

Phosphorus as growth-regulating factor relative to other environmental factors in cultured algae

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Abstract

This review considers how the regulating effect of P on the growth of phytoplankton will be modified by other environmental factors, e.g., the physical factors light and temperature as well as the nutrients N, Si, Se, Fe and C. Questions about interaction effects are considered, as well as the possibility of general description of the interaction phenomena.

The general response to nutrient limitation can be considerably altered by light and temperature. The algae use relatively more P with increasing light, and the P content of the algae increases at lower temperature. The algae are, therefore, more sensitive to P limitation at high light and low temperature. The P yield at μ seems to be relatively independent of temperature, but the lower the growth rate, the larger is the influence of the temperature.

Two opinions exist of how dual limitation of two nutrients affects the growth rates of phytoplankton: a threshold, non-interactive model where growth is controlled by a single nutrient at a time, and a multiplicative, interactive model where growth is controlled by both suboptimal nutrients simultaneously. Both models are supported by different experimental data.

Introduction

Phosphorus has a predominant role in cellular energetics as ATP (adenosine triphosphate), and also forms an important part of many structural and biochemically functional components (e.g., enzymes) required for cell growth. It is, thus, highly probable that this delicate enzymatic machinery can be influenced by both physical and other chemical factors. It has long been known that interactions exist between light, temperature and nutrient concentrations as to their effects on algal growth. In one paper, now classical, Maddux & Jones (1964) clearly demonstrated that light and temperature optima decreased when algae (*Nitzschia closterium* and *Tetraselmis* sp.) were grown in low N and P concentrations. The light optimum found in conditions of high nutrient supply was clearly inhibiting, and the

tolerance to high temperatures decreased at the lower levels of nutrient supply. Shapiro *et al.* (1969) showed that the 'phosphate sparing' effect (lake water giving much better growth of cultured algae than distilled water media with the same P concentration) was not due to any unknown organics but to lack of inorganics salts (Mg) in the artificial medium. In a later paper, Shapiro & Glass (1975) showed that the combination of phosphorus and manganese additions had a synergistic effect on algal growth (C14 experiments in raw cultures from L. Superior). It is also a common observation in mesotrophic lakes, e.g., L. Windermere and L. Erken, that the diatom maximum is often broken before all Si is used up (Lund, 1950; Pechlaner, 1970). In contrast, in more eutrophic lakes with excess P, e.g., L. Furesø & Lyngby Sø, L. Norrviken and Loch Leven, the diatoms can utilize the silica almost

completely (Jørgensen, 1957; Ahlgren, 1970 and Bailey-Watts, 1976). The level at which a certain nutrient factor becomes limiting can, thus, vary with the level of other factors.

In a review covering the literature until 1981 on utilization of phosphorus by microalgae, Cembella *et al.* (1984) stated: 'Further experimental work on light-temperature-nutrient interactions is required on several ecologically important species to provide a solid data base for the development of an interaction-based model'. From the last five years I have found another ten papers on 'factor interactions'. The question remains, however, how such interactions can be explained. Talling's (1979) penetrating discussion on this subject began with the chapter 'The classical background: Liebig's law and Blackman's principal'. Sometimes I wonder whether we really can find any better explanations than those given by the early pioneers, despite the availability of all the modern technology.

This review considers how the regulating effect of P on the growth of phytoplankton will be modified by other environmental factors, mainly the physical factors light and temperature as well as the nutrients nitrogen (N), silica (Si), selenium (Se), iron (Fe), and carbon (C). (The concept 'interaction' used in this paper has no synergistic meaning.) Three questions are considered:

1. Does any interaction exist between phosphorus and another nutrient as is shown for some nutrients and a physical factor?
2. If so, is the regulation caused by the interaction between two nutrients different from that of a nutrient and a physical factor?
3. Is it possible to give general description of these interactions in quantitative terms?

I have concentrated on studies with continuous cultures (turbidostats and chemostats), because those techniques best determine the relationships between different factors and algal growth rates.

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General theory of growth

The *absolute growth rate* can be described as the derivative, dB/dt , of an ordinary growth curve, where the biomass (B) is plotted against time (t) (e.g., Krebs, 1978). In unlimited growth (J-curve) the absolute growth rate is directly proportional to biomass

$$\frac{dB}{dt} = \mu \cdot B$$

where the proportional factor μ is defined as the *specific growth rate*

$$\mu = \frac{dB}{dt} \cdot \frac{1}{B} \quad (1)$$

Organisms growing in a limited space, e.g., in batch cultures, show restricted growth (S-curve) which means that the density gradually rises until a factor (e.g., light, different nutrients) becomes limiting. This reduces the increase of the population which finally ceases to grow (Fig. 1, solid line). The absolute growth, i.e., the growth of the whole population, is fastest at the point of inflection (Fig. 1, broken line). In contrast, the specific growth rate is half the *maximum specific growth rate*, $\hat{\mu}$, at the point of inflection (Figure 1, dotted line). A common model of restricted growth is the following differential equation

$$\frac{dB}{dt} = \mu \cdot B \left(1 - \frac{B}{K} \right) \quad (2)$$

which was first introduced by Verhulst (1838) in population dynamics. K is the carrying capacity, i.e., the maximum biomass to which the population approaches, a property governed by both the organism and the environment. A general solution (see e.g., Batschelet, 1971) of the differential

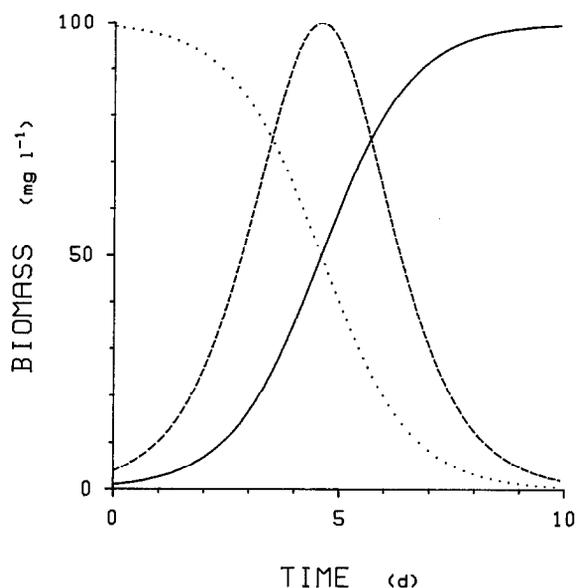


Fig. 1. Examples of batch culture characteristics. *Solid line*: Ordinary growth curve (S-curve, without lag phase). Biomass as a function of time, $B = f(t)$. *Broken line*: Absolute growth rate of the whole population, i.e., the derivative, dB/dt , of the S-curve. *Dotted line*: The specific growth rate, μ , against time. $\mu = dB/dt \cdot 1/B$.

equation (2) is

$$B = \frac{K}{1 + e^{a - \mu t}} \quad (3)$$

where $a = \ln(K/B_0 - 1)$, and B_0 = the initial biomass (inoculum). Eq. 3 is the mathematical description of the S-curve in Fig. 1. At the point of inflection it is valid that

$$B = \frac{K}{2} \quad \text{and} \quad \mu = \frac{\hat{\mu}}{2}$$

It has been shown that the specific growth rate, μ , can be described as a function of the external nutrient concentration (S) as well as the internal nutrient content (q) (Goldman, 1977a; Mickelson *et al.*, 1979). The relationships can be described by simple rectangular hyperbolas (Fig. 2) of similar mathematical expressions (for review, see Ahlgren, 1977, 1978). Concerning the μ/S relationship, Monod's equation (Monod, 1949)

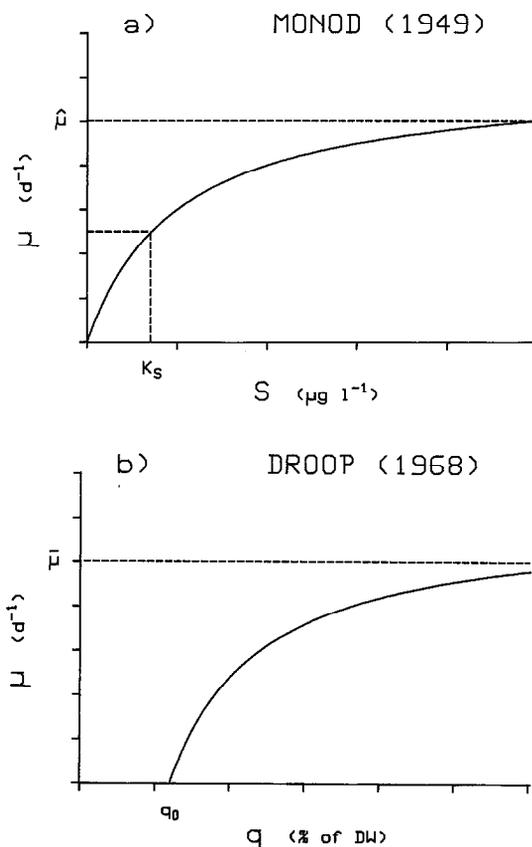


Fig. 2. Example of chemostat culture characteristics. Relationship between specific growth rate (μ) and a) external nutrient concentration (S). K_s = half-saturation constant. $\hat{\mu}$ = maximum specific growth rate, associated with S. b) internal nutrient content (q). q_0 = minimum nutrient content or subsistence cell-quota. $\bar{\mu}$ = maximum specific growth rate, associated with q.

