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Principles of the light-limited chemostat: theory and ecological applications

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Abstract

Light is the energy source that drives nearly all ecosystems on planet Earth. Yet, light limitation is still poorly understood. In this paper, we present an overview of the principles of the light-limited chemostat. The theory for light-limited chemostats differs considerably from the standard theory for substrate-limited chemostats. In particular, photons cannot be mixed by vigorous stirring, so that phototrophic organisms experience the light-limited chemostat as a heterogeneous environment. Similar to substrate-limited chemostats, however, light-limited chemostats do reach a steady state. This allows the study of phototrophic microorganisms under well-controlled light conditions, at a constant specific growth rate, for a prolonged time. The theory of the light-limited chemostat is illustrated with several examples from laboratory experiments, and a variety of ecological applications are discussed.

Introduction

Chemostats have contributed considerably to the development of general ecological theories, because they offer several convenient advantages for the pragmatic ecologist. Firstly, microorganisms typically grow in large numbers and have short generation times. Hence, many generations can be investigated within a short time-span. Secondly, chemostats provide an experimental device in which a wide variety of environmental factors can be simulated under well-controlled laboratory conditions. Thirdly, the spatial homogeneity offered by the chemostat provides an ideal testbed for many ecological models. Fourthly, the accuracy of chemostat experiments allows a precision of replication that is almost unprecedented in ecological research.

As a result, over the past decades chemostats have contributed to a firm body of ecological theory on substrate-limited growth and competition (Monod 1950; Droop 1974; Fredrickson 1977; Veldkamp 1977; Tilman 1982; Grover 1991a; Smith & Waltman 1995). The theory is supported by a wealth of chemostat experiments (Novick & Szilard 1950; Harder et al. 1977; Tilman 1977; Grover 1991b; Gerritse et al. 1992; Smith 1993; Lendenmann et al. 1996; Ducobu et al. 1998). Moreover, chemostats and related laboratory microcosms have found wide application in other fields of ecological research, including studies of food-web dynamics (Pengerud et al. 1987; Bohannan & Lenski 1997; Kaunzinger & Morin 1998), biodiversity (McGrady-Steed et al. 1997; Notley-McRobb & Ferenci 1999; Rainey et al. 2000), and evolution (Dykhuizen 1990; Rosenweig et al. 1994; Velicer & Lenski 1999).

Nearly all chemostat theory and experiments are based on the notion of one or more limiting substrates. These limiting substrates are usually supplied in the form of a mineral medium, and are distributed homogeneously throughout the culture system by vigorous stirring. This general set-up is applicable to nearly all potentially limiting substrates, including inorganic nutrients, important vitamins, and organic carbon sources. However, the set-up does not apply to one peculiar but extremely important resource. It does not apply to light, the energy source that drives nearly

all ecosystems on our planet. Firstly, photons cannot be supplied by means of a mineral medium. Secondly, and most importantly, photons cannot be mixed by vigorous stirring but form a photon flux that decays exponentially with depth.

Over the past years, our laboratory has worked on a consistent body of theory and experiments for light-limited chemostats (Mur et al. 1977; Post et al. 1984; Huisman & Weissing 1994, 1995; Matthijs et al. 1996; De Nobel et al. 1998; Huisman 1999; Huisman et al. 1999a). It turns out that the theory for light-limited growth is manageable, but differs considerably from the theory for substrate-limited growth. This paper gives an overview of our theoretical and experimental approach, with an emphasis on the theory that underlies light limitation and with a prospect for ecological applications.

Materials and methods

Phototrophic species

Light-limited chemostats are designed to study phototrophic microorganisms. For ease of communication, in the sequel we will frequently use the term 'phytoplankton' to refer to phototrophic microorganisms. Obviously, from a strict taxonomic perspective this term is not always appropriate. The theory and experiments outlined below are relevant for studies of all phototrophic microorganisms, including anoxygenic prokaryotes like the purple bacteria and green bacteria, oxygenic prokaryotes like the cyanobacteria and prochlorophytes, eukaryotic phytoplankton like green algae and diatoms, as well as cell cultures of higher plants.

