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Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis

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A compilation of data on the elemental composition of marine phytoplankton from published studies was used to determine the range of C:N:P. The N:P ratio of algae and cyanobacteria is very plastic in nutrient-limited cells, ranging from < 5 mol N:mol P when phosphate is available greatly in excess of nitrate or ammonium to > 100 mol N:mol P when inorganic N is present greatly in excess of P. Under optimal nutrient-replete growth conditions, the cellular N:P ratio is somewhat more constrained, ranging from 5 to 19 mol N:mol P, with most observations below the Redfield ratio of 16. Limited data indicate that the critical N:P that marks the transition between N- and P-limitation of phytoplankton growth lies in the range 20–50 mol N:mol P, considerably in excess of the Redfield ratio. Biochemical composition can be used to constrain the critical N:P. Although the biochemical data do not preclude the critical N:P from being as high as 50, the typical biochemical composition of nutrient-replete algae and cyanobacteria suggests that the critical N:P is more likely to lie in the range between 15 and 30. Despite the observation that the overall average N:P composition of marine particulate matter closely approximates the Redfield ratio of 16, there are significant local variations with a range from 5 to 34. Consistent with the culture studies, lowest values of N:P are associated with nitrate- and phosphate-replete conditions. The highest values of N:P are observed in oligotrophic waters and are within the range of critical N:P observed in cultures, but are not so high as to necessarily invoke P-limitation. The C:N ratio is also plastic. The average C:N ratios of nutrient-replete phytoplankton cultures, oceanic particulate matter and inorganic N and C draw-down are slightly greater than the Redfield ratio of 6·6. Neither the analysis of laboratory C:N:P data nor a more theoretical approach based on the relative abundance of the major biochemical molecules in the phytoplankton can support the contention that the Redfield N:P reflects a physiological or biochemical constraint on the elemental composition of primary production.

Key words: carbon, carbohydrate, lipid, nitrogen, phosphorus, protein, phytoplankton, Redfield ratio

Introduction

The Redfield ratio is one of the tenets of aquatic biogeochemistry. Named in honour of Alfred Redfield, this concept refers to the relationship between organism composition and water chemistry. Redfield (1958) concluded that the elemental composition of plankton was 'uniform in a statistical sense' and that variations in inorganic C, N and P in seawater were 'almost entirely as a result of the synthesis or decomposition of organic matter'. Redfield based this assertion on R. H. Fleming's 1940 data set for the C:N:P content of plankton yielding a ratio of 106:16:1 and the observations of L. H. N. Cooper and F. A. Richards showing that inorganic C, N and P concentrations in and below the main permanent thermocline varied in the proportions 105:15:1.

Geochemists and biologists define the numerical value of the Redfield ratio differently. Geochemists use a C:N:P stoichiometry 105:15:1 based on the covariation of nitrate, phosphate and non-calcite contribution to total inorganic C in deep seawater (Broecker & Peng, 1982), whereas biologists use a ratio of 106:16:1 based on Fleming's analysis of the average elemental composition of marine organisms (Goldman *et al.*, 1979). Redfield seemed to be indifferent on this matter, equating the elemental composition of organisms with that of inorganic nutrients in the deep sea.

The degree to which the C:N:P stoichiometry of marine particulate matter can deviate from the Redfield ratio of 106:16:1 is critical to our understanding of the role of phytoplankton in biogeochemistry (Falkowski, 2000). The Redfield C:N ratio is used in oceanography for calculation of export production, and for nutrient-based productivity calculations, as well as in models of ocean productivity. The Redfield N:P ratio of 16:1 is often used as a benchmark for differentiating N-

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limitation from P-limitation, and is thought to set an upper limit on the nitrate:phosphate ratio in the ocean (Falkowski, 1997; Tyrrell, 1999; Lenton & Watson, 2000). This assumes that phytoplankton is N-limited at N:P < 16, and that it is P-limited at N:P > 16. The limit of 16:1 is often attributed to P-limitation of N_2-fixation, as was first hypothesized by Redfield (1934). A modification of this approach considers Fe to be the limiting factor, but that the upper limit on N:P of plankton is still constrained by the Redfield proportions of 16:1 (Falkowski, 1997).

A recent paper by Broecker & Henderson (1998) challenges these conventions regarding the inflexibility of the average C:N:P composition of phytoplankton and marine particulate matter (Hecky *et al.*, 1993) and the N:P ratio that marks the transition between N- and P-limitation (Tyrrell, 1999). To account for biological sequestration of CO_2 in the ocean during glacial maxima, Broecker & Henderson (1998) suggested that the nitrate:phosphate ratio may have significantly exceeded 16:1, reaching a value of 25 mol N:mol P. Recently, Falkowski (2000) asked whether biologists can support or refute the N:P of 25:1 invoked by Broecker & Henderson (1998) for glacial periods. While stating that the question is still open, Falkowski (2000) asserted that 'the upper bound for N/P ratios in the dissolved inorganic phase in the oceans is almost certainly a consequence of the intrinsic chemical composition of marine phytoplankton'. He did not specify the numerical value of this upper boundary.

While the constancy of the deep water inorganic N:P ratio at a value of approximately 16 throughout the world's oceans is remarkable, generations of biochemists and physiologists have commented on the plasticity of the elemental composition of phytoplankton in the field and in laboratory cultures (see Hecky *et al.*, 1993). Can these observations of variability in elemental stoichiometry be reconciled with the original thesis put forward by Redfield (1934)? Redfield himself, aware of the plasticity in the elemental composition of phytoplankton, referred to the 'statistical composition' of phytoplankton to account for variations in elemental composition amongst various plankton communities. He later acknowledged that the constancy of the deep water inorganic N:P ratio may in fact result from a complex balance between several biological processes including nitrogen fixation and denitrification (Redfield, 1958).

Here we attempt to address the issues regarding plasticity of C:N:P of phytoplankton with a view to re-examining the evidence supporting the hypothesis that the ratio of deep water dissolved inorganic N:P is kept more or less constant by biochemical constraints on the average elemental composition of phytoplankton (Falkowski, 2000). The questions remain: What are the upper and lower limits for the C:N:P of phytoplankton in general? Is the average N:P tightly constrained to a value of 16:1 as in the conventional interpretation of the Redfield stoichiometry? Is it 25:1, as would appear to be necessary if Broecker & Henderson (1998) are correct about nutrient levels in the ocean during glacial maxima? Whether the limit is 16:1 or 25:1 or some other ratio, what is the biochemical basis for this limit?

Our approach in answering these questions is threefold. First we examine the variability in the C:N:P stoichiometry of nutrient-replete and nutrient-limited phytoplankton cultures. We determine the most commonly observed C:N:P ratio for phytoplankton grown under optimal nutrient conditions and discuss the critical ratio that marks the transition between N- and P-limitation. Second, we explore the biochemical basis for the variability in elemental composition and provide estimates of lower and upper limits for physiologically achievable C:N:P ratios. Third, the variability in the stoichiometric ratios from laboratory studies is compared with the considerable variability in the C:N and N:P ratios of marine particulate matter.

We show that the laboratory data do not support the idea of a biochemically fixed C:N:P ratio in the proportion defined as the Redfield ratio. Although the average C:N ratio of optimally growing, nutrient-replete cultures is close to the Redfield value of 6·6, the tendency for particulate N:P is much less than 16 (median = 9), most likely due to accumulation of inorganic P storage products. The data also suggest that different phytoplankton taxa are characterized by different C:N:P stoichiometry under nutrient-replete conditions. Furthermore, a very limited data set indicates that the critical N:P ratio that marks the transition between N- and P-limitation is significantly higher than the Redfield ratio. This is in agreement with a theoretical analysis based on a realistic range of biochemical macromolecules contributing to the C, N and P content of phytoplankton. Comparison of the N:P values for ocean particulate matter with the critical N:P suggests that marine phytoplankton are not severely P-limited. Because of the overlap in the lower range of N:P ratio for N-limited and N-replete cultures, the N:P ratio alone does not allow us to determine whether or not the phytoplankton is N-limited.

Elemental composition of marine phytoplankton in laboratory cultures

Differences in elemental composition can arise from interspecific variability amongst algal species with different C:N:P requirements under optimal growth conditions or from physiological acclim-

ation to growth under N- or P-limitation. We consider these sources of variability in turn. In particular, we differentiate amongst:

(i) the N:P and C:N ratios that characterize phytoplankton biomass under nutrient-replete conditions,
(ii) the range of N:P and C:N ratios observed in nutrient-limited conditions, and
(iii) the particulate N:P ratio that divides N-limited from P-limited growth regimes.