$$\mu = \hat{\mu} \frac{S}{K_s + S} \quad (4)$$

is often used in connection with P (e.g., Tilman & Kilham, 1976; Goldman, 1977a; Ahlgren, 1977, 1978a; Mechling & Kilham, 1982), but also Mitscherlich-Baule's equation (Baule, 1917)

$$\mu = \hat{\mu} [1 - e^{\ln 0.5 \cdot S/K_s}] \quad (5)$$

gives equally good or sometimes even better fits to chemostat data (Ahlgren, 1978a, 1987). (K_s = half-saturation constant, i.e., a P concentration which can support a growth rate equal to half the maximum growth rate, $\mu = \hat{\mu}/2$). These

relationships are strictly valid only at steady state growth conditions, e.g. in chemostats (Fig. 2a). Algae grown in steady state are characterized by balanced growth i.e., the uptake (V) and growth (μ) are coupled, $V = \mu \cdot q$ so the amounts of all cellular components (q) increase at the same rate, resulting in a fixed cellular composition (Eppley, 1981).

In contrast, the hyperbolas described for the μ/q data do not start at the origin but have an X-intercept which gives q_0 , i.e. the subsistence cell quota of the limiting nutrient at zero growth rate (Fig. 2b). Different mathematical expressions have been used to describe this relationship (see Ahlgren, 1978a). Fuhs' equation,

$$\mu = \bar{\mu} [1 - e^{\ln 0.5 \cdot (q - q_0)/q_0}] \quad (6)$$

where $\bar{\mu}$ = the maximum growth rate associated with the internal nutrient content (q), gives a good fit to data of the P content (q_P) (e.g., Fuhs, 1969; Ahlgren, 1985a). Droop's equation (Droop, 1968),

$$\mu = \bar{\mu} \frac{q - k_q}{q} \quad (7)$$

where k_q is the cellular content at $\mu = \bar{\mu}/2$, is also often used in connection with P (Droop, 1974; Rhee, 1973; Gotham & Rhee, 1981; Terry, 1980; Mechling & Kilham, 1982). (In Droop's equation k_q is put equal to q_0 .) It has also been established that the same type of relationship can be valid for a non-limiting nutrient (Droop, 1974; Uelinger, 1981; Ahlgren, 1985b). Models describing μ as a function of internal nutrient content permit uptake and growth to be uncoupled (Droop, 1975). Greater attention is therefore paid to these internal nutrient models in this paper, as they are more relevant to the fluctuating nutrient conditions in the field. Besides, the same hyperbolic functions are known to be valid for data on intracellular phosphorus and photosynthesis at saturating irradiance (C14 measurements, AZ-opt; Senft, 1978; Smith, 1983), which is mostly the base for growth rate estimates of populations in the field.

Interaction with light

Investigations of the effects of light under nutrient limitation mostly deal with other nutrients than P, e.g., Si (Davis, 1976), N (Rhee & Gotham, 1981b; Zevenboom *et al.*, 1980), and Vitamin B (Droop *et al.*, 1982). As nutrient limitation influences the photosynthetic apparatus, some of the combined effects of light and these other nutrients should be of general validity. A short summary of these results is thus motivated.

It seems to be a general feature that nutrient content increases at low light intensities, i.e., as shown for Si (Davis, 1976) and N (Rhee & Gotham, 1981b; Zevenboom *et al.*, 1980) as well as Vitamin B (Droop *et al.*, 1982). At least in the case of N it is a result of increased nutrient requirements when light is suboptimal, an adaptive mechanism which is associated with increases in the size of the photosynthetic units (Falkowsky, 1980). Rhee & Gotham (1981b) stated that the combined effects of light and nitrate limitation were greater than the sum of the individual effects but were neither additive nor multiplicative. It should be possible to describe these effects in a formal expression. Zevenboom *et al.* (1980) also suggested formulas for describing this interaction. Their formulas are based on Droop's (1968) equation (Eq. 7) which is solved for q together with a linear relationship found between q/q_s and I (q_s is the N content at light saturation, I_s):

$$q_s = \frac{\bar{\mu} \cdot k_q}{\bar{\mu} - \mu} \quad \text{for } I > I_s \quad (8)$$

$$\frac{q}{q_s} = a + b \cdot I \quad \text{for } I < I_s \quad (9)$$

where a and b are constants. Based on these two equations Zevenboom *et al.* (1980) constructed a 3-dimensional plot of q dependent on μ and I at $I < I_s$. At $I > I_s$, $q/q_s = 1$ (Eq. 9), i.e., q is independent of I and only dependent on μ (Eq. 8). The most interesting area is, however, the conditions

when I is close to I_s . Is there really an abrupt transition, as Zevenboom *et al.* (1980) suggested, between q being dependent on only I and then on only μ ? Combining equations 8 and 9 we get:

$$q = \frac{\bar{\mu} \cdot k_q (a + b \cdot I)}{\hat{\mu} - \mu} \text{ or written in Droop's}$$

form:

$$\mu = \bar{\mu} \frac{q - k_q (a + b \cdot I)}{q} \quad (10)$$

which means that $K_q (= q_0)$ is not a constant but varies with the factor $(a + b \cdot I)$ which is unity at $I = I_s$ (b is negative). Unfortunately, Zevenboom *et al.* (1980) showed no data close to $I = I_s$, but it looks as if the transition could be equally well or even better described as a gradual transition between the two areas $I < I_s$ and $I > I_s$ (indicated by the broken line in Fig. 3) instead of the linear relationship shown which crossed the X-axis at I close to I_s .

In contrast to both Rhee & Gotham (1981b) and Zevenboom *et al.* (1980), Droop (1982) found that q_0 for Vitamin B was not dependent on

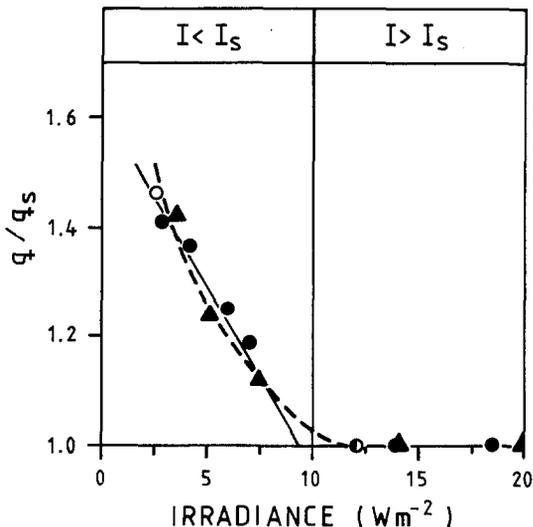


Fig. 3. The ratio between the actual cell quota (q) and the cell quota (q_s) at light-saturation (I_s) as a function of the light irradiance (I). The broken line indicates a gradual transition suggested between the two areas $I < I_s$ and $I > I_s$ (see the text). The different symbols indicate steady state conditions at different μ : $\bullet = 0.007 \text{ h}^{-1}$, $\circ = 0.015 \text{ h}^{-1}$, $\triangle = 0.019 \text{ h}^{-1}$. (After Zevenboom *et al.* 1980.)

I . Whether the different standardizations used, i.e., joule (Droop, 1982), cell number (Rhee & Gotham, 1981b) or dry weight (Zevenboom *et al.*, 1980), had something to do with the contradicting results is difficult to say. It is also possible that the reaction of Vitamin B is different to that of N.