Culture system

One of the key features of light is its essentially unidirectional nature. In aquatic ecosystems, the light flux generally enters from above, and photons that are not absorbed penetrate into the deep. Our light-limited chemostats are specifically designed to simulate the unidirectional nature of the light gradient. For practical convenience, however, we rotated the light gradient by 90°. Thus, photons do not traverse from top to bottom but from left to right (Matthijs et al. 1996; Huisman et al. 1999a).

Figure 1 shows a schematic drawing of the chemostat system. The entire chemostat system is placed in a closed box, to avoid interference with outside

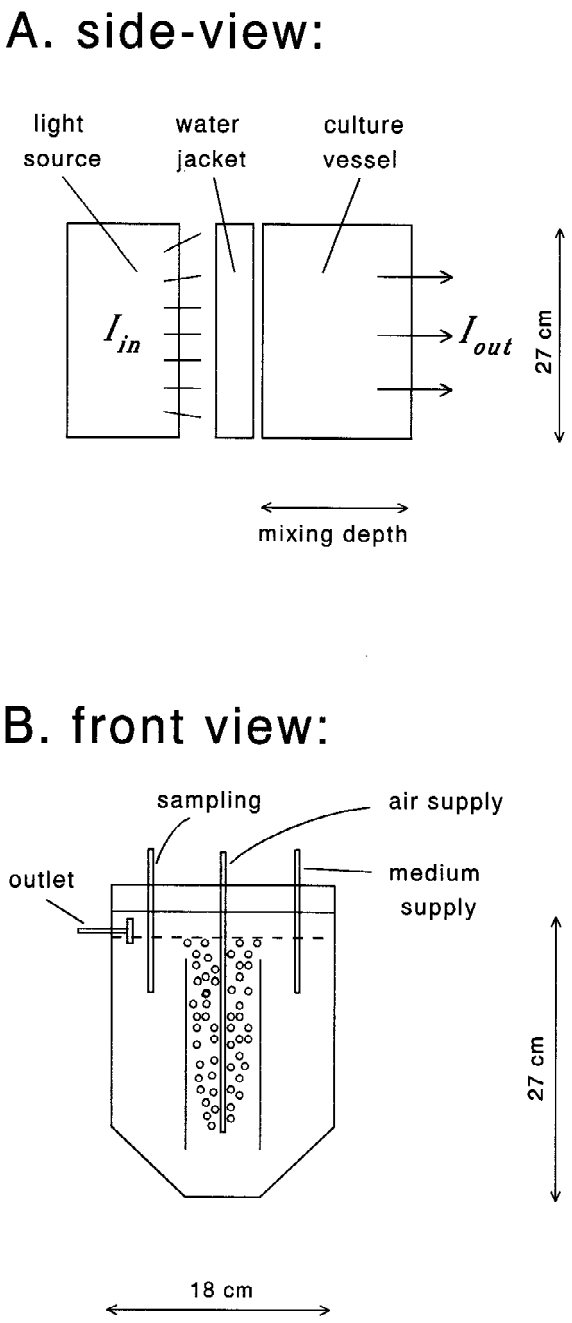

Figure 1. Schematic drawing of the light-limited chemostat. (A) Side-view with light source, water jacket, and culture vessel. (B) Front view of the culture vessel. (Modified from Huisman et al. (1999a), with permission from the Ecological Society of America.)

light sources. Each box contains a light source, a water jacket, and a culture vessel. The light source may consist of fluorescent tubes, light-emitting diodes, or electric bulbs, depending on the preferred light spec-

trum. The light sources are arranged next to each other to cover the full front of the culture vessel. A rectangular water jacket, connected to a thermocryostat, is placed between the light source and the culture vessel, to remove the heat of the lamps and to maintain the culture vessel at a constant temperature. We use culture vessels with a variety of different optical path lengths, which allows laboratory simulation of different mixing depths (Figure 1).

Sterile mineral medium, rich in nutrients, is pumped from 3-l bottles into the culture vessel using a peristaltic pump. An outlet towards a sterile waste bucket allows the mineral medium, together with the cultured organisms, to leave the culture vessel at the same rate again. The dilution rate, D, of the continuous culture is expressed as the flow rate, F, set by the peristaltic pump divided by the effective working volume, V, in the culture vessel (i.e., $D = F/V$).