Variability of N:P and C:N under nutrient-replete conditions

Nutrient-replete conditions are those in which the concentrations of inorganic nutrients in solution are several-fold greater than the half-saturation constants for nutrient assimilation. Under nutrient-replete conditions, values for N:P range from 5 to 19 mol N:mol P (Fig. 1*B*), values of C:N range from 3 to 17 mol C:mol N (Fig. 1*A*) and values of C:P range from 27 to 135 mol C:mol P (not shown). These ranges appear to arise largely from interspecific or clonal variability rather than from differences in growth conditions or analytical techniques. Different species when cultured under similar conditions and analysed with identical techniques yield a range of N:P and C:N ratios. As early as 1961, Parsons and co-workers documented a range of N:P from 5·6 to 16·5 for nine species of marine phytoplankton. More recently, Sakshaug and co-workers (1983, 1984) documented a range from 7 to 17 in six species and Burkhardt *et al*. (1999) documented a range from 5·0 to 11·8 in seven species. Despite the variability, these data indicate that most of the N:P ratios fall well below the Redfield ratio of 16:1, whereas the C:N ratios are distributed about the Redfield ratio of 6·6 (Table A2, Appendix).

The interspecific variability that is summarized in Fig. 1*A* and *B* does not account for phenotypic plasticity that may arise from differences in growth conditions. Physical/chemical factors that may affect the elemental composition of nutrient-replete phytoplankton include nutrient concentration ratios, daylength, irradiance, salinity and temperature. The few studies that have been undertaken suggest that there is some phenotypic flexibility of C:N:P within nutrient-replete cultures that may arise from variations in culture conditions. However, the magnitude of this variability is typically small relative to the observed interspecific range of N:P (Sakshaug *et al*., 1983; Terry *et al*., 1983; Nielsen & Tonseth, 1991; Nielsen, 1992, 1996). More research is required on a wider range of organisms and over a wider range of conditions to determine the limits on the variability of C:N:P in response to light, temperature and salinity.

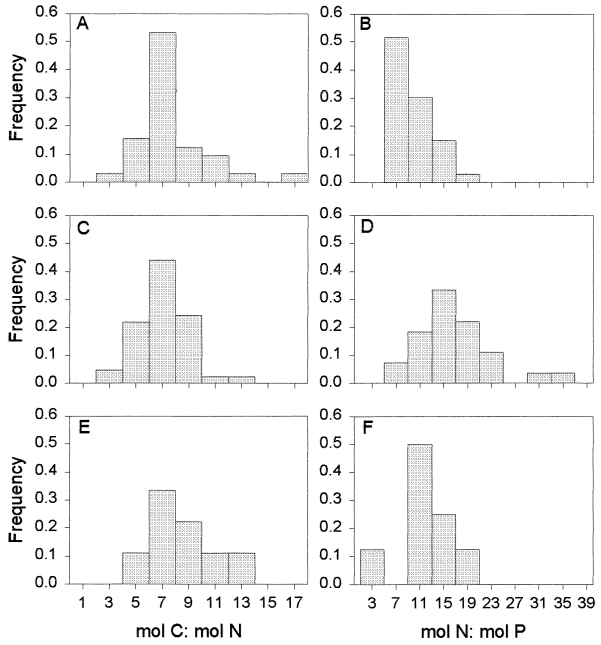

Fig. 1. Elemental composition of (*A*, *B*) nutrient-replete marine microalgal and cyanobacterial cultures, (*C*, *D*) marine particulate matter and (*E*, *F*) nutrient draw-down during phytoplankton blooms. (*C*)–(*F*) should be interpreted with caution as no attempt was made to ensure that the samples summarized here are representative of the ocean as a whole. Rather, the oceanographic observations reflect the data base that is available in the literature. Sources of information for phytoplankton cultures are Burkhardt *et al*. (1999), Goldman *et al*. (1992), La Roche *et al*. (1993), Nielsen (1992, 1996), Parsons *et al*. (1961), Sakshaug *et al*. (1983, 1984), Terry *et al*. (1983). Sources of information for marine particles are Arrigo *et al*. (1999), Banse (1974) citing data of Antia *et al*. (1963), Bishop *et al*. (1977, 1980), Christian & Lewis (1997), Copin-Montegut & Copin-Montegut (1983), Daly *et al*. (1999), Eppley *et al*. (1977, 1988, 1992), Fraga (1966), Herbland & Le Bouteiller (1983), Herbland *et al*. (1998), Karl *et al*. (1995), Menzel & Ryther (1966), Perry (1976), Rios *et al*. (1998), Sakshaug & Holm-Hansen (1986), Tanoue (1985), Tréguer *et al*. (1988). Sources of information for nutrient draw-downs are Arrigo *et al*. (1999), Banse (1974), Codispotti *et al*. (1986), Cooper (1933*a*, *b*), de Baar *et al*. (1997), Haigh *et al*. (1992), Rubin *et al*. (1998), Sambrotto & Langdon (1994), Turner & Owens (1995), van Leeuwe *et al*. (1997), Wallace *et al*. (1995). The number of observations included in the histograms is as follows (*A*) 34, (*B*) 34, (*C*) 41, (*D*) 27, (*E*) 8, (*F*) 8. Only reports that presented C, N and P contents were included in panels (*A*) and (*B*).

Variability of N:P and C:N in nutrient-limiting conditions

The range in C:N:P stoichiometry is much wider under nutrient-limited conditions than in nutrient-replete cells. Physiological variability that arises from growth under nutrient-limiting conditions can override the interspecific variability summarized in Fig. 1. The effects that have received the most attention are increases in C:N and decreases in N:P in N-limited phytoplankton and increases in

both C:P and N:P in P-limited cells. For example, Goldman *et al.* (1979) reported that the C:N of *Dunaliella tertiolecta* increased from 7·1 at 90% of its maximum growth rate to 20 at 10% of its maximum growth rate in N-limited chemostats, and the N:P of *Monochrysis lutheri* increased from 15 at 90% of its maximum growth rate to 115 at 10% of its maximum growth rate under P-limited conditions.

The physiological plasticity of phytoplankton and the phenomenon of luxury consumption allow inorganic N and P levels to be stripped to undetectable concentrations in nutrient-limited cultures of widely varying inorganic N and P contents (Goldman *et al.*, 1979). The N:P of extremely nutrient-limited phytoplankton cells equalled the N:P originally present in the medium over a range of nitrate:phosphate ratios from < 5 to > 50 (Rhee, 1974; Goldman *et al.*, 1979; Elrifi & Turpin, 1985). The ability of phytoplankton to strip inorganic nutrients to undetectable levels in chemostat cultures depends on the dilution rate (i.e. growth rate). The range of N:P is more restricted at higher dilution rates (= higher growth rates) (Goldman *et al.*, 1979).

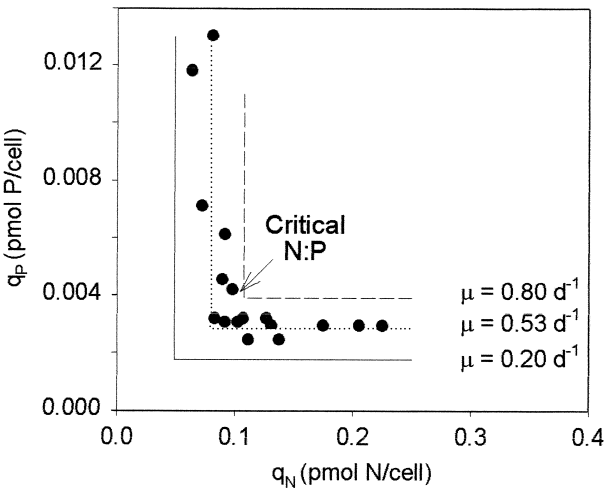

Fig. 2. Contour plot of growth rate on elemental composition for *Scenedesmus* sp. based on the Droop threshold model (based on Rhee, 1978). Contours were obtained under the assumption of a threshold interaction between N- and P-limitation, where growth rate is described by the Droop equation. The Droop equation, $\mu = \mu'(Q - Q_{min})/Q$, was solved for Q_N and Q_P at various growth rates, μ, using the values of $\mu' = 1{\cdot}38$ d^{-1}, $Q_{Nmin} = 0{\cdot}045$ pmol cell^{-1} and $Q_{Pmin} = 0{\cdot}0016$ pmol cell^{-1} determined by Rhee (1978). Also shown as circles are the experimentally determined values of Q_N and Q_P obtained under a range of nitrate:phosphate supply ratios for a growth rate of 0·59 d^{-1} obtained by Rhee (1978).