Fewer papers are found which consider how light regulates the P-metabolism of algae. However, it looks as if limitation of P gives a more complicated picture than that of N or Si. McAllister *et al.* (1964) demonstrated an interaction between light limitation and P limitation in bacteria-free cultures of *Skeletonema costatum* and *Dunaliella tertiolecta*. At P deficiency the value for light saturation (I_s) decreased about 20% by *Dunaliella* and 44% by *Skeletonema*. The P deficiency also pronouncedly reduced the rate of respiratory processes. In both the algal cultures with low P the respiration was reduced to one-fifth of the value measured at high P. In comparison, it can be mentioned that in the N limited cultures the respiration was only reduced to about 40%.

Kuenzler (1970) investigated species of marine planktonic algae under various conditions of light and nutrition. He found that the excretion of dissolved organic P mostly increased with light, but P limitation reduced the excretion in two of the examined species, and N limitation reduced the excretion in three species. The growth rates were, however, also reduced at these P and N depletions, a possible primary effect of the nutrient limitations.

Young & King (1980) demonstrated a three-way interaction between light, P and CO_2 , at the lower growth rates of *Anacystis nidulans*. In order to support a growth rate of about 0.01 h^{-1} , for example, the alga needed 10 times higher CO_2 concentration at both light and P limitation compared to when the factors were limiting separately. At higher growth rates the curves approached each other, showing that light became the primary limiting factor (Fig. 4).

Konopka (1983) studied the effect of nutrient limitation and its interaction with light on the blue-green alga *Merismopedia tenuissima* growing in batch and chemostat cultures. By following the

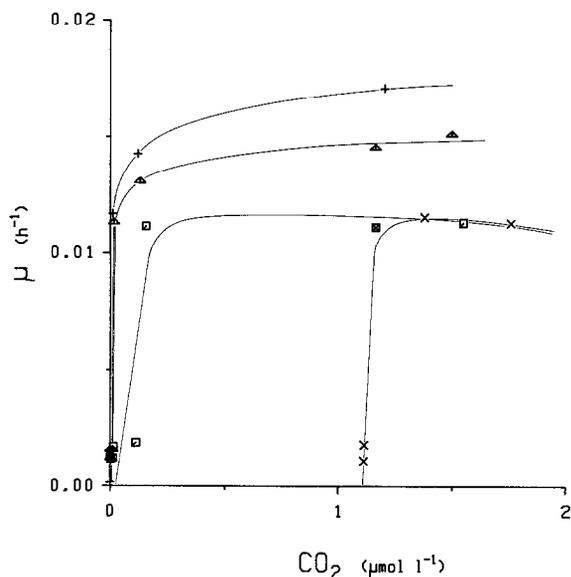


Fig. 4. Specific growth rate of *Anacystis nidulans* as a function of CO_2 for different combinations of unlimited and limited light (I) and phosphorus (P). + = non-lim., Δ = P-lim., \square = I-lim., X = I + P-lim. (Redrawn from a log/log scale from Young & King 1980.)

metabolic fate of fixed CO_2 (C14, 30-min incorporation) he found that the percentage of total fixed C14 in protein increased much more with μ under P limited conditions (20–60%) than under N limited conditions (15–25%). It is obvious that protein synthesis is obstructed by N limitation, so in that case it is more interesting to compare the P limited conditions with nutrient sufficient conditions. In earlier works, Konopka & Schnur (1980, 1981) also showed that in nutrient sufficient cultures the amount of C incorporated into protein decreased in increasing light, but in P limited conditions the same fraction was very constant at the light intensities tested. In contrast, the percentage of total fixed C14 which was found in polysaccharides decreased with μ in P limited conditions and was independent of μ in N limited conditions (Konopka, 1983). In nutrient sufficient conditions the percentage of polysaccharides increased with light (Konopka & Schnur, 1980, 1981). It is obvious that the protein and the polysaccharide syntheses should be mirror images of each other, particularly when they are measured as percentages of total fixed carbon.

Nonetheless, Konopka (1983) also plotted the percentage of C incorporated into protein against a light gradient for P limited (chemostat cultures) and nutrient sufficient algae, preconditioned at two different light intensities (Fig. 5). Below $20 \mu\text{E m}^{-2} \text{s}^{-1}$ (light limitation) the percentage of protein increased with increasing light, between the intermediate intensities of $20\text{--}100 \mu\text{E m}^{-2} \text{s}^{-1}$ (both light and P limitation) it decreased, and above $100 \mu\text{E m}^{-2} \text{s}^{-1}$ (only P limitation) it was independent of light. Both growth conditions showed the same pattern, but the P limited algae incorporated only about half the amount of C into protein as they did in non-limiting conditions. However, algae preconditioned in low light (Fig. 5, broken lines) showed smaller differences between non-limited and P-limited conditions than algae preconditioned in high light (Fig. 5, solid lines). These experiments show clearly that algae adapted to high light are more sensitive to P-limitation than algae adapted to low light. The results also suggest that there is not a sharp transition between light and nutrient limitation as was concluded by Droop *et al.* (1982), but that there may be interactions between these factors

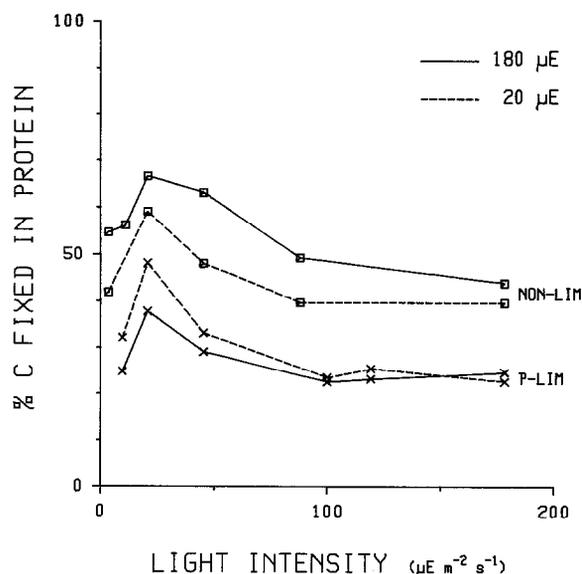


Fig. 5. Fraction of C14-incorporation into protein out of total C14-incorporation by non-limited (batch) and P-limited (chemostat) cultures of *Merismopedia tenuissima* preconditioned at 180 and $20 \mu\text{E m}^{-2} \text{s}^{-1}$. (From Konopka 1983, Figures 5a and 6.)

which affect the physiological state (Konopka, 1983, p. 408). A similar but even greater effect of light on N limited growth of *Oscillatoria agardhii* was shown by Zevenboom *et al.* (1980).

Healey (1985) investigated the interacting effects of light and nutrient (N and P) limitation on the growth rate of the blue-green alga *Synechococcus linearis* grown in chemostats. He showed that at a narrow range of irradiance, both N and light or P and light influenced simultaneously the growth rate of the alga. With decreasing irradiance the internal content of the limiting nutrient increased. The q_0 for N increased clearly with decreasing light but not the q_0 for P. The critical N/P-ratio (R_c) also increased with decreasing light, which has also been recently verified for three species of marine algae (Wynne & Rhee, 1986). (R_c = the ratio where the algae changed from being N limited to be P limited). Since μ is strongly correlated with light, this is consistent with the findings that the same ratio increases with decreasing μ (Terry *et al.*, 1985; Ahlgren, 1985b). That means that with decreasing light/ μ the algae need/use relatively more N than P. Healey's results show that at any growth rate an increasingly higher internal content of the limiting nutrient is required as other conditions become more adverse (Healey, 1985). He suggested that this way to compensate one depleted supply with another may be 'due to the fact that a physical factor, such as light, and a major nutrient may be able to substitute for one another'. This explanation differs from the classical, more probable one that the algae adapt to non-optimum conditions by increasing the concentrations of the enzymes per cell (e.g. Steemann Nielsen & Hansen, 1959; Jørgensen & Steemann Nielsen, 1965). As enzymes partly consist of essential nutrients, the increase of those nutrients is also obvious. Another explanation might be that the uptake mechanisms of N and P are less influenced by low light (in fact also shown by Healey, 1985), but transferring the nutrients taken up into real growth needs more light induced energy, which is why low light restricts the growth utilization of such nutrients.

More detailed studies of the physiological and ultrastructural responses to low light under nutrient (N and P) stress were made by Rosen & Lowe (1984). They found that under high light conditions the relative volume of chloroplasts decreased to half that under P-limitation, but in low light conditions and P limitation the volumes were about the same as in light limitation alone. The same pattern was found in the thylakoid (pigmented membranes inside the chloroplasts) surface density, as well as the chlorophyll content per cell. They also found that these ultrastructural changes in the chloroplasts were strongly correlated with the chlorophyll concentration per cell.

