〈Review〉
식물학회지 제31권 제1호
Korean J. Bot. 31(1) : 69-82(1988)

Light, Nutrient Requirements, and Optimum N:P Ratios in Unicellular Algae*

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單細胞 藻類에서 光, 營養分 要求性 및 窒素와 燐의 最適 比率

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UNDERWATER LIGHT FIELD

Limitation by mineral nutrients is one of most important factors regulating primary production in aquatic environments. In the majority of freshwater lakes P is the least abundant of the major nutrients required for algal growth and is commonly the first element to be depleted during the growing season. In off-shore and oceanic waters nitrogen is generally most scarce relative to the major nutrients. In many cases, however, the primary production is affected at the same time by many factors other than nutrients, such as suboptimal irradiance and temperature, zooplankton grazing, and parasitism. Of these overlapping factors, the light is of particular importance to primary producers because of its wide temporal and spatial variabilities in the aquatic ecosystem.

The light climate in aquatic environments is far more variable than that in terrestrial environments. As light penetrates water, radiant energy is scattered and absorbed by water molecules, and attenuates exponentially according to the Beer-Lambert Law:

$$I_d = I_o e^{-kd} \qquad (1)$$

where d is depth, I_o is the incident radiation on the surface of water, I_d is the photo flux density at the depth d, and k is the extinction coefficient. The spectral quality of light also changes simultaneously since the extinction coefficient varies with wavelength. The relative absorption in clear waters is very high in the infrared region of the spectrum, decreases rapidly in the lower wavelengths to a minimum absorption in the blue, and then increases again in the violet and especially UV wavelengths. In natural waters, light transmission is also reduced and spectral quality is altered by the optical properties of water such as the nature of suspended particulates and dissolved organic matter. Therefore, the habitat of phytoplankton is characterized by widely varying photon flux densities and diverse spectral regimes in comparison to that of terrestrial plants.

*A plenary lecture at the XVI Pacific Science Congress, Seoul, Korea, 1987.

The underwater light field is temporally dynamic. The most important temporal changes are probably those caused by vertical mixing and surface waves and other meteorological conditions such as passing clouds. The time scale of such changes ranges from a fraction of seconds, which is generated by surface wave actions, to hours associated with vertical mixing. Therefore phytoplankton must be able to adapt to a variety of light conditions in generally nutrient-limited environments. The present paper will discuss changes in nutrient requirements and growth kinetics associated with responses to rapidly fluctuating light and with light-shade adaptation which is probably the most important physiological mechanism for adjustment in the mixing layer.

RESPONSES TO LIGHT

The light fluctuations generated by surface wave actions have frequencies ranging from 0.01 to 10 Hz and can change irradiance two to three fold (Snyder and Dera, 1970; Dera *et al.*, 1975; McFarland and Munz, 1975). The maximum amplitude occurs at a certain depth below the surface because of light attenuation and the focusing effect and then decreases with depth as the focusing becomes less effective in relation to other optical phenomena (Dera *et al.*, 1975).

Such fast fluctuations at saturation intensities seem to influence the photosynthetic responses of phytoplankton near the surface. Walsh and Legendre (1982) and Quéiguiner and Legendre (1985) reported that both photosynthetic efficiency — i.e., the slope of the photosynthesis-light curve — and photosynthetic capacity (P_{max}) varied with changes in light frequencies, but with no pattern in the changes. There was little difference in cellular chl *a* content. Walsh and Legendre (1983) found the highest efficiency at 10 Hz with no change in the photosynthetic capacity. In the freshwater alga *Scenedesmus obliquus*, the efficiency was essentially the same between constant and fluctuating light (0.06 Hz), but the capacity was higher in fluctuating light, with higher cell chl *a* content (Shin *et al.*, 1987). The underlying mechansim is unclear. However, similar higher photosynthetic rates in C_3 plants under fluctuating light have been attributed to light-enhanced dark CO_2 fixation (Miyachi, 1979; Chazdon and Pearcy, 1986a; 1986b; Sharkey *et al.*, 1986).