Homogeneous mixing of the cultures is ensured by flushing small gas bubbles, at a high rate of 100–150 l/h, between two partitions within the culture vessel. The gas may consist of nitrogen gas (for anoxygenic phototrophs) or compressed air (for oxygenic phototrophs), usually with some added carbon dioxide to satisfy the carbon demands of the cultures. The gas bubbles cause an upward stream in the middle of the culture, and a corresponding downward stream at the right- and left-hand sides. This airlift system generally leads to well-mixed cultures. For species with high sinking rates, additional stirring by a magnetic stir bar may be recommended to avoid accumulation of cells at the bottom surface. The same magnetic stir bar may also be used to prevent wall growth of sticky species, by scraping the cultures at least once a day. To get rid of very sticky species, an alternative vessel might be attractive, in which the two partitions are removed and air bubbles are supplied through a sintered glass sieve at the bottom of the vessel.

The water jacket, culture vessel, and tubes for sampling, air supply, and medium supply are all made of glass, to keep the entire system as transparent as possible. The lid sealing off the vessel is laboratory built, and made of polycarbonate with three stainless-steel inlays to hold the three glass tubes. The entire culture vessel is autoclaved prior to inoculation with phytoplankton.

Nutrients

The suitability of a particular nutrient mix often depends on the kind of species that is taken into culture.

Table 1. Mineral composition of a simple nutrient-rich medium for phytoplankton in light-limited chemostats

Nutrient	Concentration (mg/l)
$NaNO_3$	1500
$K_2HPO_4 \cdot 3H_2O$[1]	80
$NaHCO_3$ [1)]	80
$MgSO_4 \cdot 7H_2O$	75
$CaCl_2 \cdot 2H_2O$	36
$Fe(III)(NH4)_3$-citrate[1]	6
Citric acid	6
H_3BO_3	4.3
$MnCl_2 \cdot 4H_2O$	2.7
Na_2EDTA	1.1
$Na_2MoO_4 \cdot 2H_2O$	0.6
$ZnSO_4 \cdot 7H_2O$	0.3
$CuSO_4 \cdot 5H_2O$	0.12
$Co(NO3)_2 \cdot 6H_2O$	0.07

[1]Autoclaved separately and added to the mineral medium after cooling to room temperature, to avoid precipitates.

Most importantly, an ample supply of nutrients is essential to obtain light limitation. As will be explained in more detail below, the expected population density under light-limited conditions is inversely proportional to the mixing depth of the culture vessel. Consequently, light-limited chemostats with a shallow mixing depth have a higher nutrient demand than light-limited chemostats with a deep mixing depth. To ensure that all nutrients are provided in ample supply, it is generally recommended to investigate whether a doubling or halving of the nutrient dosage has any effect on the steady-state population density. It is our experience that several of the mineral media published in the literature do not pass this test, and contain insufficient nutrients to support the high population densities that can be achieved in light-limited chemostats. A standard nutrient-rich medium that frequently works quite well for freshwater phytoplankton in our chemostat experiments is given in Table 1.

Inorganic carbon

The high population densities typically obtained in light-limited chemostats demand for a high supply of inorganic carbon. It is straightforward to calculate that if all required carbon would be supplied in the form of bicarbonate then the solubility products of most carbonates would be greatly exceeded. Thus, sufficient inorganic carbon can only be supplied in gaseous

form. Compressed air flushed at a high rate of 100–150 l/h often still contains insufficient carbon dioxide to satisfy the carbon demand. This holds especially true for light-limited chemostats with shallow mixing depths, which have particularly high population densities (as will be explained below). Therefore, addition of compressed carbon dioxide is recommended. Unfortunately, adding too much carbon dioxide may lead to a rapidly dwindling pH. A sufficient dosage is obtained if the pH in the culture vessel is monitored, and the carbon dioxide addition is continuously adjusted in such a way that the pH in the culture vessel remains close to the pH of the original medium.