Concluding remarks: The general response to nutrient limitation, which is lowered chlorophyll and protein contents but raised carbohydrate contents, can be altered by the irradiance level. Therefore, it may be difficult to distinguish nutrient-sufficient populations from nutrient-limited population without due considerations to the actual light conditions (Zevenboom *et al.*, 1980). This involves complications when quantifying nutrient deficiencies in natural populations in the field (Healey, 1978). The interactions between light and P are evidently partly different from interactions with other nutrients, e.g., N. It is therefore probably difficult to find a description of this interaction which is valid for all nutrients. It may be necessary to describe different models for different nutrients. The critical N/P-ratio (R_c) is inversely correlated with light and growth rate, implying that the algae use relatively more P than N with increasing light or growth rate. The algae, thus, gradually become more sensitive to P limitation than to N limitation with increasing light.

Interaction with temperature

The stimulating effects of temperature on the rates of biological processes such as growth rate (μ) have long been known, and the first attempts of biologists to express these temperature effects in mathematical rules can be traced back for many years or even centuries (e.g., Réaumur, 1735; Burckhardt, 1860; cited by Bělehrádek, 1935).

Great efforts were then devoted to analyzing the sometimes considerable variation of different temperature coefficients (μ , Q_{10} , E or A and b) for one and the same physiological process. These phenomena of 'changing temperature coefficients' were found to disappear mostly when replaced by a new temperature coefficient, b, in Bělehrádek's formula derived already in 1926 (Bělehrádek, 1926, 1935). The artificial nature of 'breaks' found when applying the temperature formula derived from chemical processes (e.g. Berthelot's, Van't Hoff's or Arrhenius' equations; see Ahlgren, 1987) to rates of enzyme reactions were also overcome by the introduction of this new formula:

$$v = a(t - \alpha)^b \quad (11)$$

where v = velocity,

α = the temperature where $v = 0$ ('biological zero'),

a, b = constants.

This formula was based on a 'physical view', which means that many biological processes are probably controlled by physical processes such as diffusion and viscosity rather than by chemical processes (Bělehrádek, 1926, 1935). When logarithmically transformed, this formula was shown to give a straight line within a wider temperature range than a semilogarithmic plot obtained from the equations of Berthelot or Van't Hoff-Arrhenius (Bělehrádek, 1935). The frequent use even today of, e.g., Q_{10} is surprising when considering the many examples given on the statistically closest fit by Bělehrádek's equation compared to the other temperature functions (Bělehrádek, 1935; McLaren, 1963; Ahlgren, 1987). Even a linear relationship of algal growth rates to temperature is often found to fit better than the more frequently used functions of exponential type, particularly when a limited temperature interval is considered. Assimilation numbers (AZ), i.e., primary production per chlorophyll, estimated for raw cultures grown in an incubator at light optimum or in the field at optimum depth can also be best described as functions of temperature by Bělehrádek or Burchardt-Harvey's (linear) equations (Ahlgren,

1987). The constants of the equations are, however, different for the different algal species and probably are species specific.

The growth rate, μ , can be described as a function of P content (q_P) (Eqs. 6 or 7). As to the influence of temperature on the P content of algae, there is little information in the literature. The few data published are quite new. Among the first to provide information in that sector was Ahlgren (1978a), who found that the mean P content of the blue-green alga *Oscillatoria agardhii*, grown in P-limited chemostats, was about doubled at 15 °C (0.56% of DW) compared to the value at 25 °C (0.26% of DW), while the mean value at 20 °C fell to half-way between these two (0.37% of DW). A year earlier, Goldman (1977b) had presented U-shaped relationships of N/cell against temperature, which was later found to be valid also for Chl/cell ratios (Goldman, 1979; Goldman & Mann, 1980). Goldman suggested that this type of response to temperature seemed to be a common feature in many species (Goldman, 1977b; Goldman & Mann, 1980). He thereby referred to Williams (1971), who presumed that minimum cell size occurs at the temperature optimum (Fig. 6, B/N). The same

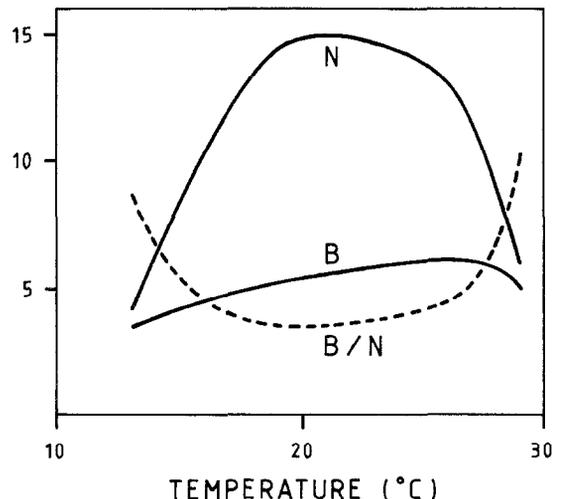


Fig. 6. Schematic representation of Maurice Blaug's chemostat results on *Chlorella*, sp., showing steady state number (N), biomass (B) and average cell size (B/N) versus temperature. The Y-axis is graded in relative units. (After Williams 1971.)

was found for other algae, e.g., *Scenedesmus quadricauda* (Komárek & Růžička, 1969). The increase of cell size above the optimal temperature for *Scenedesmus* shown by these authors was, however, steeper than the increase at low temperatures and took place only at around the lethal temperatures. The main pattern of Fig. 6 is probably general for most algal species, but with more or less moderations of the curves, particularly at the right side of the diagram.

But are the changes in different cellular constituents always proportional to each other, so the cellular composition remains fixed? According to Goldman & Mann (1980), the N- and Chl-contents of *Phaeodactylum tricorutum* were proportional, because the ratios of C/N and C/Chl were nearly constant with temperature. As regards the P content, Goldman (1979) found that the k_q for *Monochrysis lutheri* was highest (0.04 pg P/cell) at 15 °C and was reduced by half (0.02 pg P/cell) at 18.8 and 23 °C. Using the C content of 12 pg C/cell at 15 and 18.8 °C and 10 pg C/Cell at 23 °C (read out of Figs. 2, 3 and 4, Goldman, 1979), the above P contents will be about 0.33, 0.17 and 0.20% of C, respectively, or reduced by half if expressed as a percentage of dry weight (DW, Fig. 7, squares). Rhee & Gotham (1981a) investigated the combined stress of sub-optimal temperature and N or P limitation by *Scenedesmus*. They found that q_0 (N) per cell increased considerably at low temperatures, from 56 to $236 \cdot 10^{-9}$ $\mu\text{mol N/cell}$ at 20 and 11 °C, respectively. The C content per cell increased to a lesser extent, from 0.163 to $0.435 \cdot 10^{-5}$ $\mu\text{mol C/cell}$ at the two temperatures mentioned (estimated from Rhee & Gotham, 1981a, Fig. 2a, where the reciprocal cell-C is plotted against temperature). The increase with decreasing temperature of the minimum cell quota for N, expressed per cell carbon or per dry weight (DW), should, thus, be significant. (Using the C content above, the q_0 at 20 °C will be $56 \cdot 10^{-9} \cdot 14 / 0.163 \cdot 10^{-5} \cdot 12 \times 100 = 4.0\%$ N of C or about 2% N of DW, and q_0 at 11 °C = $236 \cdot 10^{-9} \cdot 14 / 0.435 \cdot 10^{-5} \cdot 12 \times 100 = 6.3\%$ N of C or about 3% N of DW). In contrast, the increase of q_0 for P with decreasing temperature found by

Rhee & Gotham (1981a) was probably not significant if the corresponding increase in cell carbon is considered; $1.6 \cdot 10^{-9}$ $\mu\text{mol P/cell}$ at 20 °C and $2.84 \cdot 10^{-9}$ $\mu\text{mol P/cell}$ at 12 °C. An estimate analogous to that above gives, in fact, higher phosphorus content at 20 °C than at 12 °C, namely 0.13 and 0.11% P of DW, respectively. Rhee & Gotham (1981a, Fig. 2a) data on the reciprocals of cell quotas for C are, however, very variable, so the estimated P contents are not very reliable (Fig. 7, triangles). Recently, results from P-limited growth experiments with *Scenedesmus quadricauda* in chemostats at different temperatures gave significantly higher q_0 for P at 5 °C than at 15 and 20 °C (Fig. 7, crosses, Ahlgren, 1987). The algae were grown under different light intensities at each temperature, corresponding to the light optimum found for the different temperatures. It is possible that the tendency of increased q_0 -values for P at 25 °C in Ahlgren's data was caused by a wrongly (too low) estimated light optimum at 25 °C. A reason why Rhee & Gotham's (1981a) q_0 -values for P did not show significantly higher values at low temperatures (Fig. 7, triangles) might also be that the light,