Critical N:P

The ability of algae to deplete inorganic N and P to undetectable concentrations over a wide range of inorganic N:P ratios in the growth medium raises the issue of how to determine the transition point between N- and P-limitation. To examine this problem we turn to the description of nutrient-limited growth rate as a function of the cellular content (i.e. the cell quota) of a single limiting nutrient (Droop, 1983; Terry *et al.*, 1985). The Droop equation (Droop, 1983) provides a good empirical description of the relationship between growth rate and cell quota of the limiting nutrient in a range of species for a range of nutrients (Morel, 1987). The dependence of the steady-state balanced growth rate on the cell quota of the limiting nutrient can be described by:

$$\mu = \mu'(Q_L - Q_{Lmin})/Q_L$$

where μ is the growth rate, μ' is a constant that is related to the maximum growth rate, Q_L is the cell quota of the limiting nutrient, and Q_{Lmin} is the minimum cell quota of the limiting nutrient.

Experiments in which the rates of supply of two nutrients have been varied independently show that there is a sharp transition point (or threshold) between N- and P-limitation (Droop, 1983). This threshold concept describes the available data, albeit these data are limited to a few studies examining interactions of nitrate and phosphate or vitamin B_{12} and phosphate (Terry *et al.*, 1985). The threshold interaction is clearly evident in a contour plot of growth rate on axes of cell N and P quotas (Fig. 2). For a given growth rate, the cell N quota (Q_N) is low and constant under N-limiting conditions but increases under P-limitation. Conversely, the cell P quota (Q_P) is low under P-limiting conditions and increases under N-limitation (Fig. 2).

Given the threshold nature of N- and P-limitation (Droop, 1983), the transition between P-limited and N-limited growth occurs at the point where the cell contents of both N and P simultaneously limit growth rate (Terry *et al.*, 1985). This is the only point where growth rate is co-limited by the two nutrients and it is defined as the critical ratio (Terry *et al.*, 1985). In order to obtain an increase in growth rate for cells with the critical N:P, the cellular contents of both nutrients must be increased. Thus, an increase in N:P above the critical ratio drives the cells from co-limitation into P-limitation, whereas a decrease in N:P below the critical ratio drives cells into N-limitation. The extent to which variability of N:P away from the critical ratio represents accumulation of inorganic nutrient reserves, low molecular weight precursors or macromolecules has yet to be fully documented (however, see Rhee, 1978).

Terry *et al.* (1985) found that the critical N:P equalled about 40–50 in *Pavlova lutheri* growing at 0·55–1·21 d^{-1}. Somewhat lower values were calculated for the diatom *Phaeodactylum tricornutum*

(25–33 at growth rates from 0 to 1·3 d^{-1}) and the freshwater chlorophyte *Scenedesmus* sp. (22–30 at growth rates of 0–1 d^{-1}) (Terry *et al.*, 1985). This limited data set for critical N:P indicates variability amongst species and with nutrient-limited growth rate. However, all the values are above the Redfield ratio of 16:1.

The critical N:P is not a biological constant, but is expected to depend on the biochemical composition of phytoplankton cells, which may in turn be regulated by environmental conditions. In particular, the critical N:P is expected to be influenced by irradiance because light-harvesting pigment–protein complexes and photosynthetic electron transfer chain components account for a large, but variable, fraction of cell mass (Geider *et al.*, 1996). Under low-light conditions, a high investment in light-harvesting and other components of the photosynthetic apparatus is essential for optimizing energy capture, whereas reduction of cell quotas of light-harvesting components under high-light conditions minimizes the potential for photo-oxidative stress. The effect of irradiance on the critical ratio is likely to be especially pronounced in the cyanobacteria, which have a high N-requirement for the light-harvesting phycobilisomes (Raven, 1984). New data are required to test these speculations.

Summary of findings for phytoplankton cultures

In summary, the available data on the elemental composition of marine phytoplankton cultures indicate that:

1. the physiological range of N:P in phytoplankton is from < 5 under severe N-limitation to > 100 under severe P-limitation,
2. the range becomes increasingly restricted as the species-specific maximum growth rate is approached,
3. the N:P ratio of nutrient-replete phytoplankton ranges from about 5 to 19, with most observations falling below the Redfield ratio of 16,
4. the critical N:P ratio that marks the transition between N- and P-limitation appears to be in the range 20–50, and thus exceeds the Redfield ratio of 16, and
5. the C:N ratio, although variable, has a typical value that is close to that of the Redfield ratio under nutrient-replete conditions.

Although we have a good general understanding of the variability of N:P and C:N in marine microalgal cultures, there are significant gaps in available data. In particular, there are few data on the C:N:P stoichiometry of marine cyanobacteria (especially nitrogen-fixing cyanobacteria and picocyanobacteria) and harmful algal species, and there is very little information on the critical N:P. In fact, critical N:P has been determined for only three taxa, and these taxa are considered by many to be laboratory weeds. In the next section we examine the range of variability in the bulk biochemical composition of both marine and freshwater algae and cyanobacteria, and attempt to use this information to place limits on the C:N:P composition.

Biochemical basis for C:N:P ratio

The variability of the C:N:P composition of phytoplankton can arise either from changes in the concentrations of N- and P-containing organic macromolecules or from the accumulation of nutrient-reserve (polyphosphate, nitrate) or energy-reserve (starch or triglyceride) pools. As there is little information on low molecular weight compounds, we consider here the effect of the major classes of organic macromolecules on the elemental C:N:P stoichiometry. While C is a significant component in all classes of organic macromolecules (24–80 % of dry weight), N and P are enriched in some compounds but notably absent in neutral lipids and carbohydrates (Table 1).

The major pools of organic N are proteins and nucleic acids. In addition, N is present in chlorophylls *a*, *b* and *c*, amino acids, and N-containing osmolytes (glycine betaine). Chitin may be an important pool in diatoms (Conover, 1978). Inorganic N may also contribute to cell N, particularly in cells with large vacuoles that can be used to store nitrate. In contrast, much of the cell organic P is associated with nucleic acids or phospholipids, while generally absent from protein except as reversibly bound to it. RNA is the most abundant P-containing macromolecular fraction in the cell, followed by phospholipid and DNA. In addition, there are smaller pools of P present as carriers of substrate, energy and information (glucose phosphate coenzymes and ATP, cAMP and IP3). P may also be present in inorganic polyphosphate reserves.

The elemental composition of the cellular building blocks is fixed by their molecular structures (Table1). Thus, C, N and P are incorporated into nucleic acids according to a C:P of about 9·6, N:P of about 3·8 and C:N of about 2·6. Based on the structure of the 21 amino acids and their relative abundance in algal proteins (Laws, 1991), one can calculate a theoretical C:N molar ratio of 3·8. Similar considerations of the molecular structure of phospholipids lead to a C:P ratio of approximately 38:1 for this class of compounds. Although this is a minimalist view of cell composition, it allows bounds to be set on the expected C:N:P stoichiometry based on the abundance of organic macromolecules (proteins, nucleic acids, total lipids, phosphoglycerides and carbohydrates).

