

MINIREVIEW

RATIONALIZING ELEMENTAL RATIOS IN UNICELLULAR ALGAE¹

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In 1934, the year Harold Urey was awarded a Nobel prize in chemistry for the discovery of deuterium, Alfred Redfield (Fig. 1), a 44-year-old animal physiologist at Harvard University, proposed that marine plankton have relatively constrained elemental ratios (Redfield 1934). The so-called Redfield ratios of 106 C: 16 N: 1 P (by atoms) were subsequently embraced by many biological oceanographers and geochemists as canonical values, comparable to such physical constants as Avogadro's number or the speed of light in a vacuum. Although the wholesale adoption of a simple chemical analysis by a large group of otherwise highly self-critical scientists was certainly not Redfield's intention, his fundamental interpretation of the causes and consequences of constant elemental ratios in the ocean has become a key foundation for biogeochemistry. Nonetheless, despite the passage of almost 70 years since his landmark paper, there is no biological understanding of elemental ratios. Although Redfield ratios per se have become an accepted empirical observation, their deeper meaning remains obscure. Here I examine how Redfield and his famous elemental ratios evolved, and present some analyses about their meaning.

Redfield's interest in elemental ratios was stimulated by the earlier work of the British marine chemist, H. W. Harvey (Harvey 1926). Redfield noted that the ratio of dissolved fixed inorganic nitrogen (nitrate) to phosphate in seawater was remarkably constant regardless of the absolute concentration of the molecules, and was similar (but not exactly equal) to the bulk N/P ratio of what he referred to simply as plankton. The original seawater analyses of Redfield were not corrected for salt; this was rectified in an article by Cooper in 1938. In 1958, Redfield used the chemical analyses for

the (average) plankton provided by Richard Fleming in an obscure (and unreviewed) publication from 1940 (Fleming 1940) to (re)derive the famous ratio (Redfield 1958). The term *plankton* was originally used by Fleming in the broadest context, and included both phytoplankton³ and zooplankton (but not bacteria or picoplankton, which at the time were not sampled). As time went on, more and more chemical analyses of seawater and both marine and lacustrine plankton (mostly phytoplankton were obtained) (Vinogradov 1953, Haug and Myklestad 1973, Copin-Montegut and Copin-Montegut 1983, Hecky et al. 1993), and the basic premise that (autotrophic) organisms utilize nitrogen and phosphorus in the proportion in which they are found in seawater, and return these elements back to seawater upon their death and decomposition, was largely embraced by geochemists (Sverdrup et al. 1942, Broecker and Peng 1982, Anderson and Sarmiento 1994). The elemental composition of seawater was therefore thought to represent the steady-state ratio of the remineralized (i.e. oxidized) organic matter. A summary of the results of chemical analyses, replicated by several workers, is provided (Table 1).

The concept of a constant elemental composition for plankton and seawater is extremely convenient for modeling ocean biogeochemistry and marine planktonic processes (Broecker and Peng 1982, Pahlow and Riebesell 2000), but the conceptual understanding of the biological basis for variability in elemental ratios is lacking. Hence, there are numerous papers in the literature showing that the bulk elemental composition and/or nutrient assimilation of various phytoplankton assemblages differ from the Redfield ratio (e.g. Menzel and Ryther 1964, Sambrotto et al. 1993). Variability in the elemental composition of plankton was certainly recognized by Fleming, who wrote (as a co-author of *The Oceans*, Sverdrup et al. 1942), "The ratios given above hold very well for the nitrate and phosphate in ocean waters, but since they represent the net effect of biological activity, marked deviations from the ratios may be found in individual types of organisms."

Redfield's fundamental insight into the chemistry of aquatic ecosystems was not dependent on a specific elemental ratio per se, but rather was related to the coupling between the chemical composition of plankton (specifically the N/P ratio) and that of seawater.

Key index words: Gaia hypothesis, iron, nitrogen fixation, oceanic productivity, Redfield ratio

Abbreviations: HNLC, high nutrient, low chlorophyll; ppGpp, guanosine 5'-diphosphate, 3'-diphosphate

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³What were called *netplankton*.



FIG. 1. Alfred C. Redfield (1890–1983). Photo by John Hahn (© Woods Hole Oceanographic Institution, reproduced with permission). A brief biography of Alfred Redfield can be found on the World Wide Web at <http://www.nap.edu/readingroom/books/biomems/aredfield.html>.