Growth rate is also faster under high-frequency lights (e.g., Shin *et al.*, 1986) and therefore, for algal mass cultures for biomass production, culture systems are generally engineered to provide oscillating light conditions (Livansky, 1979; Markl, 1980; Lee and Pirt, 1981; Laws *et al.*, 1983).

Light-shade adaptation is probably one of the most important adaptive strategies to environmental changes possessed by photosynthetic organisms. In well mixed waters, turbulent fluid motions vertically displace phytoplankton in the water column, thus exposing them to exponentially changing light intensities and concomitant shifts in spectral environments. In stratified waters, on the other hand, they may be exposed to varying light intensities for an extended period of time. In light-shade adaptation for planktonic algae, therefore, the time scale for the adaptation is of critical importance. If the mixing rate is faster than the time scale

for adaptation, the adaptation can be only minimal or partial at best. If the rate is slower than the rate of adaptation, however, adaptation may be complete, therefore photosynthetically suffering little throughout vertical transport. Light-shade adaptation has been frequently modeled by the first order kinetics. The first order kinetic constant (k) for shade adaptation for marine diatoms and green algae ranged from 1.7×10^{-2} to 5.2×10^{-3} hr^{-1}. These constants are equivalent to half-times of 41 hrs to 133 hrs, respectively [$t_{0.5}=\ln 2/k$]. However, adaptation to high light is much faster (Falkowski, 1980; Post *et al.*, 1984).

Phytoplankton adapt to light intensities by various mechanisms, such as regulating the content of photosynthetic pigments, enzymes for dark CO_2 fixation, and electron carriers. Under low photon flux densities, cellular chlorophyll *a* concentrations increasae. The increase stems from either an increase in the size of the light harvesting complexes associated with a fixed number of photosyntetic units (PSUs) or an increase in the number of PSUs in the thylakoid membrane (for reviews see Prezelin, 1981; Falkowski, 1980; Richardson *et al.*, 1983). However, other experimental observations indicate that the relationship between photosynthetic responses and the characteristics of PSUs may not be simple and direct (Richardson *et al.*, 1983).

The ratio of chl *a* to accessory pigments was in general higher at suboptimal light intensities (Van Liere *et al.*, 1979; Larkum and Barrett, 1983; Neori *et al.*, 1984; Lönneborg *et al.*, 1985) and this increase was accompanied by and increase in the light absorption by accessory pigments relative to absorption by chl *a* (Neori *et al.*, 1984; Lönneborg *et al.*, 1985).

Adaptation to low photon flux density by changing the activity of dark enzymatic processes was also found (Beardall and Morris, 1976; Senger and Fleishhacker, 1978a; Rivkin *et al.*, 1982; Chang *et al.*, 1983). In the oceanic dinoflagellates *Pyrocystis notiluca* and *P. fusiformis*, there was a parallel change in RuBPCase and P_{max} with little change in both the cellular pigment concentration and the ratio of P_{max} to K_I (the light intensity where $P=0.5\ P_{max}$) (Rivkin *et al.*, 1982). *Scenedesums obliquus* and the symbiotic dinoflagellate *Symbiodinium microadriaticum* regulate the size of electron carrier pools in the photosystems in response to light regimes (Senger and Fleishhacker, 1978b; Chang *et al.*, 1983).

The relative concentration of light-harvesting pigments changes with spectral qualities in both procaryotic and eucaryotic algae (Kohl and Nicklish, 1981; Wallen and Geen, 1971a; Jeffrey and Vesk, 1977; Vesk and Jeffrey, 1977; Humprey, 1983; Wan Liere and Walsby, 1983). In general the concentration of pigments increases in the spectral regimes that are less able to be absorbed and used. Photosynthetic products and intracellular glycerol accumulation for osmoregulation were also influenced by light spectra (Wallen and Geen, 1971a; Jones and Galloway, 1979).

Photosynthetic products are different under different photic environments. In general, the rate of protein synthesis is saturated at lower light intensities than carbohydrate production and thus, the proportion of CO_2 incorporated into protein is high at reduced light intensities in both natural assemblages and pure cultures (Konopka and Shnur, 1980; Morris, 1981). Similarly, polysaccharide synthesis in the marine cyanobacterium *Oscillatoria thiebautii* was

highest at the optimal irradiance levels, but the synthesis of protein, low molecular weight metabolites and lipids showed the opposite trend (Li *et al.*, 1980). The effects of light intensity, however, depend on the nutrient status. Under N limitation, the enhanced synthesis of protein was less pronounced (Morris, 1981). Blue light also enhances protein synthesis both in cultures and natural assemblages (Wallen and Geen, 1971a; Morris, 1981).

