifera and radiolarians, and a photosynthetic kleptoplastidic ciliate (see Supporting Information (Text S1) for more details on symbioses).

IV. Physical constraints on size, morphology and motility

1. Intracellular transport

All cells must deal with the problem of intracellular transport. Unicellular phytoplankton have a minimum cell size of just 1 μm^3, which is perhaps proximally influenced by ecological factors, but ultimately dictated by nonscalable aspects of cell structure (Raven, 1998a). Examples of nonscalable structures that could restrict further decreases in size to much below 1 μm^3 are proteolipid bilayer membranes based on C_{16}–C_{18} fatty acids (hence *c.* 8 nm thick), genomes which, for photolithotrophs, probably cannot have fewer than *c.* 1400 genes, and ribosomes. Individual proteins are also nonscalable but would not restrict decreases in cell size until much smaller hypothetical cell sizes. As cell size increases, the surface-to-volume quotient decreases and the average transport distance within the cell increases, so diffusion becomes increasingly inadequate as a means of maintaining constant solute concentrations throughout the cytosol of the cell (Nobel, 2005). The influence of cell shape on intracellulular transport is considered in the Supporting Information (Text S1).

The largest diatom cells, along with the largest desmid cells (charophyceans which are a few tens of μm thick and 0.4 mm in diameter), lack the capacity to distribute solutes using cytoplasmic streaming. Both groups have a single nucleus that is located near the centre of the cell. For diatoms, it is in the centre of the vacuole and connected to the peripheral cytoplasm by cytoplasmic strands. The movement of nuclear transcripts from a single source might create problems for the transport of mRNAs and proteins – particularly proteins with large molecular masses and hence low diffusivities – through the cytosol to appropriate sites such as plastids and mitochondria. Maintaining solute fluxes at the rates required for growth over distances of up to hundreds of μm requires larger gradients (expressed as moles per cubic metre concentration difference per metre of pathway) because average transport distance, which scales with cell volume, V, as $\propto V^{1/3}$, is not entirely offset by declines in specific growth rate with increasing cell size ($\propto V^{-\alpha}$, $\alpha < 1/3$; see Section V below). Vacuolation helps to increase the surface area for interception of photosynthetically active radiation (PAR) and for nutrient influx per unit metabolizing volume (i.e. that part of the cell not occupied by the vacuole; Raven, 1997a), but does not explicitly help with

the problems of intracellular transport from the nucleus to the extremes of the cell.

Larger transport distances may also increase the difficulty of synchronizing the expression of nuclear and organelle (chloroplast and mitochondrion) genes. The majority of endosymbiont genes that have been retained – mainly for organelle functions (Reyes-Prieto *et al.*, 2006) – have been transferred to the nucleus. Only a small minority of genes are retained in the organelle, perhaps in part because rates of mutagenesis can be relatively high (Allen & Raven, 1996). Synchronizing of gene expression is made difficult because organelle genomes are only a few μm from the site at which the resulting genes function, while nuclear genes can be a few hundred μm from the site of function in organelles. For chloroplasts, this is true regardless of whether a cell has many small chloroplasts or a single, large, reticulate chloroplast, because multiple copies of the chloroplast DNA are dispersed throughout a single large plastid (Kuroinia *et al.*, 1981).

Further quantitative work is needed to determine the extent to which these potential synchronization problems in large cells actually occur and, if they do, whether these are significant in the context of the specific growth rate of *Ethmodiscus* of $\leq 0.39\ d^{-1}$ (Villareal *et al.*, 1999). In this context, it is of interest that larger cells of colonial *Hydrodictyon*, which are planktonic algae with cell volumes (mainly vacuole) of up to several mm^3, are multinucleated and lack cytoplasmic streaming (Van den Hoek *et al.*, 1995; Graham & Wilcox, 2000). Larger, benthic differentiated unicellular (or perhaps better described as acellular) ulvophycean green algae, such as *Bryopsis* and *Caulerpa* (Caulerpales = Bryopsidales; Graham & Wilcox, 2000), are generally also multinucleate, but do possess cytoplasmic streaming (Raven, 2003). However, algae such as *Hydrodictyon* have a regular spatial arrangement of nuclei, each surrounded by a similar-sized cytoplasmic 'domain' that may be determined by the cytoskeleton, whereas the arrangement of nuclei within members of the Caulerpales is random and not associated with the cytoskeleton (McNaughton & Goff, 1990). Taken together, these findings suggest that larger cells may maintain greater numbers of nuclei as a means of reducing intracellular transport distances, but that cytoplasmic streaming may be important when the size of nuclear 'domains' is variable. By contrast, members of the Dasycladales (e.g. *Acetabularia*; Van den Hoek *et al.*, 1995; Graham & Wilcox, 2000) are uninucleated through most of their life cycle despite being relatively differentiated and having a maximum dimension of tens of mm (see Serikawa *et al.*, 2001). These organisms possess cytoplasmic streaming and it is thought that the cytoskeleton (actin filaments) is involved in transport and/or localization of mRNA (Vogel *et al.*, 2002). These roles of cytoplasmic streaming are additional to their involvement in transferring low molecular mass solutes within large differentiated organisms with spatially localized functions and resource sources (Raven, 2003).

