Limiting Factors in Phytoplankton Algae: Their Meaning and Measurement

Abstract. *There are two common responses of plants to changes in concentration of limiting factors: change in the final yield (type I response) or change in the growth rate (type II response). Type II is typical of phytoplankton algae in nature, yet some experiments have failed to show growth rate changes because of inappropriate experimental design.*

To understand and control eutrophication in natural waters it is essential to determine what factor or factors are limiting the growth of phytoplankton algae. However, in designing and interpreting experiments to determine limiting factors, aquatic ecologists have not fully recognized that there are two possible fundamental growth responses by which the plant biomass or population may increase until limited by some factor. The first, which I call type I, is that originally described by Liebig (*1*) and known as Liebig's law of the minimum: The growth rate remains unchanged but growth continues longer, and the final yield increases with increasing concentration of the limiting factor (Fig. 1). In terrestrial plants, where nutrient cycling is slow and daily predation is relatively slight, such a situation may be common. The second pattern of growth, which I call type II, is that described by Blackman (*2*): The rate of growth of the plant increases with increasing concentration or intensity of the limiting factor (Fig. 1).

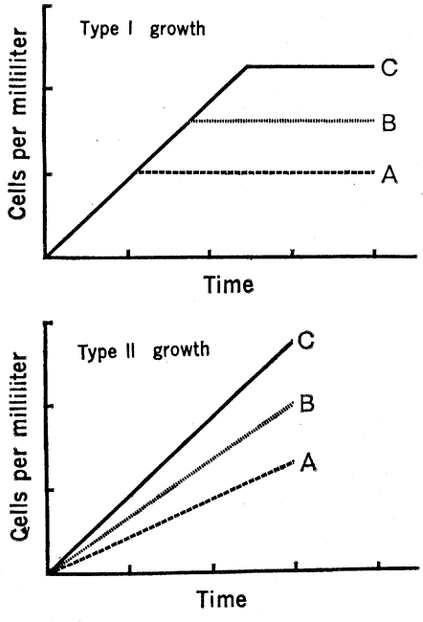

Fig. 1. Type I and type II growth patterns. The ordinate is a logarithmic scale. Increasing concentrations of the limiting factor are represented by *A*, *B*, and *C*.

Whereas only one of these growth patterns is likely to be typical of plankton algae in natural situations, both have been reported in the limnological literature (*3–6*). Because of high mortality rates and the rapid rate of nutrient cycling in aquatic situations, type II is the most likely and, in fact, many studies have conclusively shown changes in algal growth and photosynthetic rates with changes in the temperature (*7*), the intensity of light (*8*), or the concentration of vitamins (*9*), phosphorus (*4*, *5*), or nitrogen (*6*, *10*). Yet in many laboratory studies where many of these same factors have been varied, no change in growth rate has been found, but a change in final yield has been observed.

I believe that in many of these experiments one rate-limiting factor was in such low concentration that it obscured the effect which a change in concentration or intensity of other limiting factors could have had on the algal growth rate. The intensity of light in laboratory experiments could have such an effect, as could the concentration and form of carbon in the culture medium, or perhaps a lack of vitamins or other organic growth factors. Jones (*11*) has shown a distinct interaction between light intensity and nutrient concentration on the growth rate of *Carteria* sp. At low light intensities a change in the concentration of nitrogen and phosphorus had no effect on the rate of growth, whereas at medium light intensities the rate of growth was higher with a greater concentration of nitrogen and phosphorus. Such a situation may be common in laboratory studies, where it is difficult to create natural light intensities. This alone may account for the fact that many laboratory studies yield data that fit growth patterns of type I rather than of type II.

Another source of difficulty in observing a change in the rate of growth may be the experimental technique used. Laboratory bioassays involving a batch culture technique, in which a large amount of water to be tested (a batch) is incubated with a relatively small initial inoculum of a test organism, rarely show a type II pattern. With this experimental design a diminished growth rate caused by the low concentration of some limiting factor appears only as the population approaches maximum yield. However, this change in rate is almost impossible to observe, because while the population is growing exponentially the nutrient concentration is decreasing exponentially (*12*), and the population grows most of the time in a relatively high, nonrate-limiting concentration of the limiting nutrient. Only during the last day or so of the experiment does the population encounter a rapidly decreasing nutrient medium.

This can be seen in a hypothetical case developed by using the data of Golterman *et al.* (*5*), where the relationship between the rate of growth of *Scenedesmus obliquus* and the concentration of phosphorus follows a Monod-type equation (*13*). These data can be used to predict the change in the rate of growth of a population as the concentration of phosphorus changes. Figure 2 shows how such a population would grow in culture if the amount of phosphorus taken up is proportionate to the number of cells created. Notice the slight difference in the population growth as it is influenced by the concentration of phosphorus, and what the population growth would be if the growth rate remained constant and was not influenced by the concentration of