Although it had already been understood that the stoichiometric assimilation and remineralization of nutrients provided a rationale for the remarkable similarity of N/P ratios in plankton and seawater, Redfield provided a rather startling interpretation as to the causality of this relationship. He suggested that the nitrate/phosphate ratio in the ocean interior is virtually identical to that of the plankton because the *plankton determine the chemical composition of the ocean*. That rather mind-boggling thought, which preceded the formal concepts of a Gaia world by two decades (Lovelock 1979), was based on the conclusion that the concen-

tration of fixed inorganic nitrogen is biologically determined, whereas the concentration of phosphate is set by the ratio of riverine inputs from continental sources to outputs via burial in sediments. Influenced by discussions with the preeminent aquatic ecologist, G. Evelyn Hutchinson, Redfield suggested that when fixed inorganic nitrogen becomes limiting, nitrogen fixation would meet the demands of the planktonic community. Hutchinson, who literally wrote the treatise on limnology (Hutchinson 1971), understood that primary production in most northern hemisphere lakes is limited by phosphate; when nitrogen becomes limiting in such lakes, nitrogen-fixing cyanobacteria often provide a source of fixed nitrogen for the primary producers. This is an ecological analog of just-in-time parts supply in manufacturing. Hence, Redfield concluded that phosphate is the nutrient that constrains oceanic productivity. This view was embraced by geochemists for decades to come (Broecker and Peng 1982, Tyrrell 1999).

What Redfield did not anticipate was that nitrogen fixation in the ocean could itself be limited by some other factor. This proposition is supported by an increased understanding of nutrient cycles and phylogenetic diversity in aquatic ecosystems (Capone et al. 1997, Karl et al. 1997, Falkowski et al. 1998). Perhaps ironically, the concept of nitrogen limitation of oceanic primary production is most clearly supported by geochemical evidence. The concentration of fixed inorganic nitrogen in the central ocean systems is vanishingly low: in some cases, only a few nanomolar. With only two exceptions, in all ocean basins, fixed nitrogen/phosphate ratios are almost always higher in the ocean interior than at the surface (Falkowski 1997, Fanning 1992, Gruber and Sarmiento 1997). The lower N/P ratio in the euphotic zone is a consequence of preferential nitrogen assimilation by phytoplankton. If fixed nitrogen is so scarce, then why are there not more nitrogen-fixing cyanobacteria?

Our current working hypothesis suggests that marine nitrogen fixers are limited by iron (Falkowski 1997, Karl et al. 1997). The rationale for this hypothesis is based on both physiological and geochemical evidence. Marine nitrogen fixers have relatively high iron demands, not only as a consequence of the requirement for iron in nitrogenase, but also because the relative cellular concentration of photosystem I appears to be high (Subramaniam et al. 1999). In both heterocystic and nonheterocystic nitrogen-fixing cyanobacteria (the latter being the more prevalent cell type in the ocean), PSI supplies the requisite ATP for nitrogen fixation via cyclic electron transport and may also help scavenge oxygen via the Mehler reaction (Raven 1988). In the open ocean, the aeolian supply of iron is critical for meeting the high iron demand of nitrogen-fixing cyanobacteria (Falkowski et al. 1998). While identification of iron-limited areas has been confined primarily to the so-called high-nutrient, low-chlorophyll (HNLC) regions (Coale et al. 1996), there is growing evidence that large areas of some

TABLE 1. Ratios of C: N: P (by atoms) in plankton and sea water

Source	By atoms		
	C	N	P
Redfield (1934), plankton	137	18	1
Redfield (1934), seawater	—	20	1
Cooper (1938), seawater	—	15	1
Fleming (1940), phytoplankton	108	15.5	1
Fleming (1940), zooplankton	103	16.5	1
Fleming (1940), average ^a	106	16	1
Copin-Montegut and Copin-Montegut (1983), plankton and particulate matter	103	16.1	1
GEOSECS (nitrate/phosphate ratio > 500 m), in seawater	—	14.7	1

^aThis average of phytoplankton and zooplankton is the Redfield ratio (Redfield 1958).

central ocean gyres are also iron limited (Behrenfeld and Kolber 1999). Moreover, however, the distribution of nitrogen-fixing cyanobacteria appears to correspond to high aeolian fluxes of iron. Aeolian fluxes of iron are, in turn, regulated by episodic aridity and proximity to continental sources. Regions of the western tropical Atlantic receive large iron inputs from sub-Saharan Africa. Either as a consequence or by coincidence, nitrogen-fixing cyanobacteria provide so much fixed nitrogen to this region of the ocean that it becomes demonstrably phosphate limited. In contrast, the subtropical South Pacific is extremely iron limited, fixed nitrogen concentrations are low, and there is excess phosphate in the upper ocean.