2. Diffusion boundary layers

A diffusion boundary layer exists around all objects in a fluid medium; the thickness of this layer is the distance from the surface within which all solute and solvent movement normal to the surface of the organism is by diffusion (Denny, 1992). Theoretical considerations (e.g. Denny, 1992; Raven, 1998a) predict that the external diffusion boundary layer thickness is equal to the radius of the organism for a spherical cell, although the boundary layer thickness is smaller than this prediction for radii > 50 μm. Whether this layer effectively leads to growth limitation depends on the balance of nutrient concentration in the bulk medium and the rates of diffusivity and nutrient transport at the cell surface. Diffusive limitation of growth when the concentrations of nutrients such as nitrate and phosphate are low is, all else being equal, more likely for colonies composed of cells of < 50 μm radius than for unicells of the same size as the cells in the colony. This is because, as we shall see in Section VI, the potential specific growth rate decreases with increasing cell or colony size less rapidly than would maintain the relative importance of diffusive limitation of nutrient supply by the diffusion boundary layer in the overall uptake process.

The greater importance of boundary layers is consistent with the generally observed increase in the concentration of nutrient needed to give half the maximum rate of nutrient uptake with increasing cell size for unicells (Eppley *et al.*, 1969, Litchman *et al.*, 2007; cf. Smith & Kalff, 1982). Even allowing for any decrease in maximum specific growth rate with increasing cell size (Section VI) it would be expected that the proportionally greater restriction on the rate of uptake by diffusion through boundary layers would have the effect of decreasing the apparent affinity of the entry of solutes that reach saturation as the concentration increases. Such saturation can arise from either a plasmalemma-located transporter (e.g. of CO_2 or HCO_3^- in photosynthesis by most algae) or diffusive entry through nonsaturating mechanisms (e.g. by lipid solution) followed by assimilation using an enzyme (e.g. in the minority of algae that lack inorganic carbon concentrating mechanisms (CCMs)) (Raven *et al.*, 2005a,b; see also Raven *et al.*, 2008 for a discussion of NH_3/NH_4^+ entry and assimilation). There are, however, serious methodological problems in determining half-saturation constants for growth, with those measured experimentally being somewhere between the real (free of experimental problems) half-saturation constant for growth (K_g) and that for the actual transporter (K_t); K_g is likely to be very much less than K_t (Flynn, 1998). In the absence of systematic measurements of the affinity of transporters and enzymes in a more resolved system with minimal and constant restrictions imposed by diffusion of substrate, it is not possible to be sure whether the diffusion boundary layer effects account for all the apparent decrease in affinity *in vivo* with increasing cell size, or whether there is a relaxation in selection for a high affinity of the transporters or

enzymes for their substrates when such affinities cannot be fully expressed *in vivo* because of diffusive limitation or because of regulation of the transporters (Flynn, 1998).

Direct measurements of boundary layer thickness in phytoplankton organisms using microsensors have only proved possible for relatively large colonial planktonic organisms such as *Phaeocystis* (Ploug *et al*., 1999a,b), *Trichodesmium* (Carpenter *et al*., 1990), a symbiotically photosynthetic freshwater ciliate (Sand-Jensen *et al*., 1997), unicells such as planktonic symbiotic Foraminifera (Jørgensen *et al*., 1985; Rink *et al*., 1998; Köhler-Rink & Kühl, 2005), and a large centric diatom (Kühn & Raven, 2008).

The diffusion boundary layer around *Phaeocystis* colonies was found to be consistent with theoretical expectations in the study of Ploug *et al*. (1999a,b). Differences in phosphate affinity between single filaments and 'puff' and 'tuft' colonies of filamentous *Trichodesmium* also appear consistent with theory, at least qualitatively (McCarthy & Carpenter, 1979; Fu *et al*., 2005). The low affinity (here measured as the reciprocal of the half-saturation concentration, but more appropriately expressed as the substrate-saturated rate divided by the half-saturation concentration) for inorganic carbon (not saturated in air-equilibrated seawater) in diazo-photolithotrophic growth of *Trichodesmium* might also be related to diffusive boundary layers around 'puffs' and 'tufts' (Hutchins *et al*., 2007; Levitan *et al*., 2007; Ramos *et al*., 2007). This is because this marine β-cyanobacterium has a subset of the CCM components typical of freshwater β-cyanobacteria (Badger & Price, 2003; Badger *et al*., 2006), which might be expected to give saturation of photosynthesis in air-equilibrated seawater if there were no significant boundary layer limitations. Tchernov & Lipschultz (2008) reported changes in stable carbon isotope discrimination ($\delta^{13}C$) in *Trichodesmium* that are consistent with enhanced diffusion limitation of CO_2 supply in larger colonies.