Thus, responses to low light intensities require the synthesis of new pigment molecules and enzymes, shifts in metabolic products, and/or additional energy expenditure. These in turn may entail increased nutritional requirements as well as changes in relative requirements. Nutrient limitation may then result in a decrease in the rate of photoresponses, and ultimately slower growth rate. Indeed, in the dinoflagellate *Gonyaulax polyedra,* nutrient limitation delayed the onset of pigment synthesis, slowed the rate of chlorophyll *a* synthesis, and reduced the final yield of cell chlorophyll. Furthermore, some of the photoadaptive capabilities of nutrient-sufficient cells were also lost (Prezelin and Matlick, 1983). The consequence of the longer time required for photoadaptation and impaired adaptive capabilities is slower growth rates which will endanger their survial, if the rates are not fast enough to replenish losses due to death, grazing, sinking, etc. The high chlorophyll *a* content under oscillating light in some species also indicate that both absolute and relative requirements of nutrients may also be different under the light regime of near-surface waters.

It is obvious then that there is a close link between nutrient supply and photoadaptation. If photoadaptive strategies are to maintain photosynthetic and growth rates at suboptimal irradiance conditions, some important questions may be raised: (1) Can light climate alter nutrient requirements? and (2) Can suboptimal light conditions be compensated for in part by an increased nutrient supply? (3) If both light and nutrient are limiting simultaneously, would they interact in an additive, multiplicative or threshold manner? These questions may be best examined using kinetic approaches. Therefore, the kinetics of nutrient limitation will be briefly reviewed.

NUTRIENT AND GROWTH

Quantitative requirement for growth. Growth rate is a function of substrate concentration only at very low substrate levels. It is therefore difficult to establish the relationship because even moderate growth would cause a very rapid change in substrate concentration. Monod (1942) examined this relationship by measuring the change in bacterial concentration using a nephelometer and estimating the concentration of the limiting substrate as a bacterial population ceased to grow as a result of the substrate limitation. To estimate growth rate, he took tangents of the curve of population increase. From these measurements, he proposed that growth rate followed first order kinetics with a term for saturation:

$$\mu = \frac{\mu m \cdot S}{K_s + S} \qquad (2)$$

in which μ is growth rate; μ_m, maximum growth rate; S, substrate concentration; and K_s, the value of S when $\mu = \mu_m/2$. This empirical relationship shows a quantitative relationship between μ and the amount of limiting nutrients. It is quite similar to the familiar relationship between the velocity of an enzyme-catalyzed reaction and the substrate concentration for the reaction, that is, the Michaelis-Menten equation:

$$v = \frac{V_m \cdot S}{K_m + S} \tag{3}$$

where v is the reaction rate; V_m is the maximum rate; and K_m is the S value when $v = V_m/2$.

Although other asymptotic models for growth have been proposed (e.g., Baule, 1917; Teissier, 1932), the Monod relationship has been widely used largely because of the ease of linear transformations and perhaps of its analogy to the enzyme reactions.

When algal growth was examined in batch cultures, however, no such quantitative relationship appeared to exist. In P-limited medium, growth continued even long after the limiting substrate had been exhausted. Even when the substrate-growth rate relationship was examined in steady state cultures in a chemostat, the Monod-type relationship did not seem to hold for algae. Instead, an asymptotic saturation relationship was found between growth rate and intracellular nutrient concentration (see Rhee, 1980; 1982). Thus, an equation relating μ to intracellular nutrient concentration was proposed (Caperon, 1967). It formally resembles the Monod equation in which S is replaced with intracellular nutrient concetration, or cell quota (Q):

$$\mu' = \frac{\mu'_m(Q - Q_o)}{K_Q + (Q - Q_o)} \tag{4}$$

where Q_o is the minimum cell nutrient concentration, or the minimum cell quota; Q is cell quota; μ'_m is μ when Q is infinite, and K_Q is the half-saturation constant.