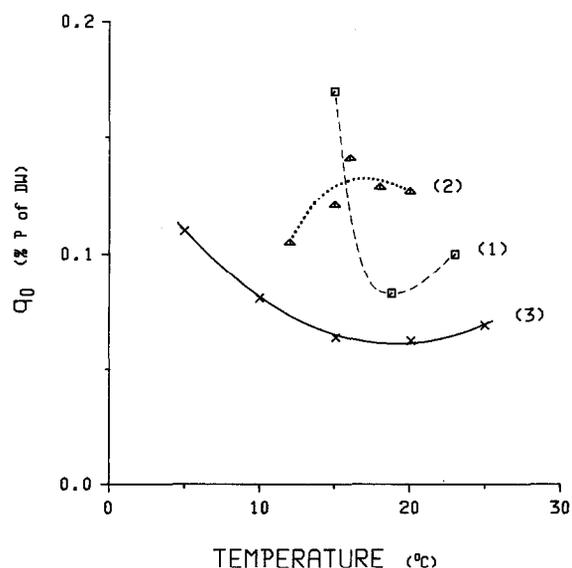


Fig. 7. Minimum cell quota (q_0) for phosphorus in percent of dry weight (DW) versus temperature. (1) *Monochrysis lutheri* (Goldman, 1979). (2) *Scenedesmus* sp. (Rhee & Gotham 1981a). (3) *Scenedesmus quadricauda* (Ahlgren 1987).

which was held constant at all temperatures, could be supraoptimal at the lower temperatures and suboptimal at the highest temperatures.

Concluding remarks: It is hard to distinguish between effects of light and temperature, as they are so intertwined and dependent upon each other (cf. Maddux & Jones, 1964). Most temperature experiments hitherto have been made under constant light conditions, and if the light then happens to be suboptimal or supraoptimal, the results may primarily be a light effect instead of a temperature effect.

Nonetheless, at a moment it is fairly correct to state that the P content as well as the N content increase at low temperatures but probably to different extents. The q_0 for P as a function of temperature can be described by a 2nd degree polynomial for the green alga *Scenedesmus quadricauda* (Ahlgren, 1987). The principal diagram (Fig. 8) suggested by Goldman & Mann (1980) has been verified by Rhee & Gotham (1981a) regarding N, by Paasche (1980) regarding Si, and by Goldman (1979) and Ahlgren (1987) regarding P.

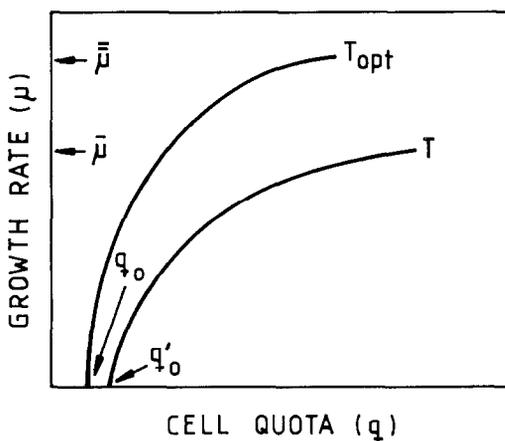


Fig. 8. Principal diagram of specific growth rate (μ) versus cell quota (q) at optimum temperature (T_{opt}) or at suboptimal temperature (T). q_0 is higher than the absolute minimum cell quota, q_0 . $\bar{\mu}$ = specific growth rate associated with q , $\bar{\mu}'$ = absolute specific growth rate. (Modified after Goldman & Mann 1980.)

Interactions with other nutrients

Non-interactive versus interactive models

Can the models describing μ as a function of the internal nutrient content (Eqs. 6 and 7) be altered by other nutrients? There are different opinions on this. The question of how dual limitation affects the growth rates of phytoplankton has developed into a question of whether there is a *threshold effect* (non-interactive), i.e., an either/or principle *sensu* Liebig, where growth is controlled by a single nutrient, or a *multiplicative effect* (interactive, Baule, 1917) where growth is dependent on all suboptimal nutrients simultaneously (cf. Rodhe, 1978; Talling, 1979).

The threshold model is supported by Droop (1974), Rhee (1978) and Terry (1980), and they suggested that algal growth rates are determined by the single nutrient which is in shortest supply and independent of all other nutrients. This opinion has become widespread and is sometimes considered to be a statement (Terry, 1980; Terry *et al.*, 1985). Droop's result (Droop, 1974), concerning the marine chrysophycean *Monochrysis lutheri* grown in a chemostat with limited P and vitamin B in different proportions, show that a threshold model (Eq. 7) agreed better with his data than a multiplicative one (extended from Eq. 7, see Ahlgren, 1985b, Table II). In contrast, interaction effects of two nutrients are often demonstrated in *uptake* experiments (Zevenboom *et al.*, 1980; Uelinger, 1981; Terry, 1982).

The multiplicative model originates from studies of the increase of harvest (various crops) in sciences related to agriculture and was supported by experiments with simultaneous fertilizing with N, K and P (see e.g., Boguslawski, 1958). For microorganisms such models were discussed from a more theoretical standpoint (e.g., Droop, 1973; O'Brien, 1974), but the validity for multiplicative dependence was not tested. However, some kind of interaction between two nutrients seemed to exist for C and N on the growth of *Enterobacter aerogenes* (Cooney *et al.*, 1976) as well as for N and P on the growth of the blue-green alga *Oscillatoria agardhii* (Ahlgren,

1985b). The first multiplicative model (Baule, 1917) was later improved by solving the original differential equation for two or more nutrients (Baule, 1953, 1956). These equations (see also Ahlgren, 1980; Eqs. 3 and 4) which, thus, stands on a better footing than the extended one-factor expressions, have only been tested hitherto on the growth of *Oscillatoria agardhii* (data from Ahlgren, 1985b). Six of the eight data sets could be fairly well described by these models. However, at the lower growth rates the external N and P concentrations were often too low to permit evaluation of the different equations tested (Ahlgren, unpubl.).

Phosphorus and nitrogen

In chemostat experiments with the green alga *Scenedesmus* sp., using a fixed dilution rate ($= \mu$ at steady state) and varying N/P-ratios in the nutrient medium, Rhee (1978) found that below an N/P-ratio of 30 (by atoms, equals to 13.5 by weight) growth was determined solely by N limitation and above 30 (R_c for *Scenedesmus*) solely by P limitation, and there was no additive or multiplicative effect of the two nutrient limitations. Besides, Rhee (1978) and Rhee & Gotham (1980) concluded that the transition from one nutrient limitation to another was abrupt and took place at 'the optimum ratio', which varies between species and could play an important role in population dynamics (optimum ratio = critical ratio, R_c , a better definition according to Terry 1980). The optimum/critical ratio was also found to be identical with the ratio of the minimum cell quotas, $q_0(N)/q_0(P)$ (Rhee & Gotham, 1980). However, as it was shown later that R_c varies with growth rate (Terry, 1980; Terry *et al.*, 1985; Ahlgren, 1985b; Turpin, 1986), an interaction may be difficult to observe at one single dilution rate, as in Rhee's case.

In chemostat experiments with the blue-green alga *Oscillatoria agardhii*, including different N/P ratios in the medium as well as varying dilution rates (growth rates), I demonstrated that interaction effects of N and P on the growth rate of

Oscillatoria very probably existed (Ahlgren, 1985b). In contrast to the results of Rhee (1978), the transition areas between N and P limitation were not sharp. They also varied with growth rate. At certain growth rates the interaction between N and P was, however, not fully described by the multiplicative models tested.

Phosphorus and silica

Growth rates under P and Si limitation have been mainly studied by Tilman and S. Kilham (Tilman & Kilham, 1976; Kilham *et al.*, 1977), and it was on the basis of results on the diatoms *Asterionella formosa* and *Cyclotella meneghiniana* that Tilman prepared his 'Resource-based competition theory' (Tilman/Tilman, 1976; Tilman, 1977), where it is postulated that two species can coexist stably only when the growth rate of each species is limited by a different nutrient (resource). *Model I* is based on the following steady state equations (derived from the general balance equations for continuous cultures when the time derivatives are set equal to zero, Tilman, 1977):

$$N = Y(S_0 - S) \quad (12)$$

$$S = \frac{D \cdot K_s}{\hat{\mu} - D} \quad (13)$$

where N = the steady state population of one species,

S_0 = the limiting nutrient in the inflow,

S = the limiting nutrient in the outflow

Y = yield coefficient.