Evidence for such boundary layer effects has, however, been mixed. For example, specific growth rates have been found to be higher for phytoplankton colonies than for unicells in the studies of Veldhuis *et al*. (2005: see Section VI for further work on *Phaeocystis*) and Wilson *et al*. (2006). It is noted that, in these studies, growth was probably not nutrient-limited. Nutrient transport capacity develops rapidly with nutrient stress (e.g. Flynn *et al*., 1999), when substrate limitation is important, and this compounds problems of measuring half-saturation constants (Flynn, 1998). Overall, these examples highlight the need for further investigation of the biological implications of the decreased diffusive conductance of the boundary layer around colonies in comparison with those around isolated unicells from colonies.

Larger colonies or organisms with flagella, such as *Volvox*, can have significant reductions in diffusive limitation on nutrient flux at the surface of the colony or organism as a result of advection by the flagella (Short *et al*., 2006; Solari *et al*., 2006a,b). Any restrictions on solute fluxes between the colony surface and the bulk medium would favour the retention of extracellular inorganic or organic storage compounds of low molecular mass, if these are truly storage (re-used) rather than simply accumulated compounds (Raven, 1982, 1997a). However, the possibility of long-term (more than a doubling time) storage of such compounds seems remote (Reynolds, 2007; but see Flynn & Gallon, 1990) in view of the known diffusivities of low molecular mass materials in the mucilage and the permeability of the pellicle of *Phaeocystis* to compounds at least as large as sucrose (Hamm *et al*., 1999). Flynn & Gallon (1990) present evidence consistent with short-term (over a light/dark cycle) storage of free amino acids in mucilage surrounding the unicellular diazotrophic cyanobacterium *Gloeothece*. We shall see later that the potential for storage of low relative molecular mass (M_r) compounds is greater in vacuoles, which are found predominantly in larger cells (Raven, 1995b; Menden-Deuer & Lessard, 2000), because vacuoles are much less intrinsically leaky (flux of solute per unit area per unit driving force acting on the solute) than are extracellular matrices, or pellicles.

The modelling by Yoshiyama & Klausmeier (2008) of optimal cell sizes for optimal nutrient uptake by planktonic micro-organisms integrates external diffusion, membrane transport and intracellular assimilation in relation to the size of resource molecules. As Yoshiyama & Klausmeier (2008, p. 68) point out, the application of this model to large phytoplankton unicells requires explicit consideration of the large fraction of the volume occupied by vacuoles in these organisms, which impacts not only on the balance of the two transport components relative to the assimilation term in the model but also on intracellular transport, resource storage, vertical motion and the package effect (Raven, 1984, 1997a).

3. Package effect

The 'package effect' involves a decrease in the specific absorption coefficient of a pigment molecule when it is aggregated versus homogeneously distributed. Theory and observation indicate that photon absorption per unit pigment will decrease with cell volume in unicells for any fixed pigment concentration (Duysens, 1956; Agusti, 1991). The occurrence of cells in a colony (or multicellular organism) relative to the same cells occurring in isolation would be expected to increase both the package effect with regard to the absorption of PAR and UV (Kirk, 1975a,b, 1976, 1994; Raven, 1984, 1991; Garcia-Pichell, 1994) and the restriction on nutrient uptake from low external concentrations as a result of the greater thickness of the diffusion boundary layers around larger objects (Raven, 1989, 1998a). Other factors being equal, both of these effects would have the same impact on a colony as on a giant unicell or multicellular organism of the same size and shape (Kirk, 1975a,b, 1976, 1994; Raven, 1984, 1989, 1998a; Garcia-Pichell, 1994).

Theoretical predictions regarding the package effect are consistent with empirical data on the effects of organism size

obtained for *Microcystis* in a comparison of colonies with isolated unicells (Ganf *et al.*, 1989), and with empirical data on the effects of varied chromophore concentrations obtained for *Phaeocystis* in a comparison of colonies grown at different irradiances (Moisan & Mitchell, 1999). These studies specifically addressed absorption of PAR where, within limits, 'more is better'. However, the same physical principles apply to the absorption of UV-B radiation (Raven, 1991; Garcia-Pichell, 1994) where 'less is better'. Extracellular polysaccharides as part of colony structure, whether homogenously dispersed or in a pellicle, do not absorb significant UV-B, but could potentially facilitate the attachment of UV-B screening compounds.