Droop (1968) independently proposed a two-parameter equation:

$$\mu = \mu'_m\left(1 - \frac{Q_o}{Q}\right) \tag{5}$$

The Droop equation is equivalent to the above equation if $K_Q = Q_o$. Indeed, most experimental data showed that K_Q is little different from Q_o in most cases.

Eq. 4 and 5 were later shown to be equivalent to the Monod function in steady state (see Rhee, 1980; 1982). The seeming lack of a relationship between μ and S in steady state chemostat cultures observed earlier was later found to stem from the fact that the steady-state substrate level was below the analytical limit of detection. Careful studies using radioisotopes (see Button, 1985) and mathematical derivations (see references in Rhee, 1982) showed that the Monod-type relationship exist beteeen μ and S in steady state.

The cell quota equation has an advantage of being able to describe nonsteady state growth, such as that in batch cultures (Droop, 1975) and natural poplations (Jones *et al.*, 1978). It is also

useful in determining the nutrient requirement in terms of Q and Q_0. That is, if Q at a given growth rate increases at a subsaturating light intensity, it is clear proof that higher amounts of nutrients are required to maintain that growth rate at the intensity.

Relative requirement and optimum ratio. Historically, an important question regarding nutrient limitation has been how growth rate is related to nutrient concentration in natural waters when more than one nutrient are present in limiting quantities. The theories ranged from the multiplicative formulation of factor interactions represented by Baule (1917) and Rodhe (1978), to an either-or principle by Blackman (1905). The multiplicative theory predicts growth rate as the product of growth functions for each nutrient which may be limiting simultaneously. On the other hand, the either-or, or threshold, model of Blackman states "when a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the slowest factor." This threshold theory is similar in concept with Liebig's Law of Minimum (1855) which held that the crop yield is proportional to the amount of nutrient present in minimum relative to the crop's requirements.

The controversy of possible multi-nutrient limitation was first experimentally examined only in the mid-1970's. In our laboratory, the unicellular alga *Scenedesmus obliquus* was grown in a chemostat at a constant growth rate with mineral media containing N and P at various ratios. The N:P atomic ratio of the media was varied by changing N concentrations while holding the P level constant. When steady-state cell numbers were plotted against N:P atomic ratios, the cell number increased linearly with the ratio up to a ratio of 30, indicating growth limitation by N. Then, the stady state cell number abruptly leveled off at all ratios above 30 and remained at a constant level (Rhee, 1974; 1978). The results indicated that there was no simultaneous limitation by two nutrients; growth was limited by a single nutrient in shorter supply. This conclusion was also found when cell N and P levels were plotted against N:P ratio: cell N remained constant up to an N:P ratio of 30 and then increased. On the other hand, cell P decreased to the N:P ratio 30, but it remained constant above the ratio. Droop (1974), using different approaches, also came to the same conclusion in his investigation of *Monochrysis lutheri* for their P and vitamin B_{12} limitations, and Terry (1980) also reported an additional confirmation. In the threshold-type limitation the nutrient which has smaller $Q:Q_0$ ratio is the limiting nutrient and the Q_0 value of limiting nutrient is independent of the concentration of the other nutrient.

The threshold type nutrient limitation introduced the concept of relative nutrient requirement. In our work, the transition point between N and P limitation was called the optimum N:P ratio. Our study later found that the optimum ratio varied among different species, thus prompting us to suggest a simple conceptual framework which explained the coexistence and competitive exclusion of phytoplankton (Rhee and Gotham, 1980). This nutrient competition theory has later been expanded and advanced by Tilman *et al.* (1982) and they were able to predict the area of competitive exclusion and coexistence for competing species.

Therefore, any alteration in the relative requirement of nutrients, or the optimum ratio, is ecologically as important as changes in absolute requirements. It is defined as the cell quota

ratio of two nutients at the same growth rate, when either nutrient is limiting (Rhee, 1980) and has been found to change with growth rate (Turpin, 1985). Therefore, any change in the optimum ratio by irradiance can be determined on the basis of the cell quota model for growth by investigating changes in the quota ratios by the environmental factor of interest.