Table 1. Approximate elemental composition of biochemical classes. The percentage of cell mass (% cell mass) associated with different biochemical fractions reflects the range observed in algae and cyanobacteria under both nutrient-replete and nutrient-limited conditions (see Tables 2–6)

	Elemental composition	% cell mass	gC g^{-1} DW	gN g^{-1} DW	gP g^{-1} DW
Amino acids	–	0–12	–	–	–
Protein[a]	$C_{4{\cdot}43}H_7O_{1{\cdot}44}N_{1{\cdot}16}S_{0{\cdot}019}$	30–65	0·53	0·16	–
RNA[b]	$C_{9{\cdot}5}H_{13{\cdot}75}O_8N_{3{\cdot}75}P$	3–15	0·34	0·155	0·091
DNA[c]	$C_{9{\cdot}75}H_{14{\cdot}25}O_8N_{3{\cdot}75}P$	0·5–3	0·36	0·16	0·095
Lipids[d] (other than phosphoglycerides)	$C_{40}H_{74}O_5$	10–50	0·76	–	–
Phosphoglycerides[e]	$C_{37{\cdot}9}H_{72{\cdot}5}O_{9{\cdot}4}N_{0{\cdot}43}P_1$	5–15	0·64	0·008	0·043
Chlorophyll *a*	$C_{55}H_{72}O_5N_4Mg$	0·2–5	0·74	0·063	–
Chlorophyll *b*	$C_{55}H_{70}O_6N_4Mg$	–	0·73	0·062	–
Chlorophyll *c*	$C_{35}H_{29}O_5N_4Mg$	–	0·69	0·092	–
Carotenoids and xanthophylls[f]	$C_{39-48}H_{52-68}O_{0-8}$	0·2–5	0·80	–	–
ATP	$C_{10}H_{16}O_{13}N_5P_3$	< 0·1	0·24	0·14	0·18
Carbohydrates	$C_6H_{12}O_6$	5–45	0·40	–	–

[a]Based on the structure of the 21 amino acids and their relative abundance in algal proteins (Laws, 1991).
[b]Assumes equal moles of deoxyadenylic, deoxycytidylic, deoxyguanylic and deoxythymidic acids.
[c]Assumes equal moles of adenylic, cytidylic, guanylic and uridylic acids.
[d]This is the composition assumed by Laws (1991).
[e]Assumes equal moles of P in phophatidylinositol, phophatidic acid, phophatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylserine.
[f]Range of elemental compositions taken from Jeffrey *et al.* (1997). The typical value for gC g^{-1} DW given in the table falls within the range 0·75–0·90 with the lowest values observed in some xanthophylls and highest value in ß-carotene.

There are relatively few studies that have attempted to apportion the mass of C, N or P in a phytoplankton cell amongst classes of biochemical compounds. Inconsistencies and ambiguities in such an undertaking may arise because many observations of biochemical composition are based on assays with varying degrees of specificity and accuracy. None-the-less, these ambiguities have been partially alleviated here by reporting observed ranges in concentrations of various organic macromolecules.

Protein

Protein is one of the most abundant macromolecules in the cell, constituting approximately 30–60 % of the cell mass under nutrient-replete conditions (Tables 2, 3). The largest proportion of organic N in phytoplankton is contained in protein. Laws (1991) suggested that under N-limiting conditions, roughly 85 % of the N in phytoplankton cells is allocated to protein. This estimate compares favourably with the recent analysis of Lourenco *et al.* (1998) for 10 marine microalgae in which amino acid residues accounted for 63–88 % of cell N in exponentially growing and CO_2-limited cultures. However, other observations for N-limited and ammonium-replete cultures suggest that proteins account for only 45–80 % of cell N (Table 4). The difference between the results of Lourenco *et al.* (1998) and the others reported in Table 4 may arise in part from free amino acids, which can make up a substantial proportion of cell N. As much as 6–12 % of cell N is found in amino acids in N-replete cultures, the proportion dropping linearly with N-limited growth rate to nil at zero growth in chemostat cultures (Rhee, 1978; Maske, 1982; Lohrenz & Taylor, 1987).

Carbon-rich macromolecules

Carbohydrates and neutral lipids can be major macromolecular components of cells, but are expected to accumulate mainly under nutrient-limited conditions. Both can range between 10 % and 50 % of the dry weight of the cell (Tables 3, 5). Carbohydrates can be divided into structural components that are found in cell walls, and storage components that can accumulate inside or outside of the chloroplast. Structural carbohydrates will account for a higher proportion of cell mass in cells with high cellulose contents in their cell walls. Storage carbohydrates accumulate under light-saturated and nutrient-limited conditions. Similarly, lipids can be divided into polar lipids that play key roles in the cell membranes and neutral lipids that serve as energy storage reserves.

In a comprehensive survey, Shifrin & Chisholm (1981) found that lipids accounted for an average of 17 % of dry weight in log-phase chlorophytes, and 24·5 % of dry weight in log-phase diatoms. Assuming C : lipid of 0·76 (Table 1), and given C : dry weight of 0·47 in the green algae and 0·41 in the diatoms (Shifrin & Chisholm, 1981), this indicates

Table 2. Protein, carbohydrate and lipid contents (in g g^{-1} dry weight) of microalgal and cyanobacterial cultures under nutrient-replete conditions. For some of the entries in this table, dry weight was calculated from organic carbon assuming 1 g C corresponds to 2 g ash free dry weight. See original papers for methodology

Species	Growth conditions	Protein	Carbohydrate	Lipid	RNA	DNA	Reference
Four marine and freshwater diatoms	Log phase	0·27–0·45	–	0·20–0·30	–	–	Renaud *et al.* (1994)
Eight marine and freshwater chlorophytes	Log phase	0·30–0·68	–	0·20–0·31	–	–	Renaud *et al.* (1994)
Twelve strains of freshwater N_2-fixing cyanobacteria	Log phase	0·37–0·52	0·16–0·38	0·08–0·13	0·056–0·096	0·009–0·022	Vargas *et al.* (1998)
Ten marine microalgae and cyanobacteria		0·32–0·59	–	–	0·005–0·075	0·0025–0·028	Lourenco *et al.* (1998)
Eighteen freshwater and 11 marine species	Log phase	–	–	0·13–0·46	–	–	Shifrin & Chisholm (1981)
Six *Scenedesmus* species		0·09–0·56	0·04–0·28	0·05–0·50	–	–	Tahiri *et al.* (2000) from various sources
Emiliania huxleyi	Log phase	0·25–0·32	0·13–0·21	0·45–0·56	–	–	Fernandez *et al.* (1996)
Isochrysis galbana	Log phase	0·38–0·45	0·08–0·10	0·22–0·33	–	–	Fidalgo *et al.* (1998)
	Early stationary phase	0·34–0·36	0·09–0·11	0·34–0·42			
	Late stationary phase	0·28–0·33	0·11–0·14	0·31–0·42			
Tetraselmis suecica	Nutrient-replete at $\mu = 1{\cdot}0\ d^{-1}$	0·64[a]	0·15–0·21[a]	0·18–0·21[a]	–	–	Fábregas *et al.* (1995)

[a]Assumes protein + lipid + carbohydrate sum to 1·0.

that on average lipids accounted for about 27% of cell C in the green algae and 45% of C in the diatoms. These values are somewhat higher than the range of 10–30% of particulate organic ^{14}C that is found in the lipid fraction (chloroform/methanol fraction) after 12–24 h ^{14}C-labelling experiments (Table 3). The accumulation of large pools of these C-rich storage compounds will have pronounced effects on C:N and C:P ratios but will not affect the N:P ratio.

The relative contribution of neutral lipids and carbohydrates to the total dry weight should be highest in nutrient-limited cells with low protein content. It is not clear whether neutral lipids and carbohydrates should vary independently, but the contribution of these two components to the total ash-free dry weight should be inversely correlated with that of protein.

Polar lipids

The cellular abundance of phosphoglycerides, a special class of lipids rich in P, can have a significant effect on the N:P ratio. Phospholipids can account for $< 10\%$ to $> 50\%$ of total lipids (Tables 3,5). Given the role of triglycerides as energy storage products that accumulate particularly under light-saturated or nutrient-limited conditions, and that phosphoglycerides are structural components that depend mainly on the quantity of biological membranes present in the cell, it is reasonable to expect an inverse relationship between the contribution of total lipids to cell mass and the contribution of phospholipids to total lipids. For example, phospholipids accounted for 37% of total lipids in nutrient-replete *Chaetoceros gracilis* but $< 10\%$ in P-stressed cells (Lombardi & Wangersky, 1991).

The proportion of cell P contained in phospholipids can be evaluated for the freshwater chlorophyte *Ankistrodesmus folcatus*. Lipid accounted for 52% of the dry weight of nutrient-replete *A. folcatus* with phospholipids accounting for 13·5% (Kilham *et al.*, 1997). Given a particulate C:P ratio of 80 mol C:mol P in nutrient-replete cells of this alga (Kilham *et al.*, 1997) and the elemental composition for phospholipids from Table 1, we calculate that phospholipids accounted for 36% of cell P in this species. Similar calculations can be made for *Stephanodiscus minutulus* (Lynn *et al.*, 2000), in which phospholipid can be calculated to account for 34% of cell P in nutrient-replete cells.