In general, extracellular compounds are better able to screen the protoplast than intracellular compounds. *Phaeocystis* colonies appear to have extracellular as well as intracellular UV-B screening compounds (Riegger & Robison, 1997; Moisan & Mitchell, 2001). However, the effectiveness of the screening compounds in these colonies is not sufficient to make them less sensitive to UV-B than co-occurring unicellular diatoms (Riegger & Robison, 1997); overall effects of UV-B are not just a function of screening compounds, but also of tolerance and damage repair (see e.g. Heraud & Beardall, 2000).

Any differences in the size dependence of the package effect and intracellular pigment composition between unicellular and colonial organisms could contribute to differences in the size scaling of light-limited or photoinhibited photosynthesis and growth between these growth forms.

4. Vertical movement

Upward and downward movement of cells is affected by a variety of factors. Vertical movement is important for phytoplankton because it can influence access to light and nutrients, as well as the eventual loss by sinking of all nonmotile cells that are denser than the medium. All else being equal, a larger object is expected to move more rapidly through a medium than a smaller one (formalized in Stokes' Law). Although this is a complex area to investigate experimentally (Walsby & Holland, 2006), it is pertinent to our consideration of coloniality, as it means we might expect a colony to move more rapidly than its constituent cells.

Many other properties of phytoplankton can also affect their vertical movements. For nonflagellate phytoplankton, downward movement is favoured by increasing the 'ballast' through storage of carbohydrate (Boyd & Gradmann, 2002) or mineralization. Upward movement in some cyanobacteria is achieved using gas vesicles, with downward movement occurring when this buoyancy is more than offset by polysaccharide ballast (Walsby, 1994). *Phaeocystis* colonies have been observed to be buoyant in seawater (Peperzak *et al.*, 2003) by an unknown mechanism, presumably involving manipulation of solute content (Boyd & Gradmann, 2002) as eukaryotes lack gas vesicles.

In freshwater eukaryotes, the capacity to rise relative to the immediate environment probably cannot be achieved by decreasing density by either changing intracellular (vacuolar) solutes or decreasing the 'ballast' provided from storage of carbohydrate (see Boyd & Gradmann, 2002). Positive buoyancy cannot readily be achieved by accumulating lipids even as a large fraction of the biomass, although this can occur in some strains of the green alga *Botryococccus* with up to 75% hydrocarbon in the dry weight (Young *et al.*, 2004). The 'genus' *Botryococcus* mainly contains strains from Trebouxiophyceae, although two strains occur in different clades within the Chlorophyceae (Serousy *et al.*, 2004).

The capacity to move upwards relative to the surrounding water can be attained, and is certainly most readily controlled, by flagellar motility. Small freshwater colonies and unicells of the size of individual cells of *Volvox* can only swim at up to *c.* 150 $\mu m\ s^{-1}$ (e.g. the freshwater *Cryptomonas phaseolus* has an ESD of *c.* 10 μm; Pedrós-Alió *et al.*, 1987). However, large *Volvox* colonies of > 1 mm ESD can swim at speeds of > 1 $mm\ s^{-1}$ as a result of decreased friction relative to the available energy per unit volume to power flagella. Limits on this available energy for a spherical structure come from the necessarily superficial disposition of the flagellate cells and the package effect, which limits the possibility of increased PAR absorption by the light-harvesting apparatus as fractional absorption approaches 1.0 (Raven, 1984).

The ecological and evolutionary relevance of possible cyclic upward and downward movement by phytoplankton seems to relate to inverse gradients of PAR (highest at the surface) and nutrients such as nitrogen (N) and phosphorus (P) (highest at depth), combined with a small vertical component of water movement in the upper mixed layer/epilimnion. These ecological conditions are met, at least as far as gradients are concerned (see modelling by Flynn & Fasham, 2002), for very large unicellular diatoms and colonies of large diatom cells (e.g. species of *Coscinodiscus*, *Ethmodiscus* and *Rhizosolenia*), prasinophyte phycomata, large dinoflagellates in parts of the oligotrophic ocean, dinoflagellates in fjords, and *Volvox* in lakes (Swift & Meunier, 1976; Kahn & Swift, 1978; Raven & Richardson, 1984; Kirk, 1998; Raven, 1998b). Migrations generally occur on a diel basis (near the surface during the day; deeper at night), but can take several days in the oligotrophic ocean. The vertical migration of *Microcystis* has been modelled by Rabouille *et al.* (2005) and Rabouille & Salençon (2005), and that of the dinoflagellate *Alexandrium* by Flynn (2002). For *Trichodesmium* in an oligotrophic region of the north Pacific, modelling suggests the possibility of migrations between the upper euphotic zone and the phosphocline, but only for colonies above a threshold size (White *et al.*, 2006). However, it must be remembered that these vertically migrating phytoplankton organisms share their habitat with photolithotrophs that do not show such migration and also with potential predators that often migrate upwards during darkness: as usual in ecology, one size does not fit all.