Theory and experiments

The light gradient

The light intensity incident upon the culture vessel (I_{in}, in μmol photons m^{-2} s^{-1}) can be adjusted by tuning the intensity of the lamps. This is not always the recommended approach, however, because many lamps change their spectral composition with a change in intensity. Alternatively, the incident light intensity can be conveniently adjusted without spectral changes by means of neutral density filters placed between the light source and the water jacket. Further fine-tuning is achieved by changing the distance between the light source and the culture vessel.

The incident light is partly absorbed by phytoplankton and partly by the mineral medium. Let $I(z, t)$ indicate the light intensity at depth z and time t. According to Lambert-Beer's law, light absorption (i.e., the change of light intensity with depth) is proportional to the concentrations of the light-absorbing substances and to the local light intensity:

$$\frac{\partial I}{\partial z}(z, t) = -\sum_{j=1}^{n} k_j \omega_j(t) I(z, t) \tag{1}$$

where summation is over all light-absorbing substances (including phytoplankton, dissolved material, and the water itself), the proportionality constant k_j is the specific light attenuation coefficient of light-absorbing substance j, and $w_j(t)$ is the concentration of light-absorbing substance j. It is useful to distinguish between light absorption by phytoplankton and light absorption by all non-phytoplankton components in the water column. To reduce the number of model parameters, we therefore summarize light absorption by all non-phytoplankton components into a single term, which we call the background turbidity. Thus, if phytoplankton is labeled by the subscript 1, and the other light-absorbing substances by the subscripts 2 to n, the background turbidity is defined as:

$$K_{\text{bg}} = \sum_{j=2}^{n} k_j \omega_j(t) \tag{2}$$

For notational convenience, we drop the subscript 1. Accordingly, Equation (1) becomes:

$$\frac{\partial I}{\partial z}(z, t) = -(k\omega(t) + K_{\text{bg}}) I(z, t) \tag{3}$$

where k is now the specific light attenuation coefficient of the phytoplankton, and $w(t)$ is the population density of the phytoplankton. Equation (3) can be solved, which yields an equation for the light intensity I at depth z and time t:

$$I(z, t) = I_{\text{in}}\, \text{e}^{-(k\omega(t)+K_{\text{bg}})z} \tag{4}$$

Thus, Lambert-Beer's law states that light intensity decays exponentially with depth. We note from Equation (4) that the light gradient is not static but dynamic. Any change in phytoplankton population density will result in a concomitant change in the light gradient.

We note that Lambert-Beer's law is an approximation of reality. For instance, our formulation above neglects reflection and scattering of photons as well as the spectral distribution of light. For more comprehensive treatments of the underwater light field, the interested reader is referred to the optics literature (e.g., Kirk 1994).

Useful statistics and measurements

The light gradient can be summarized by several useful statistics. Light penetration, I_{out}, is a key parameter defined as the light intensity that penetrates through the culture:

$$I_{\text{out}}(t) = I_{\text{in}}\, \text{e}^{-(k\omega+K_{\text{bg}})z_{\text{m}}} \tag{5}$$

where z_{m} is the mixing depth (optical path length) of the culture vessel. Given a unidirectional light gradient, light penetration is easily measured by mounting a quantum sensor at the back surface of the culture vessel. To estimate the spatial variation in light intensity on the plane perpendicular to the unidirectional light flux, we commonly measure light penetration at 10 regularly spaced positions at the back surface. This

reveals that, in our chemostat systems, the spatial coefficient of variation of I_{out} is generally less than 8%. Mirrors placed against the side surfaces of the culture vessel help to reduce losses of light by sideward scattering. Furthermore, all those parts at the front surface of the culture vessel that should not be illuminated can be covered by black paper. Addition of mirrors and black paper reduces the spatial coefficient of variation of I_{out} even further, to less than 5%. This illustrates that a truly unidirectional light gradient can indeed be achieved in the laboratory.

Measurement of I_{in} and I_{out} is all that is required to estimate the background turbidity, the specific light attenuation coefficient of the phytoplankton, the fractional light absorption, and the average light intensity. More specifically, the background turbidity can be estimated from a chemostat filled with mineral medium but without phytoplankton, according to the equation:

$$K_{bg} = \frac{\ln(I_{in}) - \ln(I^0_{out})}{z_m} \tag{6}$$

where I^0_{out} denotes light penetration through a culture vessel without phytoplankton.