The phospholipids contain a small amount of N (Table 1). In addition, polar compounds such as chlorophylls are likely to co-purify with the lipid fraction in physical/chemical fractionation schemes. N accounts for about 6% of the mass of chlorophylls *a* and *b* and 9% of the mass of

Table 3. Proportion of organic carbon incorporated into defined macromolecular classes during ^{14}C-labelling. For methods, see original papers

Location	Protein	Carbohydrate + nucleic acid	Lipid	Low molecular weight compounds	Phospholipid /Total lipid	Reference
Phaeodactylum tricornutum	0·30–0·40	0·10–0·15	0·30–0·35	0·10	–	Terry *et al.* (1983)
Log phase phytoplankton cultures	0·39–0·52	0·13–0·17	0·21–0·31	0·11–0·16	–	Laws (1991) from various sources
Eastern North Atlantic	0·35–0·50	0·10–0·20	0·15–0·50	0·10–0·40	–	Fernández *et al.* (1994)
Sargasso Sea phytoplankton	0·35–0·50	0·30–0·38	0·09–0·11	0·07–0·16	0·21–0·59	Smith & D'Souza (1993), Smith *et al.* (1997)
Lake Huron	0·25–0·45	0·34–0·46	0·10–0·16	0·11–0·13	0·38–0·58	Furgal *et al.* (1998)

Table 4. Protein content of marine phytoplankton cultures as a proportion of cell N. For methods, see original papers

	Growth conditions	Protein N/ Total N	Reference
Ten species of marine microalgae	Nitrate-replete	0·65–0·85	Lourenco *et al.* (1998)
Six species of marine microalgae	Nitrate-replete and nitrate-limited	0·67	Dortch *et al.* (1985)
Nannochloris atomis	Ammonium-limited	0·45–0·60	Lohrenz & Taylor (1987)
Phaeodactylum tricornutum	N-replete	0·58	Terry *et al.* (1983)
Skeletonema costatum	Ammonium-limited	0·52–0·82	Maske (1982)

Table 5. Lipid and phospholipid contents of marine and freshwater algal and cyanobacterial cultures. For some of the entries in this table, dry weight was calculated from organic carbon assuming 1 g C corresponds to 2 g ash-free dry weight. For methods, see original papers

	Growth conditions	Total lipid/ Dry weight	Phospholipid/ Total lipid	Reference
Ankistrodesmus folcatus	Nutrient-replete	0·52–0·59	0·23–0·26	Kilham *et al.* (1997)
	P-limited			
	N-limited			
Chaetoceros gracilis	P-replete log phase and P-limited stationary phase	0·10–0·14	0·06–0·25	Parish & Wangersky (1990), Lombardi & Wangersky (1991)
Dunaliella tertiolecta	Log and stationary phase	–	0·25	Lombardi & Wangersky (1995)
Isochrysis galbana	Log phase	0·22–0·33	0·31–0·35	Fidalgo *et al.* (1998)
	Early stationary phase	0·34–0·42	0·16–0·21	
	Late stationary phase	0·31–0·42	0·10–0·13	
Phaeodactylum tricornutum	N-limited	–	0·12	Parish & Wangersky (1987)
	N-replete		0·35	
Stephanodiscus minutulus	Nutrient-replete	0·45	0·36	Lynn *et al.* (2000)
	Si-limited	0·55	0·26	
	N-limited	0·39	0·22	
	P-limited	0·48	0·27	

chlorophyll *c* (Table 1). For C:Chl *a* = 20, C:N = 7·7 (by weight), we calculate that chlorophyll *a* would account for about 2·4% of cell N.

Nucleic acids

There is considerable variability in RNA content between phytoplankton taxa, with RNA accounting for 2·5–13% of dry weight (Tables 2, 6), or 0·4–5% of cell C (based on an RNA:C of 0·01 to 0·15; see Tables 2, 6). Within several species that have been examined, RNA contributes a constant proportion of cell organic matter under nutrient-replete conditions at both light-limiting and light-saturating irradiances (Mann & Carr, 1974; Laws *et al.*, 1983*b*; Thomas & Carr, 1985). This contrasts with bacteria, where RNA content varies in concert with growth rate (Churchward *et al.*, 1982). Cell RNA content

Table 6. RNA, DNA and protein contents of marine and freshwater algal and cyanobacterial cultures. For some of the entries in this table, dry weight was calculated from organic carbon assuming 1 g C corresponds to 2 g ash-free dry weight. For methods, see original papers

	RNA ($g\ g^{-1}$ DW)	DNA ($g\ g^{-1}$ DW)	RNA/DNA ($g\ g^{-1}$)	Protein ($g\ g^{-1}$ DW)	Protein/RNA ($g\ g^{-1}$)	Reference
Agmenellum quadruplicatum[a]	0·030 0·049	–	–	–	–	Laws *et al.* (1983*a*)
Amphidinium carterae	0·025	0·007	3·6	0·65	26	Thomas & Carr (1985)
Amphidinium carterae[a]	0·033	0·013	2·5	–	–	Laws *et al.* (1983*a*), Jones *et al.* (1995)
Anabaena variabilis	0·081	0·012	6·8	0·51	6·2	Healey & Hendzel (1975)
Anabaena variabilis	0·13	0·016	8·1	0·34	2·6	Fontes *et al.* (1992)
Anacystis nidulans	0·055	0·006	9·1	0·40	7·3	Parrott & Slater (1980)
Cylindrotheca sp.[a]	0·037 0·050	–	–	–	–	Laws *et al.* (1983*a*)
Dunaliella tertiolecta[a]	0·025	0·015	1·7	–	–	Laws *et al.* (1983*a*), Jones *et al.* (1995)
Euglena gracilis	0·05	0·006	8·3	–	–	Cook (1963)
Pavlova lutheri[a]	0·068	0·032	2·1	–	–	Laws *et al.* (1983*a*), Jones *et al.* (1995)
Phaeodactylum tricornutum	0·08	–	–	0·30	3·8	Fidalgo *et al.* (1995)
Scenedesmus quadricauda	0·033	0·007	4·7	0·37	11	Healey & Hendzel (1975)
Synechococcus sp.	0·08	–	–	–	–	Kramer & Morris (1990)
Thalassiosira weissflogii[a]	0·075	–	–	–	–	Laws *et al.* (1983*a*)

[a]Converted to dry weight basis on the assumption that carbon contributes 50 % of ash-free dry weight.

and RNA:DNA ratios have been reported to decline under nutrient-limiting conditions. For example, RNA:C declined under N- and P-limiting conditions in *T. weissflogii* (Laws *et al.*, 1983*b*), but the decline may be attributed largely to the accumulation of energy reserve polymers that contributed to cell C but not to cell P. DNA accounts for 0·6–3·2 % of dry weight (Table 6), or 0·2–1·1 % of cell organic C based on a DNA:C of 0·006–0·03 g DNA/g C (Jones *et al.*, 1995). Although the RNA and DNA contents of phytoplankton co-vary when cells of widely different sizes are considered, the RNA-to-DNA ratio shows considerable variability, ranging from 1·7 to 9·1 $g\ g^{-1}$ (Table 6).

Data provided by Laws *et al.* (1983*b*) suggest that RNA can account for virtually 100 % of the cell P in P-limited *Thalassiosira weissflogii*. Specifically, we calculated a C:P of 190 to 390 mol/mol from the measured RNA:C of 0·075–0·15 $g\ g^{-1}$ using the elemental stoichiometry for RNA reported in Table 1. These calculated values of C:P, based on RNA as the only intracellular P compound, are almost identical to the range of measured particulate C:P of 190–380 (Laws *et al.*, 1983*b*). Nucleic acids can also make significant contributions to cell N. N makes up about the same proportion of mass of protein, RNA and DNA (Table 1). Thus, at the lowest protein:RNA of 2·6 observed in microalgae (Table 6), RNA would contain roughly 38 % of the N contained in protein. This would drop to < 4 % at the highest observed protein:RNA of 26 (Table 6).