D = dilution rate $= \mu$ at steady state conditions.

K_s = half-saturation constant,

$\hat{\mu}$ = maximum specific growth rate.

Eq. (13) is Monod's wellknown equation (Eq. 4) but solved for S. The boundary between growth rate limitation of one species by two nutrients (i.e., Si and P) is most easily estimated in the following way (Tilman, 1976; Tilman, 1977 p. 347:

When the Si and P concentrations cause equal growth rates we get from Monod's equation:

$$\hat{\mu} \frac{Si}{K_{Si} + Si} = \hat{\mu} \frac{P}{K_P + P}$$

$$\frac{Si}{P} = \frac{K_{Si}}{K_P} \quad (14)$$

The boundary for *Asterionella* is thus given by the K_{Si}/K_P -ratio = $3.94/0.02 = 197$ (Tilman, 1977). The same boundary for *Cyclotella* was $1.44/0.25 = 5.6$. The K_s -values for the two algae imply that *Asterionella* is a better competitor at low P concentrations and *Cyclotella* at low Si concentrations. The $\hat{\mu}$ -values are about the same for the two algae. For ratios >197 *Asterionella* should be P-limited and ratios <197 Si-limited and analogously, for ratios >5.6 *Cyclotella* should be P-limited and for ratios <5.6 Si-limited.

Tilman (1977, Models I and II) estimated these boundaries by setting the biomass obtained from resource 1 and resource 2 equal for a species and derived the following equation from Eq. (12) (for simplicity I here use Si and P as examples instead of Tilman's indices):

$$\frac{S_0 - Si}{P_0 - P} = \frac{Y_P}{Y_{Si}} \quad (15)$$

Tilman (1977, Eq.5) solved for S_0 and got the following equation:

$$S_0 = Si + (P_0 - P) \frac{Y_P}{Y_{Si}} \quad (16)$$

from which he estimated the S_0 or P_0 for which the species would be equally limited by both resources by using the given constants and the concentrations of phosphate and silicate used in the experiments. Other authors have also practised the same equation (16) (Holm & Armstrong, 1981), but both they and Tilman (1977) used a fixed Y_{Si}/Y_P -ratio, obtained from experiments where μ was 0.5 d^{-1} and 0.1 d^{-1} , respectively. Since the yield as well as the biomass change with growth rate, the use of a constant Y_{Si}/Y_P -ratio

may not be adequate, however, unless the variation with growth rate is exactly proportional for the two nutrients. Since this is very improbable, a better way to calculate these boundaries would be to solve for the ratio S_0/P_0 in Eq. 15 which can be done in the following way: To simplify we put

$$\frac{Y_P}{Y_{Si}} = y \quad \text{and} \quad \frac{K_{Si}}{K_P} = k$$

From Eq. (14): $Si = k \cdot P$ (put into 15)

$$\frac{S_0 - k \cdot P}{P_0 - P} = y$$

$$S_0 - k \cdot P = y \cdot P_0 - y \cdot P$$

$$S_0 = P_0 \cdot y - P \cdot y + P \cdot k$$

$$S_0 = P(k - y) + P_0 \cdot y$$

Dividing both sides with P_0 we get

$$\frac{S_0}{P_0} = \frac{P}{P_0} (k - y) + y \quad (17)$$

or with the original notations

$$\frac{S_0}{P_0} = \frac{P}{P_0} \left[\frac{K_{Si}}{K_P} - \frac{Y_P}{Y_{Si}} \right] + \frac{Y_P}{Y_{Si}} \quad (18)$$

Eqs. 17 or 18 tell us that:

If $P \ll P_0$, which is the case at low growth rates, the S_0/P_0 ratio approaches y or Y_P/Y_{Si} . If $P = P_0$, which is the case when $\mu = \hat{\mu}$ (outflow), the S_0/P_0 became equal to k or K_{Si}/K_P . It follows that if μ is between 0 and $\hat{\mu}$, the ratio S_0/P_0 should be intermediate to the two ratios Y_P/Y_{Si} and K_{Si}/K_P .

Tilman's (1977) Model II is based on Droop's equation (Eq. 7). In fact, the boundaries he estimated by using three equations turn out to be the ratio of $q_0(Si)/q_0(P)$, which is consistent with the finding that

$$q = \frac{1}{Y} \quad (19)$$

at steady state, since the excretion of a soluble organic fraction of the nutrient in question is mostly missing or so low that it can be ignored. The above calculation, as well as the approach in Tilman (1977) are approximations of the actual mathematics, which were more exactly specified in an isocline model by Tilman (1980 and 1982).

The boundaries for a species between being limited by one nutrient (1) or by another (2) can thus be estimated by the three ratios:

$$\text{I) } \frac{K_s(1)}{K_s(2)}; \quad \text{II) } \frac{Y(2)}{Y(1)}; \quad \text{III) } \frac{q_0(1)}{q_0(2)}$$

Which one is most correct to use? According to the derivation above, the first ratio should be valid at high growth rates close to $\hat{\mu}$, and the other two at low growth rates close to zero. The three boundaries between Si and P estimated for *Asterionella* and *Cyclotella* from constants given by Tilman (1977) and Holm & Armstrong (1981) are:

	I)	II)	III)	
	$\frac{K_{Si}}{K_P}$	$\frac{Y_P}{Y_{Si}}$	$\frac{q_0(Si)}{q_0(P)}$	
	$(\mu \rightarrow \hat{\mu})$	$(\mu \rightarrow 0)$	$(\mu \rightarrow 0)$	Range
<i>Asterionella</i>	197	87(0.5)	169	80–200
<i>Cyclotella</i>	5.8	6.2(0.5)	15	5–15
<i>Asterionella</i>	42	94(0.1)	94	40–100

(The figures within brackets below II are the μ -values used). Tilman's results from 76 competition experiments in semi-continuous cultures with different Si/P ratios in the medium and at various dilution rates agreed better with the predictions of Ratio II than Ratio III. (His Ratio II was, as already mentioned, estimated by using a fixed growth rate). These boundaries, thus, vary with growth rate and also depend on how exactly the growth constants are determined. They are, therefore, hardly sharp and a range as above may be the most appropriate for application purposes.

Tilman (1982, p. 134) argued that the expected outcome of competition between two species is almost independent of whether the growth iso-

clines have rounded corners (interactive) or right-angle corners (non-interactive). Whether or not this is valid is probably difficult to prove experimentally. The paper of Tilman & Kilham (1976) did not include conditions with low concentrations of both nutrients at the same time. In Tilman (1981) three different combinations of P and Si concentrations in the medium (Si/P = 1.2, 71 and 1045) were tested in competition experiments with six pairs of diatoms. The outcomes of the predictions were mostly successful but in some cases the model predicted higher biomasses than were actually measured (Tilman, 1981; Fig. 5c and 8c). It may be that in those intermediate cases, *Tabellaria* was limited by both the nutrient, which is not predicted by Tilman's model.

Can these boundaries predict the succession of the algae in the field? Tilman (1977) said that the Monod model (Ratio I = K_1/K_2) explained 75% of the variance in the relative abundance of *Asterionella* and *Cyclotella* in data from Lake Michigan. As far as I know this model had not been tested against other field data until lately by Sommer (1986, Si and N in Antarctic waters). Sommer (1986) demonstrated that nutrient limitation can exist in Antarctic waters. The abundance of two diatoms with high K_{Si}/K_N -ratios also showed positive correlations with the Si/N-ratios in the water. It was difficult, however, to demonstrate the presence of any competition as the concentrations of both Si and N were too low to support maximum growth rates, and the algae with low K_{Si}/K_N -ratios were too rare for counting. This points to the main difficulty of applying the resource competition theory to complex systems in nature. Nutrients must be limiting and different algae should be limited by different nutrients. In addition, a prerequisite for theories based on Monod's equation should be that the algae are growing under steady state conditions. That is not always the case in the field.

Phosphorus and selenium

In studies of the effects of the four factors N, P, Si and Se in raw cultures from L. Erken, Eriksson

(1983, Eriksson & Pettersson, 1984) found, by using factorial combinations, that the contribution to the variance of the biomass maximum from Si and Se was small but significant. During the beginning of the diatom bloom the contribution of combined N and Si was significant, and after the diatom peak combined P and Se was significant. The contribution of P was, however, always much larger, so it is probable that the algae in the lake were primarily P-limited.