Ecological interpretation of these different characteristics of phytoplankton organisms is complicated by properties of the

physical environment. For example, turbulence is a determinant of the rate of sinking. Recent findings indicate that turbulence can increase the average settling velocity of phytoplankton cells, rather than decreasing it, as had earlier been believed (Ruiz *et al.*, 2004), although the generality of these findings remains to be tested there may also be more rapid formation of cell aggregates under turbulent conditions; aggregates sediment very much more rapidly than single cells (Jackson, 1990). Also, the mixing depth affects the distribution of nutrients in the water column, and the probability of cells migrating to the nutricline before they become light (energy) limited (see simulations in Flynn, 2002).

5. Turgor pressure and turgor-resisting cell walls

Some attributes of colonial and multicellular algae are shared to varying degrees by large, vacuolate unicells. Here the thicker cell walls (Raven, 1982, 1995a) of those with turgid cells replace the extracellular materials within the colony as having the greater potential for increased screening of UV-B, compared with individual small cells. The only photosynthetic cells of this kind are eukaryotic (e.g. diatoms, dinoflagellates, prasinophytes and phycomata), although some sulphide-oxidizing chemolithotrophic bacteria have 'eukaryotic' nongaseous vacuoles (Raven, 1982, 1997a). According to the Laplace relationship, turgid eukaryotic cells devote the same fraction of their volume to cell wall material, regardless of cell size, given a particular cell wall material, turgor pressure and mechanical safety factor (Raven, 1982, 1995a). This relationship applies also to the turgor-resisting peptidoglycan layer of cyanobacterial cell walls (Hoiczyk & Hansel, 2000). The nonturgor-resistant theca of dinoflagellates was not found to significantly increase the carbon (C) per unit volume when thecate and athecate species were compared over a large size range (Menden-Deuer & Lessard, 2000). Villareal *et al.* (1999) showed that the previously determined relationship between silicon (Si) content and cell volume for diatoms up to a volume 10^6 μm^3 applied to cells up to a volume of 10^9 μm^3, so that there is a tight linear relationship between cell volume and cell Si content, as predicted by the Laplace relationship. These relationships apply to cells growing under near-optimal resource supply conditions; under resource limitation the Si content is increased (see Raven & Waite, 2004). For a given fraction of the maximum growth rate, the ballast effect of SiO_2 is the same for a large unicell as for a colony of the same volume. However, because larger cells have a larger fraction of the cell volume occupied by vacuole than do smaller cells (Raven, 1995b; Menden-Deuer & Lessard, 2000), any moderating effect of changed vacuolar composition (Boyd & Gradmann, 2002; Raven & Waite, 2004) on overall cell density would be greater in a large unicell than in a colony of the same volume, assuming no greater contribution of extracellular polysaccharide, which is not part of the regular cell wall, in colonies than in unicells. Even vacuolate diatoms that might be expected to have adequate storage volume for dissolved low M_r solutes store high-density polyphosphate (Diaz *et al.*, 2008). In addition, the amount of Si deposited in the diatom cell wall increases markedly with non-Si induced growth limitation (Martin-Jézéquel *et al.*, 2000).

However, large three-dimensional colonies typically have less than half of their volume occupied by cells, so that there is less wall material per unit colony volume than there is in a cell of the same size. Particularly in cyanobacteria, the rest of the volume contains extracellular polysaccharides other than turgor-resisting cell wall material; this extracellular material can bear UV-B-screening compounds such as scytonemin (Proteau *et al.*, 1993). The question of a turgor-resisting wall does not arise in cells with flagellar motility, as the necessarily wall-less flagella are in hydraulic connection with the main cell (Raven, 1982, 1995a).

V. Elemental stoichiometry

The elemental stoichiometry of colonies and multicellular organisms compared with large or small unicells is poorly understood. Similarity of growth conditions is a major problem when comparing the contents of the elements commonly investigated in this context (C (organic and inorganic forms), N, P and Si). What is known is considered in the Supporting Information (Text S1), and shows that there is only limited information from which comparisons of the compositions of large unicells and of colonies can be drawn, and even less for symbioses.