LIGHT-NUTRIENT INTERACTIONS

Interactions of subsaturating light with nutrient limitation. To study light-nutrient interactions, it is essential to understand the effect of an individual factor when the others are non-limiting. Light-limited growth of *Scenedesmus obliquus* under nutrient sufficiency showed a typical saturation curve (Fig. 1). These light-limited but nutrient-sufficient cells showed that cell N, among other cell constituents, increased with decreasing irradiance. These cell N values were the maximum concentrations the cells could have at those intensities.

Since the growth curve showed that light was no longer limiting at 17 Wm^{-2}, nutrient limited growth was studied at this irradiance with N as the limiting nutrient. Then, N-limited cultures were grown at two low intensities, 11 and 8 Wm^{-2}. The growth rate-cell quota for N-limited cultures at the 3 different irradiances could be described by the Droop equation (Fig. 2). When the minimum cell quota, Q_o, was calculated from the regression of 1/Q vs. μ, the values were significantly different between different irradiances. The value was highest at the lowest light level 8 Wm^{-2} at 100.5 $(+3.8, -4.1)\times10^{-9}$ μmol $cell^{-1}$; intermediate at 11 Wm^{-2} at 71.2 (+5.9, −5.2); and lowest at 17 Wm^{-2} at 48.9 (+2.9; −2.7). These variations in Q_o indicated that nutrient requirements increased at lower light intensities and that the interaction of N and light limitation was not in an either-or fashion. Such an increase in Q_o at lower light

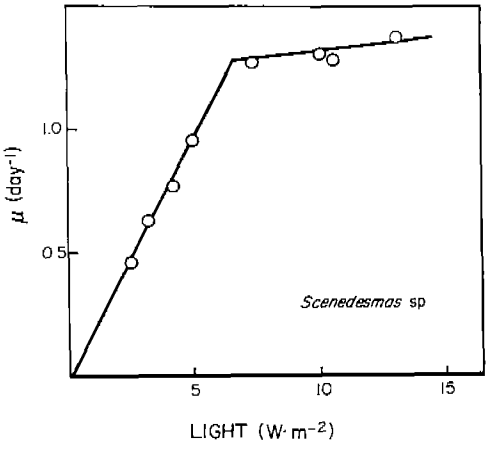

Fig. 1. Irradiance-growth rate relationship in *Scenedesmus obliauus* under nutrient-sufficient conditions. (From Rhee and Gotham, 1981)

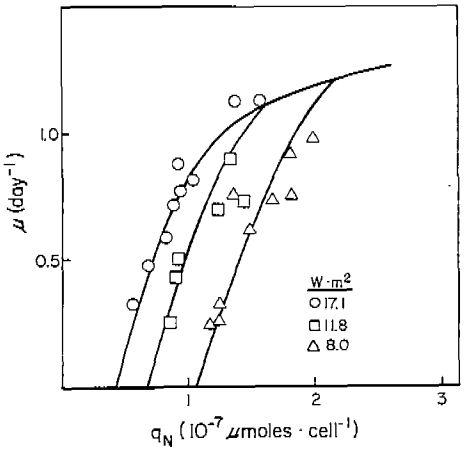

Fig. 2. Cell N quota-growth rate relationship in N-limited *Scenedesmus obliquus* at 3 different irradiance levels. (From Rhee and Gotham, 1981)

Table 1. Light, cell N, and growth rates

Light ($W \cdot m^{-2}$)	Cell N ($x10^{-7}$ μmol $\cdot$ $cell^{-1}$)	μ (day^{-1})
17*	0.65	0.40
12	2.23**	1.34
12	0.65	0.01

* Saturation irradiance

**Saturation concentration at 12 $W \cdot m^{-2}$.

intensity was also reported in N-limited *Synechoccus linearis* (Healey, 1985), *Dunaliella tertiolecta, Thalassiosira pseudonana, Phaeodactylum tricornutum,* and *Prymnesium parvum* (Wynne and Rhee, 1986), under limiting light intensities (Healey, 1985; Wynne and Rhee, 1986).