From an experimental perspective, it may be extremely difficult to prove directly that iron limitation of nitrogen fixation occurs in the open ocean. Unlike the situation in HNLC regions, where the direct injection of iron resulted in dramatic and unambiguous stimulation of primary production, iron enrichments in low nutrient, low chlorophyll waters would have to be sustained for weeks or possibly even months for marked changes in nitrogen fixation to occur. Such an experiment may be technically feasible (e.g. using a surplus supertanker to squirt the ocean surface continuously with small quantities of ferrous sulfate), but the potential political and economic constraints are formidable. Consequently, in the near term, a chimera between experimental, phytoplankton, biochemical ecologists, and trace metal geochemists will be required to either support or refute the hypothesis that iron limits nitrogen fixation in marine ecosystems.

The single largest biochemical constituent of most unicellular algae is protein (Parsons et al. 1961). To quote Jack Myers, the essential features of unicellular algae are "organisms small in size with a minimum of skeletal crud, rich in protein as cellular machinery and with potential for an intrinsically high metabolic rate" (Myers 1980). As proteins require a significant investment in reduced nitrogen, the relatively low C/N ratios of unicellular algae reflect the relative abundance of protein. The single largest investment of phosphorus is nucleic acids, specifically ribosomal RNA. Is there a biologically constrained ratio of nucleic acids to proteins in unicellular organisms (Elser et al. 1996)? Is there some canonical ratio of, for instance, ribosomes to protein within eukaryotic cells that is highly constrained? For example, when cells are growing at an optimal rate, does the ratio of ribosomal RNA to protein conform to a common value for all prokaryotes and eukaryotes? Many investigators have noted a strong correlation between bulk RNA and growth rate (i.e. the rate of protein synthesis) (Sutcliffe 1965, Dorch et al. 1983, Kemp 1995). In prokaryotes, there is strong experimental evidence that this relationship is causal. It is linked to the synthesis and abundance of guanosine 5'-diphosphate, 3'-diphosphate (ppGpp), a molecule that influences the initiation of rRNA synthesis and protein elongation (Ryals et al. 1982, Rojas et al. 1984, Glass et al.

1986). The pool of ppGpp is negatively correlated with growth; under conditions of nutrient limitation, ppGpp increases as rRNA synthesis decreases and growth rates decline. In eukaryotes, other signal molecules regulate the feedbacks between rRNA and protein syntheses.

The main purpose of these brief comments is to stimulate a discussion on scaling geochemical concepts to biological experiments. The original data used to derive the ratios were sparse, and subsequent analyses have shown that the classical ratios are generally valid for the ocean and large lakes, but not for small bodies of water (Downing and McCauley 1992). Redfield et al. (1963) also clearly realized that phytoplankton taxa with cellulose walls may have higher C/N or C/P ratios than cells with silicate frustules and that cells deprived of or limited by N or P may accumulate carbohydrate or lipids. Like the Dole effect for oxygen isotopic fractionation in the atmosphere (Berry 1992), the resulting elemental stoichiometry that we call the Redfield ratio is a result of nested processes that have a molecular biological foundation but are coupled to biogeochemical processes on large spatial and long temporal scales (e.g. ocean basins and thousands of years). Although on the scale of ecosystems, interactions between processes such as nitrogen fixation and denitrification become critical, molecular biological and biochemical studies may help provide a clearer insight into the factors that constrain elemental ratios from a mechanistic perspective. It is perhaps ironic that a paper by Broecker and Henderson (1998) hypothesizes that N/P ratios are basically unconstrained and could rise to 25 during glacial periods (when iron fluxes, and hence nitrogen fixation, might have been high). Can biologists support or refute such a hypothesis with hard data or fundamental understanding? At present the answer is simply "No," although it is conceptually difficult to understand why such a large deviation in elemental ratios would occur. Experimental phycologists, however, hold an important key to the puzzle of the Redfield ratios. 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. This upper bound appears to rest on processes that couple protein synthesis to ribosome abundance in photosynthetic eukaryotes. There is yet work to be done.

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