VI. Allometry of specific growth rates and specific metabolic rates

Among diverse organisms, specific growth rate has been observed to decline as a function of body size. This has been argued to relate functionally to the tendency of larger organisms to have lower mass-specific rates of metabolism, which in part fuels growth. Such relationships are often characterized using allometric scaling, where the dependence of a trait (T) on body size (V) is represented using the form $T = cV^{\alpha}$. Here c is a constant, and α is a 'scaling exponent,' which is positive if T is greater in larger organisms, and negative if T is greater in smaller organisms (as is often the case for specific growth and metabolic rates). The allometric scaling of specific growth and metabolic rates has been characterized in phytoplankton, with considerable data for unicells, rather less for colonies, and none that we could find for symbioses (see Supporting Information, Text S1). Data considered do not permit a definite conclusion as to whether there is a difference in allometric scaling with organism size between unicells and colonies. The situation is complicated by variations in scaling factor among clades of unicellular phytoplankton for resource-saturated growth and the differences in scaling factor for resource-saturated and resource-limited growth. A further complication is introduced

by the reported variations in specific growth and metabolic rates for different organisms as a function of colony size, with some reports showing the 'expected' (on physical grounds) decrease in growth and metabolic rates with colony size while others indicate an increase in these rates with increasing colony size.

There is a general positive correlation of cell volume, nuclear DNA content and the number of copies of the rDNA operon within and among clades (Zhu *et al.*, 2005; Mendell *et al.*, 2008; von Dassow *et al.*, 2008). Although the rDNA operon copy number also correlates with the maximum specific growth rate, for phytoplankton the scaling of maximum specific growth rate and cell size within a clade shows that the predominant correlation is with cell size (Zhu *et al.*, 2005).

VII. Trophic interactions

We have already mentioned trophic interactions between symbionts in diazotrophy and phototrophy (Section II). Phagotrophy by hosts underlies these symbioses, for each generation when there is horizontal transmission of symbionts, and at the origin of the symbiosis when there is vertical transmission. Phagotrophs in extant photosynthetic phytoplankton also include organisms that can ingest solid particles as immediate food sources as well as, or instead of, using photosynthetic prey items as symbionts (Laybourn-Parry *et al.*, 1997; Raven, 1997b; Laybourn-Parry & Marshall, 2003; Unrein *et al.*, 2007). Mixotrophy is not further considered here.

Turning to more widespread trophic interactions, that is, the consumption of planktonic phototrophs by grazers, the fossil record gives little information as to the nature of grazers on phytoplankton and only fragmentary information on colonial and multicellular planktonic primary producers (Tomitani *et al.*, 2006; Butterfield, 2007). However, there are data for the extant freshwater green alga *Scenedesmus* (the generic name used by the authors of the various papers, although some of the organisms may now be classified as *Desmodesmus*; Johnson *et al.*, 2007) and for *Phaeocystis* and *Microcystis* showing that there are phenotypic (acclimatory) responses of colony size to the presence of grazers. *Scenedesmus* and *Desmodesmus* can form colonies with one, two, four or eight cells. *Scenedesmus acutus* switched from unicellular to colonial (four to eight cells in a colony) upon exposure to the cladoceran grazer *Daphnia magna* (Lampert *et al.*, 1994). This work has been extended to other species of *Scenedesmus*, to *Chlorella* and to other grazers (Lürling *et al.*, 1997; Boraas *et al.*, 1998; Lürling & Van Donk, 2000; Mayeli *et al.*, 2004; Verschoor *et al.*, 2004; Lürling, 2006; Verschoor *et al.*, 2007), showing that the response is relatively widespread, but that the formation of colonies in the presence of grazers does not restrict grazing by all grazers tested, and that the strategy can be undermined by nutrient limitation of the alga. For *Phaeocystis* and *Microcystis* there is a much greater range of colony sizes than in *Scenedesmus*, as there is no fixed number of cells per colony. Jakobsen & Tang (2002), Tang (2003) and Nejstgaard *et al.* (2007) showed that there was an increase in colony size in the presence of grazers in *Phaeocystis globosa.* Subsequent work on this organism showed that the presence of ciliate grazers that cannot feed on large colonies significantly enhances colony formation, while colony formation is significantly depressed in the presence of copepod grazers that only feed on colonies; chemical cues are involved here (Long *et al.*, 2007). *Microcystis aeruginosa* also shows an increase in colony size and mucilage production as a result of grazing by flagellates (Yang *et al.*, 2008).

Mucilage production is not restricted to colonial or multicellular organisms, and is found in phytoplankton over a wide size range. Mucilage acts as a feeding deterrent, through a direct physical effect and/or its influence on food quality as a result of the high C:N and C:P ratios.