The effects of different combinations of P and Se on the growth of *Stephanodiscus hantzschii* v. *pusillus* – the dominant species during the spring bloom in L. Erken – were studied separately (Eriksson, 1982). At high levels of P, a doubling of Se in the medium increased both μ and biomass maximum significantly. Se increased the consumption of both the external and the internal surplus P of the alga, which means that the alga might use the available P more effectively when Se was in excess.

Phosphorus and iron

Fe is one of the most important trace metals for phytoplankton, and Fe deficiency in natural waters should be common, considering the very low amount of Fe which is mostly available in true solution. Enrichment experiments in the laboratory with natural waters have also occasionally identified Fe as limiting nutrient. Lin & Schelske (1981) showed by excluding Fe in multi-nutrient enrichment experiments that the growth rate (measured as increase of chlorophyll *a*) was significantly slower in water from L. Huron than in tests with complete additions. However, excluding P always reduced the growth much more, which is why the natural phytoplankton in L. Huron was primarily P-limited. In contrast, in enrichment experiments with raw cultures from the eutrophicated L. Norrviken, Fe was the only nutrient which gave a significant increase of the algal biomass (Ahlgren, unpubl.).

Results of Fe-limited assays in chemostats show that the P content of the blue-green alga *Oscillatoria agardhii* increased considerably up to

three times their normal values (Ahlgren, unpubl.). Preliminary results from chemostat experiments with the green alga *Scenedesmus quadricauda* also show an increased P-content at Fe limitation (Ahlgren, unpubl.). Because of the very pronounced influence of Fe-limitation on the P-content it is possible that studies with simultaneous limitation of both Fe and P should give more detailed information on the interaction phenomena.

Phosphorus and carbon

Studies of the limitation effects of P and C on chemostat grown green alga *Selenastrum minutum* were recently performed by Turpin (1986). Using a fixed C/P-ratio in the inflow medium he convincingly demonstrated how the transition from C to P limitation took place only as a result of increased dilution rate (Fig. 9). The critical ratios (R_c) found for *Selenastrum* were

$\mu(d^{-1})$	R_c (C/P by weight)
0.5–0.6	137
1.1–1.2	70

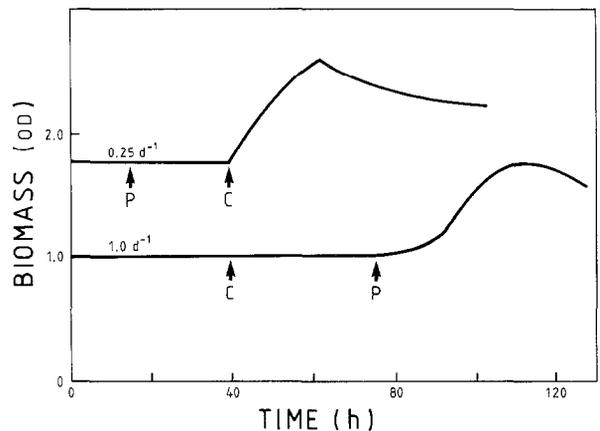


Fig. 9. The response of the biomass (optical density measured at 720 nm) to addition of P and C in chemostat cultures of *Selenastrum minutum* grown at two different growth rates. The inflow C/P-ratio was 137 (by weight). (From Turpin 1986, Figures 6 and 7.)

which confirms that the algae need relatively more P at higher growth rates. These results, I think, definitely prove that the critical ratios are dependent on growth rate. The dependence is, however, different for different nutrient pairs, and probably also different for different species, i.e., species specific.

Concluding remarks

There is now convincing evidence that the critical ration, R_c , (the ratio where the algae change from being limited by one nutrient to the other) is not constant but varies with growth rate (Terry, 1980; Terry *et al.*, 1985; Ahlgren, 1985b; Turpin, 1986). The variation for a certain nutrient pair of this 'growth rate dependent variable' is also probably species specific. It is, thus, probably necessary to run assays with a gradient of nutrient-ratios for every dilution rate (a task which takes a lot of time) in order to prove a possible interaction effect.

Two different opinions exist of how a dual limitation affects the growth rates of algae; a non-interactive model (Droop, 1974; Rhee, 1978; Terry, 1980) or an interactive model (Cooney *et al.*, 1976; Ahlgren, 1985b). The interaction demonstrated for N and P was not fully described by the multiplicative model tested (Ahlgren, 1985b). Talling (1979) also pointed out that these multiplicative expressions predict growth rates which are seriously underestimated; they 'merely combine or blend in the outcome and do not strictly interact'. The essentials of this interaction, thus, remain to be explained. Droop's (1974), Rhee's (1978) as well as Ahlgren's (1985b) conclusions were mainly based on relationships between the internal P content of the algae and growth rate (Eqs. 6, 7 or related equations, see Ahlgren, 1985b). It is possible that the improved equations which include the external nutrient concentrations (Baule, 1953, 1956) will give a better description of the interaction phenomena.

Biomass and yield as a function of phosphorus

How much biomass can a certain amount of P give rise to? If Shapiro's diagram (Shapiro, 1979; see also Ahlgren *et al.*, 1988; Heyman & Lundgren, 1988), i.e., biomass (chl) as a function of total P, had been constructed with results from culture experiments, there would also be a certain variance. This variance would, however, be explainable if the growth rates were known. It is μ which controls the amount of biomass produced. At low growth rates the same amount of P will give rise to a higher biomass than at higher μ (Fig. 10), resulting in a varying P content of the algae. The growth constant, Y (yield), which is defined as the biomass produced divided by the nutrient consumed:

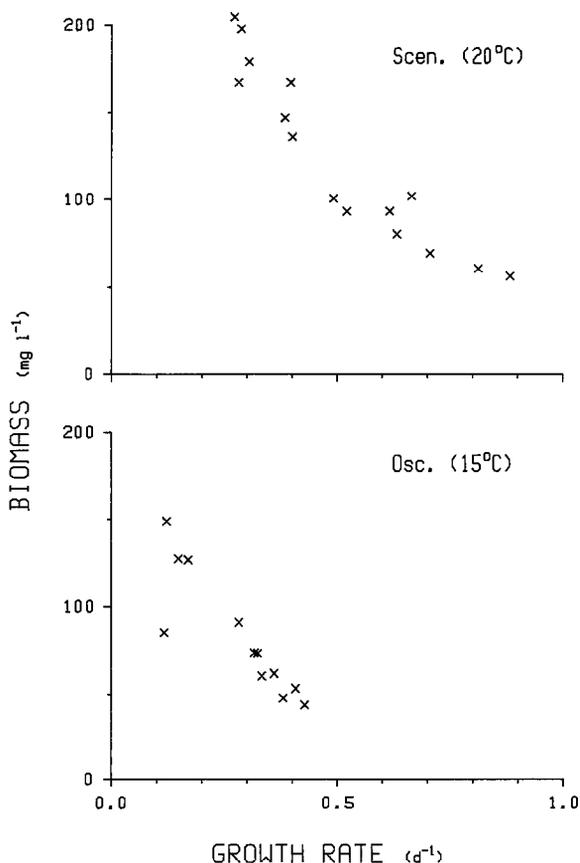


Fig. 10. Biomass (B) versus specific growth rate (μ) from P-limited chemostat experiments with *Scenedesmus quadricauda* at 20 °C and with *Oscillatoria agardhii* at 15 °C. (Data from Ahlgren 1987 and 1985b.)

$$Y = \frac{B}{S_0 - S_1} \quad (20)$$

should be a useful tool in this connection. This yield coefficient, Y , which originates from agricultural plant physiologists dealing with the harvest of crops (e.g., Mitscherlich, 1909; cf. Ahlgren, 1977), is unfortunately considered only in exceptional cases in the algal literature today (e.g., Shelef *et al.*, 1971). Y varies with μ in the same way as the biomass. At low μ the alga 'need' less nutrient than at higher μ in order to produce a certain amount of biomass.