Low molecular weight compounds

Compared with RNA and DNA, individual P-containing metabolites contribute a relatively small proportion of cell mass. For example, the mass of RNA was found to be 15–75 times that of ATP in six marine microalgae examined by Laws *et al.* (1983*a*), whereas the mass of DNA was 10–30 times that of ATP in eight marine microalgae examined by Jones *et al.* (1995). In contrast, low molecular weight compounds can contribute a significant proportion of cell N. As already noted, amino acids can account for as much as 6–12 % of cell N. The N-rich osmolyte glycine betaine can also make a significant contribution (Keller *et al.*, 1999*a*, *b*). Inorganic N may also contribute to cell N, particularly in cells with large vacuoles that can be used to store nitrate. Inorganic ammonium is a small proportion ($< 1·6$ %) of cell N in N-limited and N-replete diatoms (Dortch, 1982). Nitrate is also a low percentage of cell N in N-limited cells, but can reach about 50 % of cell N in nitrate-replete *S. costatum* (Dortch, 1982).

Partitioning of cell N and P contents amongst biochemical classes

A comprehensive examination of intracellular N partitioning in 10 species of marine phytoplankton (Lourenco *et al.*, 1998) showed that 65–85 % of cell N was associated with proteins and free amino acids, 1·3–13 % with RNA, 0·4–5 % with DNA and

0·2–3% with chlorophylls. A large contribution (6–36%) of inorganic ions to total intracellular N in this study may have arisen from the high concentrations of inorganic N (about 5 mM) in the growth medium. Significantly, Lourenco *et al*. (1998) were able to achieve a mass balance for cell N. Between 80% and 107% of cell N measured directly could be accounted for by the sum of protein, nucleic acid, pigment plus inorganic N.

Although there is a good database for estimating the contributions of RNA, DNA and phospholipids to cell biomass and C content, there are considerably fewer data for estimating the contributions of these compounds to cell P content. Perhaps the most comprehensive attempt to partition cell P amongst these different biochemical fractions is Rhee's (1973) analysis of P-distribution in *Scenedesmus*, which was based on the physical/chemical separation into biochemical components. In P-replete *Scenedesmus*, about 30% of cell P was associated with RNA, < 5% with phospholipid, but most (40%) was associated with polyphosphate. RNA and polyphosphate contents declined markedly in P-limited cells, but a large proportion of cell P still remained in polyphosphates at all P-limited growth rates (Rhee, 1973).

Structural limits on biochemical composition

Although bulk biochemical composition varies widely, there are limits on the (RNA + DNA + chlorophyll *a*)-to-protein ratio that arise from the role of proteins as structural elements in ribosomes, chromosomes and pigment–protein complexes. For example, an equal mass of protein (histones and chromatin proteins) is associated with DNA. Ribosomes are about 35% protein and 65% RNA by weight. Therefore, we might expect proteins and nucleic acids to co-vary and to be most abundant in rapidly growing cells.

Pigment–protein complexes typically contain 2–6 kg of protein per mole of chromophore in eukaryotes, and 6–16 kg protein/mol chromophore in cyanobacteria (Raven, 1984). If we assume that chlorophyll *a* accounts for 40% of cell pigment content, then the protein:chlorophyll *a* ratio of these complexes can be calculated to be 5·6–16 g protein per gram chlorophyll *a*. Thus, for a cell in which protein accounted for 45% of dry weight, RNA for 10% of dry weight and DNA for 1% of dry weight, then about 10% of the protein will be bound to the nucleic acids. If chlorophyll *a* accounts for 1% of dry weight, as it might in cyanobacteria, then a further 12–35% of cell protein will be associated with pigments. If chlorophyll *a* accounts for 4% of dry weight, as it might in light-limited chlorophytes, then 18–50% of protein may be associated with pigments. Clearly, the protein content cannot vary completely independently of the nucleic acid and pigment contents of cells.

Biochemical composition, the Redfield ratio and the critical N:P

It is possible to calculate N:P ratios at various concentrations of RNA, protein and phospholipid using the observed elemental composition and physiological range (Table 1). The Redfield proportion falls along a diagonal separating relatively protein-rich from relatively nucleic-acid rich cells (Fig. 3*A*, *B*). The contribution of phospholipids within the physiologically realistic range of 5–15% of cell mass can affect significantly the calculated N:P (compare Fig. 3*A*, *B*), but not the C:N ratio (Fig. 3*C*, *D*). However, these calculations, and the discussion that follows, should be treated with caution since low molecular weight compounds are not taken into account.

The available biochemical data for nutrient-replete cells, with nucleic acids accounting for 4–15% of cell mass and protein accounting for 30–60% of cell mass, do not greatly constrain the value of N:P (Fig. 3*A*, *B*). The N:P ratio is expected to range between 10 and 40 at low values of phospholipids (Fig. 3*A*). At higher concentrations of phospholipids, significantly lower N:P ratios (5 to 25) were calculated (Fig. 3*B*). While the upper level of the N:P ratios observed in nutrient-replete cultures (Fig. 1) can easily be achieved within the commonly accepted range of the major macromolecules, the lower value of 5 can be obtained only by invoking either high phospholipid content (towards the upper limit of the measured range), lower than physiologically acceptable protein content, or significant accumulation of polyphosphate. The calculated range of N:P (Fig. 3) is also considerably smaller than the range observed in severely N- or P-limited cultures (from < 5 to > 100). This analysis shows that we need more information about the role of storage products in determining the elemental composition of nutrient-replete cells. It also points out that the extreme N:P ratios observed under nutrient limitation are likely to be achieved by the accumulation into storage of the nutrient (either N or P) that is not limiting.

The value of C:N is fairly well constrained by the biochemical data, ranging between 6 and 9 for typical protein, nucleic acid and phospholipid contents of nutrient-replete cells (Fig. 3*C*, *D*). The Redfield C:N ratio of 6·6 is achieved when cells contain about 45% protein and 10% RNA plus DNA when phospholipids account for 5–15% of cell mass (Fig. 3*C*, *D*). Very high values of C:N (> 12) are observed only when protein declines below about 25% of cell mass.

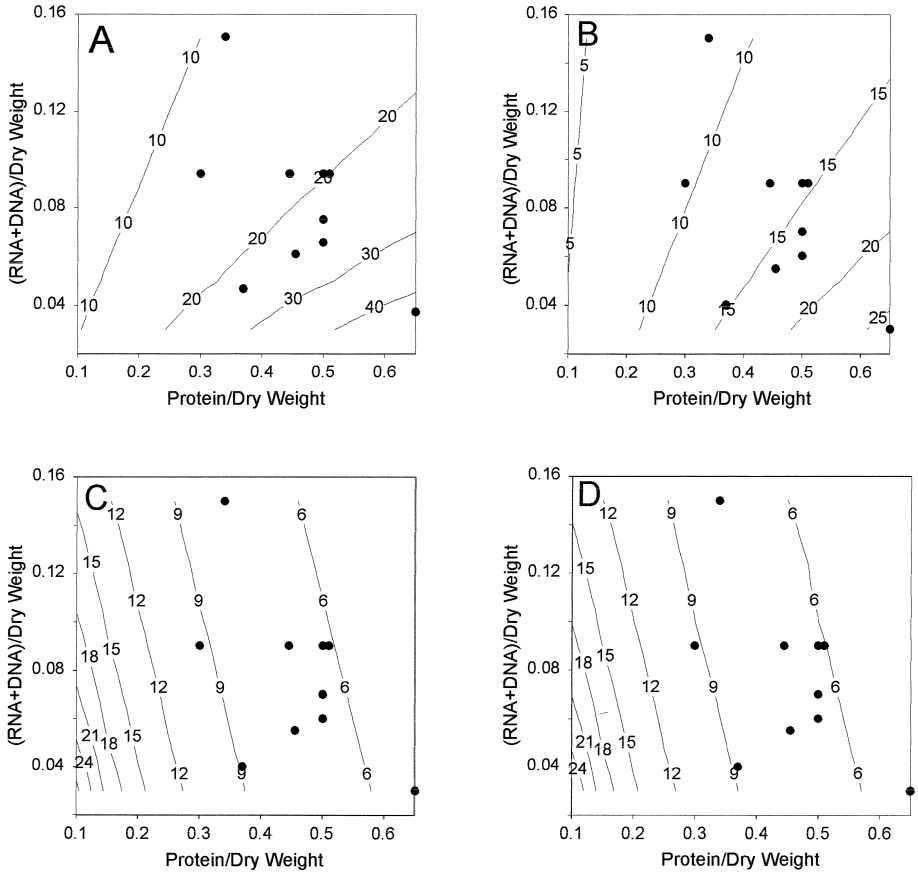

Fig. 3. Contour plots of N:P (*A*, *B*) or C:N (*C*, *D*) on nucleic acid and protein contributions to cell organic matter. Contours are based on the elemental stoichiometries given in Table 1 and the assumptions that phospholipids contribute 5% (*A*, *C*) or 15% (*B*, *D*) of cell organic matter and that there are no other N- or P-containing compounds in the cells. Superimposed on the contours are the observed nucleic acid and protein contents from Tables 2 and 6 for nutrient-replete cells.