The pattern of light and N limitation can be seen more clearly in Table 1. When light is saturating in an N-limited culture, *Scenedesmus* sp. grew at 0.4 day^{-1} at a Q value of 0.65×10^{-7} μmol $cell^{-1}$ (Fig. 2). When N was sufficient (2.23×10^{-7} μmol N $\cdot$ $cell^{-1}$) at an irradiance of 11.8 Wm^{-2}, this species grew at 1.34 day^{-1} (Fig. 1). However, when Q is 0.65×10^{-7} μmol $cell^{-1}$ and light is 11.8 Wm^{-2}, growth rate was 0 (Fig. 2). These results show that light-nutrient interaction is not of threshold type. If it were threshold type, the growth rate would have been either 1.34 or 0.40 day^{-1}, instead of 0 day^{-1}. This is also another indication that the interaction is neither multiplicative nor additive, since the growth rate of cells with an N quota of 0.65×10^{-7} μmol $\cdot$ $cell^{-1}$ at 11.8 Wm^{-2}, 0 day^{-1}, was neither the sum nor product of 1.34 and 0.40 day^{-1}.

At a given growth rate, the quota at that growth rate was higher at lower light intensities (Fig.2). This indicated that low light level was compensated for by high cell nutrient to maintain that same growth rate. A similar compensatory relationship has also been reported in both N-and P-limited *Synechococcus linearis* (Healey, 1985).

Droop et al. (1982) used a different approach to investigate the type of interactions between light and nutrient limitations. They measured the biomass in a calorific value of joules, Thus, a population of a million cells per ml would have a biomass of joules per ml. The use of this unit allowed them to express the growth rate and the rate of light absorption in the same unit as μ W per joule with a dimension of $time^{-1}$ and biomass in an energetic term. 1 μW per joule is equivalent to a specific rate of 0.0864 per day.

They investigated the marine flagellate *Monochrysis lutheri* with vitamin B_{12} as the limiting nutrient. The results showed that in vitamin B_{12}-limited cells grown at limiting irradiances of 12.7, 8.5, and 4.0 Wm^{-2}, no significant difference in Q_o was found, while in light-limited but vitamin-sufficient cells, Q_o increased in an inverse proportion to the incident irradiance. Thus, they concluded that light and nutrient limitation interact in a threshold rather than multiplicative manner.

The reason for the contrasting results between Droop's on one hand and our work and Healey's study (1985) on the other is unclear. Even if we measured biomass in the energetic term of joules, Q_o values would still change because cell C was inversely related to irradiance.

However, the different results might have been due to the kind of nutrients examined for the interaction. In case of P, Q_o did not vary with light intensity in *Synechococcus* and 5 species of marine planktonic algae, but with N, the cyanobacteria and the marine algae all showed an inverse relationship between Q_o and irradiance (Healey, 1985; Wynne and Rhee, 1986). It is possible that any difference in the Q_o of vitamin B_{12} and P at different light intensities might have been too small to be analytically detectable whereas changes in N requirements, for the increased synthesis of pigment-protein complexes and enzymes, among others, were large enough to be reflected as different Q_o values.

The contrasting results may also have been due to possible differences in light adaptation mechanisms between the species examined. Although the mechanism for *Scenedesmus obliquus* is known (Senger and Fleishhacker, 1978a; 1978b), it is not known for *Monochrysis lutheris* and *Synechococcus linearis*.

The compensatory relationship between light and limiting nutrients suggests that in natural waters nutrient requirements may increase with depth due to vertical light attenuation. Thus, slower growth or lower biomass at deeper layers may not be due solely to light-limited photosynthesis, but the consequence of light limitation coupled with increased nutrient requirements.

Interaction of light flickers with nutrient limitation. Growth rates of many unicellular algae have beeen known to be higher under fluctuating light than steady light (Sager and Giger, 1980; Laws *et al.*, 1983; Shin *et al.*, 1987). When *Scenedesmus obliquus* was grown under fluctuating light at a frequency of 0.06 and 0.38 Hz in a P-limited chemostat, P requirement was about 33% less than that under steady light at the same saturating photon flux density (Shin *et al.*, 1987). In a N-limited chemostat, on the other hand, the N requirement was about 5% higher fluctuating than under steady light. The increased N requirement appeared to stem in part from the higher cellular concentration of chl *a*, since it was also higher in P-limited cultures under fluctuating light. (Rhee and DeNucci, in preparation). It seems therefore that the light fluctuation at high frequencies in the near-surface waters may change nutrient requirements, but whether it is for an increase or decrease depends on the kind of nutrients. It is unknown whether the change is also a function of frequencies.