Coloniality can provide protection from viral attack in *Phaeocystis pouchetii*; colonies had no viral infections under conditions in which unicells were infected (Jacobsen *et al.*, 2007). It is not clear if this protection of colonies is related to the pellicle identified by Hamm *et al.* (1999). Even if viral infection occurs within a colony there is the possibility of chemical cues from the infected cells inducing apoptosis in other, genetically identical cells in the colony. This would diminish the virus burst size from the colony, and other kin cells in other colonies would be protected by a lower viral density in their environment. So, of course, would hosts of that virus that had different genotypes from those whose apoptotic response decreased the number of viral particles, so the mechanism works best in natural selection terms with very host-specific viruses. At the other extreme of organism size, very small unicells could be immune from infection by large viruses because the burst size from the limited resources in a small cell would not be sufficient to maintain the viral population (references in Raven, 2006). However, the smallest known oxygenic photolithotroph, *Prochlorococcus*, has cyanophages, so the burst size argument would only apply to significantly larger viruses than the known cyanophages. These considerations, like those of apoptosis, apply to viruses with restricted host ranges (see Raven & Waite, 2004).

VIII. Global significance of large unicells, colonies and multicellular organisms

Large phytoplankton unicells are relatively uncommon in terms of habitats in which they are significant contributors to biomass and productivity. The giant diatom *Ethmodiscus* is found in oceanic gyres and only occurs at up to 1–5 cells m^{-3}, or 0.48–2.4 μmol organic C m^{-3} (Villareal *et al.*, 1999). Estimates of the specific growth rate of *Ethmodiscus* in natural conditions range from 0.06 to 0.39 d^{-1} (Villareal *et al.*, 1999).

Colonies of the marine diazotrophic *Trichodesmium* can reach densities of 140 per m^3 in the subtropical North Pacific Ocean; with 182 filaments per colony and 52.7 ng C per

filament, there is 112 μmol C in *Trichodesmium* colonies per m^3 (Letelier & Karl, 1996). Here, colonies only account for 12% of the total number of *Trichodesmium* filaments, so the total biomass of this cyanobacterium in terms of C is 933 μmol C m^{-3}. Capone *et al.* (1997) cite trichome densities equivalent to 3510 μmol C m^{-3} for the Southwest Tropical Atlantic Ocean, and a specific growth rate of 0.14–0.23 d^{-1} in culture.

The colonial *Phaeocystis* blooms in relatively eutrophic cooler austral and boreal waters, and the organic C of this alga can reach 160 mmol organic C m^{-3} (Tungaraza *et al.*, 2003; Gypens *et al.*, 2007), while in other areas single cells of *Phaeocystis* dominate, with up to 17 mmol C m^{-3} in colonies and 264 mmol C m^{-3} in cells (Wassmann *et al.*, 2005). Veldhuis *et al.* (2005) found specific growth rates of up to 0.83 d^{-1} for single cells in nature and in culture, and up to 1.53 d^{-1} for cells in colonies.

For the multicellular *Volvox* from relatively eutrophic freshwaters, the organic C content in colonies in their natural habitat can reach 115 mmol C m^{-3} (Kirk, 1998; Desnitski, 2000).

The values for organic carbon in large phytoplankton unicells colonies and muticellular organisms should be considered in the context of the general lack of size scaling of phytoplankton organic carbon biomass with *cell* size (Finkel, 2007; contrast the conclusions of Shuter, 1978 on the N and P minimal cell quotas discussed in Section V of Supporting Information (Text S1)). The argument relies on the observation of Belgrano *et al.* (2002) that temporally and spatially averaged phytoplankton cell abundance (A; cells per m^3) scales as cell volume (m^3) V as $\sim V^{-0.75}$, while carbon per cell (C; mol C per cell) scales as $V^{0.75}$. Accordingly, B, the carbon biomass in the water column (mol C per m^3), which is C/A, scales as $V^{(0.75-0.75)}$ or V^0. Thus the small values of V for the smallest cells are approximately offset by the large values of A, and vice versa for the largest cells.

It would be useful if this section could have ended with a survey of the contributions to global biomass and productivity of large phytoplankton cells, colonies and multicellular organisms, with a comparison with the contributions of smaller unicellular phytoplankton cells. However, we have not found sufficient data on which to base such a comparison. It is hoped that the need for such information informs the design and execution of further measurements of the biomass and productivity of aquation environments.

IX. Significance of colonies and multicellular organisms relative to large unicells in the phytoplankton

Clearly colonies, pseudocolonies, multicellular organisms and large unicells co-exist in the plankton, globally if not necessarily in the same habitat. Comparisons of the attributes of the three life forms are now considered (Table 3).