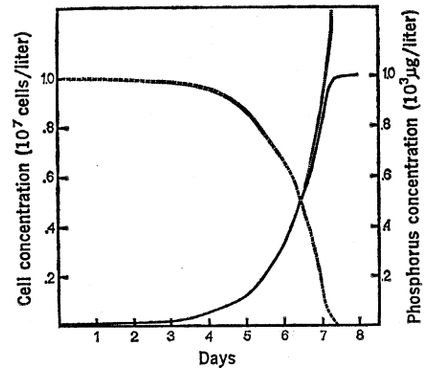

Fig. 2. Hypothetical population growth curve. The dashed line represents the decrease in inorganic phosphorus. The dotted line represents the population increase of phytoplankton algae uninfluenced by phosphorus concentration. The solid line represents the population increase as affected by the change in phosphorus concentration. Notice the slight change in growth rate in response to a low phosphorus concentration.

phosphorus. The decrease in growth rate is abrupt, and is extremely difficult to detect. The rate of growth changes only slightly during the first 6 days and decreases by less than 20 percent on the following day, but in the next 8 hours it decreases from 80 percent of the maximum rate to 0. Such a mode of growth is considerably different from what might be expected from the logistic growth model.

Other studies have failed to show a change in growth rate with differing concentrations of important nutrients because the concentrations of nutrients are very high (*14*). If the relationship suggested by Monod (*13*) and shown by Golterman *et al.* (*5*) to hold for *Scenedesmus* is valid, then changes in nutrient concentration dramatically alter the rate of growth or photosynthesis only at rather low nutrient concentrations.

There are natural situations in which a type I growth pattern may seem to occur. The study of *Asterionella formosa* over a number of years by Lund (*15*) shows that the reduction in the concentration of silica dissolved in the water coincides with the increase in *A. formosa*, and that each year growth ceases at a silica concentration of about 0.5 mg/liter, with the final yield of *A. formosa* being determined by the concentration of silica. This type of response may be determined by the slow rate of silica turnover or by the fact that silica plays no role in cell metabolism. However, as demonstrated in Fig. 2, it is extremely difficult to detect changes in the rate of growth when the population density is increasing and, consequently, the nutrient concentrations are decreasing rapidly.

It seems certain that changes in the concentration or intensity of most factors that have been identified as limiting to phytoplankton algae cause changes in the growth rate, but not necessarily in the final yield. Experimenters working on eutrophication problems and limiting factors must recognize the basic difference in the two growth patterns and design experiments that will test for changes in growth rate. Workers must be especially careful in interpreting changes in yield as definitive when they are determining whether a particular factor is limiting in nature.

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10 NOVEMBER 1972

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$$\mu = \hat{\mu}\left(\frac{S}{K_s + S}\right)$$

where μ is the specific growth rate; $\hat{\mu}$ is the maximum growth rate; S is the concentration of the substrate limiting the growth rate; and K_s is the half-saturation constant, equal to the substrate concentration where the specific growth rate is equal to one-half the maximum growth rate.
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16. Supported in part by NSF grant BO-15665 and grant 3305-5038 from the University of Kansas General Research Fund.

14 April 1972; revised 18 September 1972

Prolonged Survival of Second Human Kidney Transplants

Abstract. *Rejection of kidney transplants in 264 patients, followed by retransplantation from cadaver donors, resulted in a 1-year survival rate of 51 ± 3 percent (rate ± standard error) as compared to 51 ± 1 percent for first transplants. If the first transplant immunizes the patient or is rejected by immunologically responsive patients, second grafts into the same patients would be expected to be rejected at a higher rate. Only those reject who reject first grafts hyperacutely or between 1 to 3 months were found to have low second graft survival rates. Patients who rejected transplants after 3 months tended to have second transplant survival rates which were higher than their first graft survival rates.*

Second kidney transplants should be more rapidly rejected than the first according to the classical concept of transplantation immunity as established by Medawar. Human kidney transplants from cadaver donors appear to run counter to this rule. Survival of 257 second grafts almost exactly paralleled the survival of 1497 first grafts (*1*) (Fig. 1A). This confirms the findings of the Kidney Transplant Registry and those of Hume *et al.* (*2*). Of course, since the donors are not the same for the second graft, it could be argued that the diversity of HL-A antigens is so great that the chances for immunization to apply to a random second donor would be slight. Yet with cross-reaction it would be anticipated that, averaged over a large series of second transplants, second grafts should be rejected more rapidly than first grafts. Moreover, if immunologically responsive patients reject grafts, those who are selected out as rejectors by the first graft should more rapidly reject their second grafts. Yet most patients who are retransplanted have a *longer* survival time for their second than for their first graft. This study was undertaken to investigate this paradoxical effect.

Data of kidney transplant patients were kindly made available to us by 58 U.S. and Canadian transplant centers. Survival rates of 264 second grafts from cadaver donors transplanted between January 1967 and December 1971 were computed by actuarial methods (*3*) in different subsets as shown in Figs. 1 and 2. All patients had lost their first transplants either from related or cadaver donors. In none of the studied subsets could a significant difference be found in second graft survival after failures of first transplants from either related or cadaver donors.

Subdividing the patients into those who rejected their first grafts at different time periods, we found that three distinct types of second graft survival rates exist. As shown in Fig. 1B, patients who lose their first grafts within 1 month (hyperacute rejections excluded) have a second graft survival rate of 48 ± 6 percent (rate ± S.E.) at 1 year, a figure very similar to the