Biochemical characterization of cells should place a robust constraint on the critical N:P. This is because the critical N:P is observed only under nutrient-limiting conditions where the macromolecules that are included in our calculations (Fig. 3), rather than storage products, are likely to dominate the intracellular N and P pools. For example, to obtain a critical N:P > 30 requires that protein account for $> 50\%$ of cell mass, RNA plus DNA < 4–6% of cell mass and phospholipids about 5% of cell mass. These proportions are unlikely to be greatly affected by amino acids and nucleotides (ATP, ADP, etc.), which are expected to account for a small fraction of cell mass under conditions where N and P simultaneously limit growth rate.

Unanswered questions

Available measurements of the biochemical composition of marine and freshwater phytoplankton (Tables 2–6) raise fundamental questions about the extent of genotypic and phenotypic variability in the elemental composition of marine phytoplankton. For example, is there systematic variation in the critical N:P and, if so, can it be accounted for in terms of nucleic acid and protein contents? Do RNA:protein and DNA:protein ratios of cyanobacteria differ from those of eukaryotic phytoplankton? Are there differences in RNA:protein and DNA:protein ratios amongst higher taxa of eukaryotic phytoplankton that may contribute to success in different environments? Does RNA:protein vary with growth conditions? Is RNA:protein higher in rapidly growing cells? Is RNA:protein lower in shade-adapted (as opposed to acclimated) taxa due to the higher content of light-harvesting components? The available data do not allow us to answer these questions. However, these questions need to be answered before we can develop a mechanistic understanding of the constraints on the N:P ratio of phytoplankton in the sea. Related questions can be raised for the proportion of phospholipids relative to other cellular macromolecules.

Elemental composition of marine particulate matter

The preceding sections considered the plasticity of C:N:P in phytoplankton cultures and the limits that macromolecular composition place on phytoplankton C:N:P. Here we turn to the values of C:N:P for marine particulate matter.

Elemental composition of particulate organic matter in nature: variability of the C:N and N:P ratios of marine particulate matter

The most comprehensive analysis of the C:N:P composition of marine particulate matter was undertaken by Copin-Montegut & Copin-Montegut

(1978, 1983). Based on 782 pairs of particulate organic carbon (POC) and particulate nitrogen (PN) analyses, 508 pairs of POC and particulate phosphorus (PP) analyses and 315 pairs of PN and PP analyses, they obtained overall stoichiometries of 5·6 C:N, 106 C:P and 16·5 N:P for marine particulate matter. This extensive data set is consistent with Redfield's assertion regarding the average C:N:P stoichiometry of marine particulate matter. However, within their data set, Copin-Montegut & Copin-Montegut found significant regional differences. The particulate N:P ranged from 6·4 in one region of the Southern Ocean to 18·3 in the Mediterranean Sea. Under oligotrophic conditions the N:P of particulate matter tended to exceed the Redfield ratio of 16 and values of 40–50 N:P have been observed in *Trichodesmium* colonies (Mague *et al*., 1977; Letelier & Karl, 1996, 1998; Sanudo-Wilhelmy *et al*., 2001). In contrast, the C:N ratio is much more constrained (Fig. 1*C*).

The Copin-Montegut & Copin-Montegut (1978, 1983) data sets have been supplemented with other observations of C:N, C:P and N:P (Fig. 1*C*, *D*). The data suggest that low particulate N:P ratios are found in regions where inorganic N and P are replete whereas ratios closer to the Redfield ratio are found in regions where inorganic N and P are depleted. For example, Menzel & Ryther (1966) reported a particulate N:P of 5 for the western North Atlantic in January when deep mixing would have introduced nutrients to the surface waters, but an increased ratio of 11–15 by April when the spring bloom would have reduced nutrient levels. High values of N:P (> 20) are found in oligotrophic regions of the equatorial Atlantic and Panama Basin (Bishop *et al*., 1977, 1980).

Measurements made on isolated phytoplankton cells are consistent with the trend identified in the total particulate matter for low N:P to be associated with nutrient-replete cells. The average N:P of a late winter population of the large diatom *Coscinodiscus wailesii* was 11·5 (Tada *et al*., 2000). Significantly, *C. wailesii* represented a large proportion of the phytoplankton biomass, contributing up to 67% of total chlorophyll *a* in the samples examined (Tada *et al*., 2000). The average N:P of the vertically migrating diatom *Ethmodiscus rex* was 9 (Villareal & Carpenter, 1994). Although collected from the oligotrophic Atlantic Ocean and Caribbean Sea, the *E. rex* cells contained significant amounts of nitrate and phosphate, indicating that these populations were N- and P-replete, probably due to mining of nutrients from below the nutricline (Villareal & Carpenter, 1994).

The N:P ratio varied from 5 to 34 in marine particulate matter (Fig. 1*D*), a greater range than that observed in nutrient-replete phytoplankton cultures (Fig. 1*B*) but much lower than the extreme values observed in N-limited or P-limited cultures subjected to extreme N:P supply ratios. The highest values of N:P observed within the marine plankton are within the range of the measured critical N:P that marks the transition point between N- and P-limitation in algal cultures.

In contrast to the wide range of N:P, C:N of marine particulate matter appears to be less variable, with a range of 3·8–12·5. Significantly, the mean C:N of 7·3 (95% confidence limits 6·8–7·8) is close to the Redfield ratio. Furthermore, the limited data on *Trichodesmium* suggest an even narrower range of 4·1–7·3 (Mague *et al*., 1977; Letelier & Karl, 1996, 1998). One can speculate, despite the limited data, that the low C:N ratio observed in this species is related to its ability to fix dinitrogen gas. The C:N ratios for phytoplankton cells isolated from oligotrophic regions tended to exceed the Redfield ratio slightly. They ranged from 7·2–9·7 in *Rhizosolenia mats*, 14·4 in *Ethnodiscus rex* and 10 in *Pyrocystis noctiluca* (Villareal & Carpenter, 1994). However, these cells are relatively rare within the oligotrophic plankton and may not be representative of values in the more abundant cyanobacteria.

Our analysis (Fig. 1*C*, *D*) is based on published values of regional means or the slopes of linear regression analyses and thus may underestimate the full extent of variability of N:P and C:N. For example, the range of C:N within data sets can be quite large. Sharp *et al*. (1980) report a range for C:N from 6·9 to 48 (mean 13·1) for samples from the North Pacific Central gyre, Tanoue & Handa (1979) report a range from 3·9 to 17·5 (mean 8·2) for the North Pacific and Bering Sea, and Banoub & Williams (1973) report a range from 10 to 28 (mean 14·6) for the Mediterranean Sea. Unfortunately, these ratios are based on unreplicated samples and therefore confidence limits cannot be placed on the extreme values.

Variability in the $TCO_2:NO_3^-$ and $NO_3^-:PO_4^{3-}$ draw-down ratios

The draw-down of total inorganic carbon (TCO_2), nitrate and phosphate during phytoplankton blooms (Fig. 1*E*, *F*) can deviate significantly from the Redfield ratio of 106:16:1 for particulate matter. Inorganic N:P draw-down ratios ranged from 4·4 to 19 based on co-variation of nitrate and phosphate in upper ocean waters and bioassay experiments (Fig. 1*F*). Although representing a very limited sample of the ocean, seven of eight reported values fall below 16:1. These results are by default for nutrient-replete (at least N- and P-replete) phytoplankton since the observations require detectable concentrations of nitrate and phosphate and the half-saturation constants for nitrate

and phosphate assimilation for growth are near the detection limits of the techniques employed to measure these nutrients (Morel, 1987).