LIGHT AND RELATIVE REQUIREMENT OF N AND P (OPTIMUM N:P RATIOS)

At lower light intensities, the minimum cell quotas for N in the 4 species of marine algae *Dunaliella tertiolecta, Thalassiosira pseudonana, Prymnesium parvum,* and *Phaeodactylum tricornutum* generally increased whereas no pattern of change was seen for the minimum quota for P (Wynne and Rhee, 1986). Thus, the optimum N:P ratio in general increased at subsaturating light intensities. Healey (1985) also reported similar increases in the blue-green alga *Synechococcus linearis*.

The optimum N:P ratio in the above four species of marine algae also vaied with light spctra, but the pattern of change varied from species to species. When the minimum cell N and

Table 2. Optimum N:P ratios under steady and oscillating lights

	Cell N $\mu=0.6$	Cell P $\mu=0.6$	N : P
	(x 10^{-9} μmol · cell^{-1})		
Steady Light	47.89 (±0.16)	1.56 (±0.04)	30.70
Oscil. Light	50.30 (±0.80)	1.07 (±0.04)	47.01

minimum cell P under various intensities and spectra were pooled and plotted against the optimum ratios, a positive correlation was found with the cell N, but not with cell P. Therefore, the change in the optimum ratio was directly related to cell N and, since the cell protein content was proportional to cell N, to cell protein contents.

As mentioned elsewhere, when *Scenedesmus obliquus* was grown in an N-limited medium under light oscillating at 0.38 Hz at the saturation intensity, the cell N quota when $\mu=0.6$ day^{-1} was about 5% lower than under constant light of the same photon flux density. However, cell P quota at the same μ in P-limited culture was about 33% lower than that under constant light (Rhee and DeNucci, in preparation). Therefore, the optimum N:P ratio at this μ, which is the ratio of the above N and P quotas, was significantly higher under light oscillation at 47 than under constant light at 30(Table 2). The optimum ratio 30 under constant light was identical to the ratio determined in my earlier studies (Rhee, 1974; 1978).

The drastic increase of the optimum N:P ratio would change the kind of limiting nutrients or degree of nutrient limitation in the surface layer of light fluctuation at high frequencies. For example, an increased optimum N:P ratio may make the surface layer N-limited, which under constant light would have been P-limited. Therefore, it is theoretically possible that in the euphotic zone of stratified freshwaters, surface populations may experience N limitation whereas the same populations in the light-saturation layer of steady light may face P limitation. Those in light-subsaturation deep layers may be limited by N, since the optimum N:P ratio is also higher at low light intensities.

In seawaters where N is most often limiting, the degree of N limitation would vary as the optimum N:P ratio changes with depth. Even at the same N concentration, the populations near the surface and deeper layers would experience a greater degree of limitation relative to those in the midlayer of steady light at saturating intensities.

CONCLUSIONS

Nutrient requirement increased under subsaturating light conditions. There was a compensatory relationship between light and nutrient requirements within a certain limit. Therefore, increased amounts of nutrients could compensate for suboptimal light and vice versa. Nutrient requirements also varied under fluctuating light at high frequencies; the requirement for P decreases whereas that for N increases.

The relative requirement for N and P, or the optimum N:P ratio, increased under

subsaturating light, primarily as a result of an increased cell N requirement. The optimum N:P ratio also increased under high-frequency fluctuating light. This increase was due to decreased requirements for P and increased requirements for N. These light-induced variations in the optimum N:P ratio may modify the degree of nutrient limitation or kind of limiting nutrients vertically for phytoplankton in the euphotic zone.

The combined effects of simultaneous limitation of light and nutrients were greater than the sum of individual effects. Thus, their interaction was not of threshold or either-or type. It was neither additive nor multiplicative.

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(Received September 20, 1987)