The three life forms share the presence of a larger package effect and a thicker diffusion boundary layer around the organism in comparison with otherwise similar small unicells. All three life forms have representatives that can perform N fixation (symbiotic in the case of large unicells), and vertical migration upwards as well as downwards relative to the surrounding water. Can nonflagellate organisms achieve diel vertical migrations over a vertical distance of up to 20 m, as can some dinoflagellates

Table 3 Advantages and disadvantages of colonies/multicellular organisms relative to single small or large cells, summarized from data and syntheses in the text and the Supporting Information (Text S1)

Aspect	Evolutionary advantages of colonies/ multicellular organisms vs single small cells	Ecophysiological advantages of colonies/multicellular organisms vs single large cells of same mass	Disadvantages of colonial and multicellular life forms
Predation	• Avoidance of small grazers	• Only one cell from a colony needs to survive predation for subsequent reproduction	• More visible than single small cells
	• Colonies reduce encounter rate so reduce predation	• When a predator feeds on a colony, the colony can fragment into smaller individuals that may now be too small for the predator	• Predator can get everything from a concentrated food source
	• Allelopathic compounds diffuse away rapidly from single cells, whereas diffusion limitation leads to a build-up of chemicals around a colony. Far easier for colony to produce a threshold of allelopathic chemicals		
	• Anti-predator advantage of mucilage		
Viral mortality	• Colonies reduce encounter rate so reduce viral transmission compared with many individual cells		• Infection of one cell can result in infection of entire colony
	• Avoidance of some viruses and parasites if a pellicle is present		

Table 3 *continued*

Aspect	Evolutionary advantages of colonies/ multicellular organisms vs single small cells	Ecophysiological advantages of colonies/multicellular organisms vs single large cells of same mass	Disadvantages of colonial and multicellular life forms
Resource requirements		• Net nutrient acquisition and thus growth rates could be better in a colonial form than a single large cell	• Colony may have greater total resource requirements than a single large cell (may have more mitochondria, DNA and mucilage, as well as cell wall) package effects because of self-shading of pigments (minimized by cells in colonies concentrated on its surface compared with large vacuolate unicells)
		• More efficient transport of nutrients among cells in a colony compared with a large single cell. Oxygen and nutrient transport in large cell is a constraint	• Total nutrient requirements higher for colonies than for unicells of same total size (?)
			• Diffusion limitation of inorganic nutrient acquisition in colonies more likely than in isolated cells from those colonies
Specialization	• Division of labour/specialization is more efficient and only possible with multiple cells acting together (multicellular organisms not colonies)	• Division of labour/specialization is more efficient and only possible with multiple cells acting together (multicellular organisms not colonies)	
Genome replication		• Genome replication rate slower for larger cells compared with colonies of small cells • More energy and P needed to replicate genome in a large cell • More copies of genome in a colony compared with a single large cell	
Structural advantages	• Potential for greater C accumulation resulting in increases in day-integrated metabolic rate compared with isolated cells • UV-B screening compounds contained in extracellular material • Greater total mass allows greater motility and vertical migration		

C, carbon; P, phosphorus.

and *Volvox*, or are they mechanistically constrained to longer cycling times? Whether colonial and multicellular forms can effectively store low molecular mass nutrients such as nitrate and phosphate in their matrix material, as do large vacuolated diatoms, is not yet clear. There is the question of leakiness for extracellular storage. However, *Chlamydomonas* has recently been shown to contain polyphosphate in its cell walls (Werner *et al.*, 2007), which makes P storage in the 'apoplasm' of colonies such as *Volvox* in a form that will not immediately diffuse out seem more plausible, while still leaving open the question of how polyphosphate in the cell wall is synthesized and broken down. Even vacuolar storage of NO_3^-, for example, does not account for a significant fraction of the total N in the organism and arguments for selective advantage based upon such accumulations have been questioned (Flynn *et al.*, 2002).

Are there advantages for one or another life form in terms of avoiding a particular size of grazer, granted similar sizes for representatives of the three life forms? There are different alleged selective advantages for colonial, multicellular and large unicell life forms in different cases (e.g. protecting nitrogenase from oxygen in nostocalean differentiated organisms and in the colonial *Trichodesmium*, but not in other cases of multicellularity or coloniality). There is also a possible advantage of variations in organism size through top-down control: avoidance of small grazers by being large, and of large grazers by being small. The potential for large phenotypic variations in size is greater for many colonial algae (and some multicellular algae) than for unicells, and is realized for several genera of colonial algae.

Finally, we must remember that the discussion here, in outlining some possible constraints and opportunities associated

with a number of life forms of phytoplankton organisms, namely, small unicells, large unicells, pseudocolonies, colonies and multicellular organisms, provides only a framework for further work. It is vital that further work aims to provide evidence as to the significance of these features in the fitness of the organisms in a near-natural environment.

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Additional supporting information may be found in the online version of this article.

Text S1 Discussion of topics relating to Sections II, III, IV, V and VI of the main text.

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