The draw-down ratio for TCO_2-to-nitrate ranges from 8·6 to > 20 in the open ocean (Fig. 1*E*). All these values exceed the Redfield ratio of 6·6. One explanation for this difference is release of dissolved organic matter (DOM) with a high C:N ratio (Banse, 1994). However, the DOM data available to Sambrotto *et al*. (1993) suggested that the high C:N draw-down ratio could not be ascribed exclusively to production of DOM. Conversely, the utilization of nitrogen sources other than nitrate, such as a labile component of dissolved organic nitrogen, urea or ammonium, could compensate for the nitrate deficiency (Palenik & Henson, 1997). There is growing evidence that some phytoplankton species, including the bloom-forming *Emiliana huxleyi*, can grow very well on organic nitrogen compounds such as acetamide (Palenik & Henson, 1997). Another possible explanation for the high TCO_2:nitrate draw-down ratio is nitrogen fixation (Walsh, 1996), although detectable concentrations of inorganic nitrate and ammonium should suppress nitrogenase activity during the 'high' nutrient conditions that support phytoplankton blooms and allow draw-down ratios to be measured. Finally, elevated TCO_2:nitrate draw-down ratios may reflect growth of those phytoplankton species with a C:N ratio that exceeds 6·6. Several of the entries in Fig. 1*A* show C:N ratios for nutrient-replete phytoplankton cultures > 10, although most fall within the range 5–8. In this context, it is useful to have C:N:P of particulate matter on the same time scale as the draw-down of nutrients during blooms. Arrigo *et al*. (1999) found that the C:N ratio calculated from the draw-down of nutrient was comparable to that of particulate matter in the case of *Phaeocystis* (7·8 versus 7·7), but not in the case of diatoms (9·2 versus 6·4). This can perhaps be attributed to a difference in excretion of dissolved organic carbon between the two groups.

In summary:

1. the overall mean N:P of particulate matter is about 16,
2. however, the N:P ratio of particulate matter from N- and P-rich waters and the nitrate:phosphate draw-down ratio tend to be < 16, whereas the N:P of marine particulate matter from nutrient-poor oligotrophic regions tends to be > 16,
3. the C:N of particulate matter does not show a systematic variation with the inorganic N and P concentrations, and has a mean value that is close to the Redfield ratio, whereas
4. the limited data on the TCO_2:nitrate draw-down ratio are consistent with the Redfield ratio (mean 9·7, median 8·6, 95% confidence interval 6·2–13·2).

Discussion

It is often asserted that the C:N:P composition of marine plankton is relatively uniform (Goldman *et al*., 1979; Hecky *et al*., 1993), and many theoretical investigations of primary productivity and marine geochemistry invoke this uniformity at the Redfield proportions (i.e. Falkowski, 1997; Tyrrell, 1999). Here we have reviewed the available data on C:N:P elemental composition from nutrient-replete and nutrient-limited marine phytoplankton cultures, dissolved inorganic nutrient draw-down during marine phytoplankton blooms and marine particulate organic matter. Our analysis suggests caution in application of the Redfield ratio in theoretical biogeochemical analyses and as a conversion factor in field studies. In fact, the evidence for biochemical or physiological constraints imposing a N:P ratio of 16:1 or C:P of 106:1 on phytoplankton production is weak at best. On the contrary, the available data from nutrient-replete cultures and the experimentally determined critical N:P ratios point to the potential importance of values of N:P either lower or greater than 16:1, respectively. In contrast, the C:N ratio is much more tightly constrained by the data (Fig. 1) and theoretical analysis (Fig. 3) to a value near the Redfield ratio of 6·6.

Biochemical constraints on the N:P ratio

Mass balance calculations, based on the physiologically achievable range of the dominant biochemical macromolecules in the cell, point to a range of N:P from 10 to 40 (Fig. 3). The lower boundary of this range in N:P can be raised to 16 at a protein content of 40–50% of organic matter, which is expected in nutrient-replete cells, and at an intermediate range of phospholipid and nucleic acid. The lowest N:P ratios observed in the nutrient-replete cultures (Fig. 1*B*) can be reconciled with the biochemical analysis (Table 1, Fig. 3) only by invoking the intracellular storage of P. The critical N:P ratio will be observed when cells are simultaneously N- and P-limited, and is expected to reflect a biochemical composition that is dominated by nucleic acids, phospholipids and protein but where no N and P storage products are present. Based on protein contributing 40–60% of organic matter, one expects the critical N:P to take a value between 10 and 50 (Fig. 3*A*, *B*) depending on the nucleic acid and phospholipid contents. The ratio could be higher for cells with significant contents of N-containing osmolytes.

Geochemical implications of the Redfield ratio

We have used multiple approaches to determine whether or not the C:N:P ratio of phytoplankton is

constrained to values approaching the Redfield ratio. None of our analyses has suggested that the average elemental composition of phytoplankton is fixed, or should have remained invariant over past geological times. In fact N:P values of 25, as suggested by Broecker & Hendersen (1998), are well within the limits that we have estimated on the basis of biochemical composition. As clearly articulated by Redfield in his 1958 article, the similarity in the elemental composition of organic matter and deep nutrient ratios reflects the continuous recycling of elements between those two pools. However, our analysis of algal plasticity suggests there is little evidence that a biochemical requirement for Redfield proportions in C:N:P of phytoplankton biomass can be invoked to be responsible for maintaining the Redfield ratio over geological, glacial and interglacial timescales. As a consequence, our analyses suggest that other biological processes, such as a change in denitrification relative to nitrogen fixation (Redfield, 1958; Falkowski, 1997), may well lead to variations in the N:P ratio of both deep water nutrients and organic particulate matter.

Appendix: Statistical summary of C:N:P data

Distributions of C:N, N:P and C:P for phytoplankton cultures and marine particles were tested for normality using Kolmogorov–Smirnov (K–S), Lilliefors and Shapiro–Wilks tests. In all cases, the results of the K–S test were consistent with normal distributions. The Lilliefors and Shapiro–Wilks tests indicated that the marine particulate ratios were normally distributed, but the culture data were not. Based on these tests, we conclude that, overall, the particulate data from the field fit a normal distribution, and because the field particulate data are normally distributed, we can say that the Redfield ratio applies within the 95% confidence interval of the mean for C:P and N:P (Table A1). However, the mean C:N slightly exceeds the biologist's Redfield ratio for particulate matter of 6·6, but not the geochemist's Redfield ratio for dissolved nutrients of 7·0. Summary statistics are presented for the phytoplankton cultures (Table A2), despite the facts that Lilliefors and Shapiro–Wilks tests indicated that the data were not normally distributed. Non-parametric tests were employed to compare the C:N, N:P and C:P ratios between phytoplankton cultures and marine particles. Application of the Mann–Whitney test indicated significant differences between the mean values for cultures and marine particles for N:P ($p < 0{\cdot}001$) and C:P ($p < 0{\cdot}005$), but not C:N ($p = 0{\cdot}84$). Similarly, the K–S test indicated that N:P ($p < 0{\cdot}001$) and C:P ($p < 0{\cdot}05$) ratios were significantly different between cultures and marine particles, but C:N ($p > 0{\cdot}1$) was not. Thus, we conclude from the non-parametric statistics that the N:P and C:P ratios from the culture data are different from the particulate field data and therefore are not Redfield.

Table A1. C:N, N:P and C:P ratios of marine particulate matter (based on data from sources listed in the legend to Fig. 1)

	C:N	N:P	C:P
Mean (standard deviation)	7·3 (1·7)	16·4 (6·2)	114 (45)
Median	7·6	15	101
95% confidence limits	6·8–7·8	14·0–18·9	94–134
Range	3·8–12·5	5–34	35–221
n	41	27	23

Table A2. C:N, N:P and C:P ratios of nutrient-replete phytoplankton cultures (based on data from sources listed in the legend to Fig. 1)

	C:N	N:P	C:P
Mean (standard deviation)	7·7 (2·6)	10·1 (3·9)	75 (31)
Median	7·1	9·0	75
95% confidence limits	6·8–8·7	8·7–11·5	64–86
Range	4–17	5–19	27–135
n	34	34	34

The results of this statistical summary should be interpreted with caution. Each of the values included in our data summary (Fig. 1, Tables A1, A2) was a typical value (mean or slope of a linear regression) for a region of the ocean. The original papers cited in the legend of Fig. 1 should be consulted for the data analysis that was employed to obtain these typical values. We do not report the variance around this mean, although in some cases it could be considerable. The physical boundaries of the regions were determined by the investigators who reported the results. There is likely to be temporal variability within a region that may not have been adequately sampled. Moreover, the data that we included in our analysis were based on data sets of varying size. We did not examine whether the values at the ends of the range of C:N, N:P and C:P ratios differ significantly from the Redfield ratios without access to the raw data (we employed the published means or slopes of regression analyses). Such an analysis of the published and unpublished data should be undertaken.

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