It is argued that there is almost no ecological importance in the distinction between non-interactive versus possible interactive resources. That

may be true concerning competition between two species, but hardly for the biomass and yield of the individual species. Data from Ahlgren (1985b) clearly show that the harvest (dry weight per litre per day) was lowest at a ratio of $N/P = 14$ in the inflow medium, indicating an influence of N and P simultaneously (Fig. 11b). Experiences from the field also point to distinct ecological effects of the concentration levels of two nutrients. The production of algal biomass in two eutrophic lakes was not particularly influenced when either N or P were reduced, but when both nutrients were reduced, the biomass decreased very clearly (Ahlgren, 1978b, 1988). The biomass yield calculated for one nutrient can also be influenced by the concentration of the other nutrient. That can be demonstrated very clearly, even without statistics,

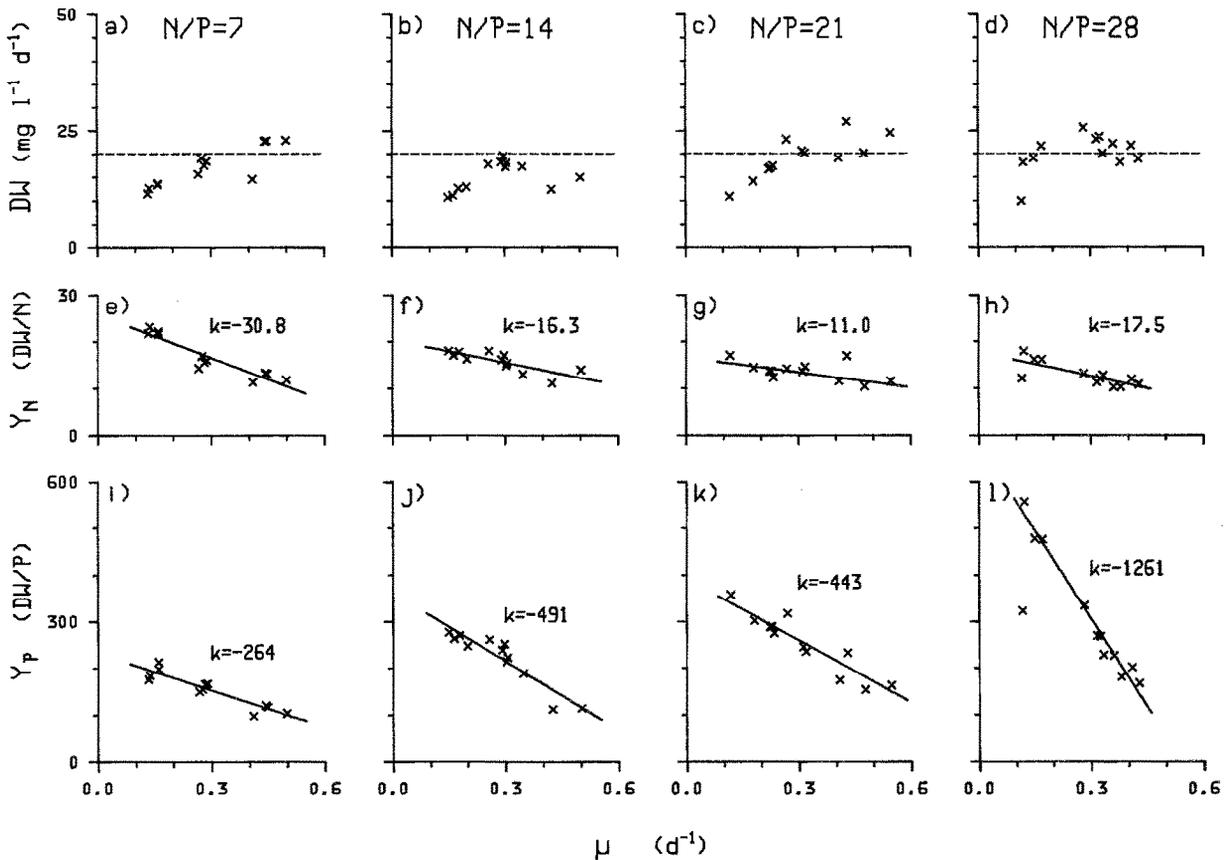


Fig. 11. Results of chemostat experiments with *Oscillatoria agardhii* with different N/P-ratios in the medium: 1st row: Harvest (mg DW l⁻¹ d⁻¹) versus growth rate (μ). 2nd row: N yield (biomass produced/N consumed) versus μ . 3rd row: P yield (biomass produced/P consumed) versus μ . (From Ahlgren 1985b).

with an argumentation analogous to that for the N- and P- contents of the algae (Ahlgren, 1985b): It was established that the alga was solely N-limited at $N/P = 7$ and P-limited at $N/P = 28$ in the inflow medium (Fig. 11). The non-interactive model says that the algae can be either N- or P-limited. According to the slopes (given in the figures), it is clear that the data in Figure 11f and g are different from those in Figure 11e. The intermediate N/P-ratios ($N/P = 14$ and 21) in the inflow cannot, thus, be N-limiting. However, the slope in 11i is also different from the slopes in Figure 11k and j, indicating that the same intermediate N/P-ratios cannot be P-limiting either. An either/or principle is, thus, not likely in this case. Instead, the yield of *Oscillatoria agardhii* must have been influenced by N and P simultaneously, i.e., an interactive model is more likely.

Y at μ -max was found to be relatively constant with temperature (Shelef *et al.*, 1971; Topiwala & Sinclair, 1971; Ahlgren, 1987). However, at lower μ , Y increases with temperature and can be described by a 2nd degree polynomial:

$$Y = A + B \cdot \mu + C \cdot \mu^2 \quad (21)$$

It was found that the constants A, B and C have different values at different temperatures (Ahlgren, 1987). A, which represents Y at $\mu = 0$, increases with temperature, while B (slope) and C (bend) merely decrease with temperature. Fig. 12 shows Y as a function of μ at four different temperatures. These results mean that when the algae grow at μ -max, which is lower at lower temperatures, they need about the same amount of P irrespective of temperature. But if for some reason they are growing at $\mu < \mu$ -max, the P-yield decreases with temperature. The lower the growth rate, the larger is the influence of the temperature (Fig. 12). These results have hitherto only been demonstrated in experiments using one algal species *Scenedesmus quadricauda*, so it is too early for generalizations. However, Y is often put equal to $1/q$, which is valid when the excretion of the soluble organic nutrient in question is missing or so low that it is negligible. As the temperature influence on the μ/q relationship was demon-

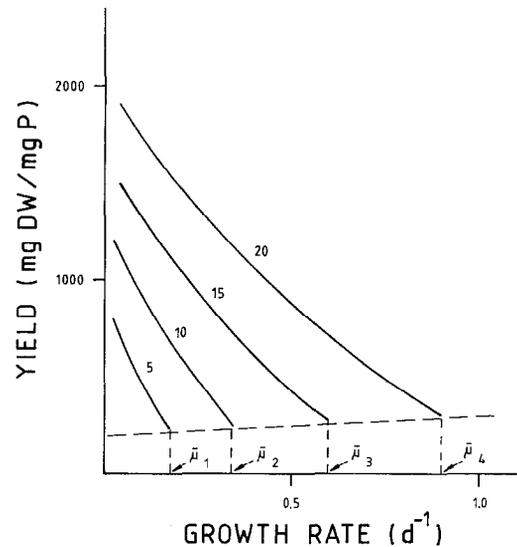


Fig. 12. Principal diagram of the P-yield (biomass produced/P consumed) versus specific growth rate (μ) at four different temperatures; 5, 10, 15 and 20 °C. $\bar{\mu}_1$, $\bar{\mu}_2$, $\bar{\mu}_3$ and $\bar{\mu}_4$ indicate the maximum specific growth rates at the different temperatures. (Data of the green alga *Scenedesmus quadricauda*, Ahlgren 1987.)

strated for several algal species, it is possible that Fig. 12 also shows a more general picture.

Conclusions

Light, temperature and nutrient factors greatly influence the P utilization of algae. Although it is difficult to separate the effects of physical factors from those of nutrients, there seem to be no principal differences between these two types of factors.

Present knowledge of combined effects of physical and nutrient factors can be summarized:

1. With increasing light/growth rate the algae need relatively more P, which means that high light adapted algae are more sensitive to P limitation than low light adapted algae.
2. Below optimum temperature the minimum P content (subsistence quota) is inversely correlated with temperature. It is still unclear whether the relationship concerning the whole temperature range is U-shaped or not.

3. The transition between light- and P-limitation as well as between N- or C- and P-limitation (R_c) varies with growth rate. The critical ratios (R_c) should be viewed as growth rate dependent variables and not as constants.
4. Two different opinions prevail on how dual limitation of two nutrients affect the growth rates of phytoplankton; either a non-interactive model or an interactive model. At present some results show better agreement with the non-interactive model. There are, however, also certain occasions when an interactive model better describes the experimental data.

In other words: when growth conditions depart from the optimum for a physical factor, the internal content of the limiting nutrient (and sometimes also non-limiting nutrients) increases. More studies are needed to find out how these light-temperature-nutrient interactions really work. It is also necessary to explain these interactions in quantitative terms to establish more general models which can be used in long-term predictions of algal development in lakes. This work is still in its early stages.

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