With knowledge of the background turbidity, the specific light attenuation coefficient of the phytoplankton can be calculated from a chemostat containing phytoplankton, according to the formula:

$$k = \frac{1}{\omega}\left(\frac{\ln(I_{in}) - \ln(I_{out})}{z_m} - K_{bg}\right) \tag{7}$$

We note that the specific light attenuation coefficient is a species-specific parameter.

The fractional light absorption, F, is defined as the fraction of the total light absorption that is absorbed by phytoplankton. It is expressed as (Tilzer 1983; Kirk 1994):

$$F(t) = \frac{k\omega}{k\omega + K_{bg}} \tag{8}$$

where k and K_{bg} have been estimated according to Equations (6) and (7), respectively. We note that the remaining fraction, $1 - F$, corresponds to the fraction of the total light absorption that is absorbed by the background turbidity.

The mean light intensity, $\hat{I}$, is defined as the depth-average of all light intensities in the water column. Using Lambert-Beer's law, the mean light intensity

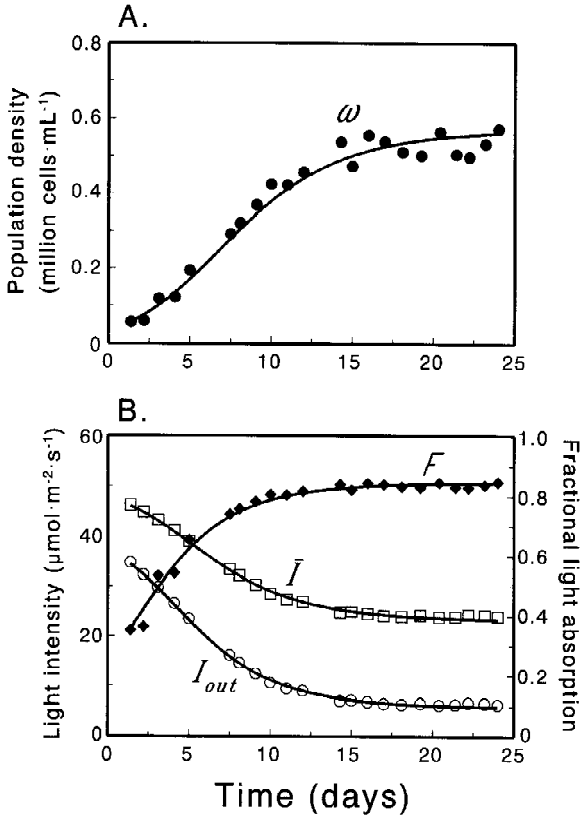

Figure 2. (A) Time course of the green alga *Scenedesmus protuberans* in a light-limited chemostat. (B) The accompanying time courses of light penetration, I_{out}, fractional light absorption, F, and mean light intensity, $\hat{I}$. Light penetration was measured directly on the culture vessel. Fractional light absorption and mean light intensity were calculated according to Equations (8) and (9), respectively. Solid lines indicate the model predictions made by Equations (18a,b). System parameters: I_{in}=60 μmol photons m^{-2} s^{-1}; z_m=5 cm; K_{bg}=0.072 cm^{-1}; D = 0.015 h^{-1}. Species parameters: μ_{max} = 0.070 h^{-1}; H = 107 μmol photons m^{-2} s^{-1}; $k = 0.7 \times 10^{-6}$ cm^2 cell^{-1}.

can be conveniently expressed as:

$$\bar{I}(t) = \frac{1}{z_m}\int_0^{z_m} I(z,t)\,\mathrm{d}z = \frac{I_{in} - I_{out}}{\ln(I_{in}) - \ln(I_{out})} \tag{9}$$

As an illustration, Figure 2 shows results of an experiment with the green alga *Scenedesmus protuberans* Fritsch. The incident light intensity was 60 μmol photons m^{-2} s^{-1}. In 3 weeks time, population densities increased from 50 000 cells/ml to more than 500 000 cells/ml. As a consequence, fractional light absorption by *Scenedesmus* increased from 0.35 to 0.85, the mean light intensity within the culture vessel decreased from 54 to 23 μmol photons m^{-2} s^{-1}, and

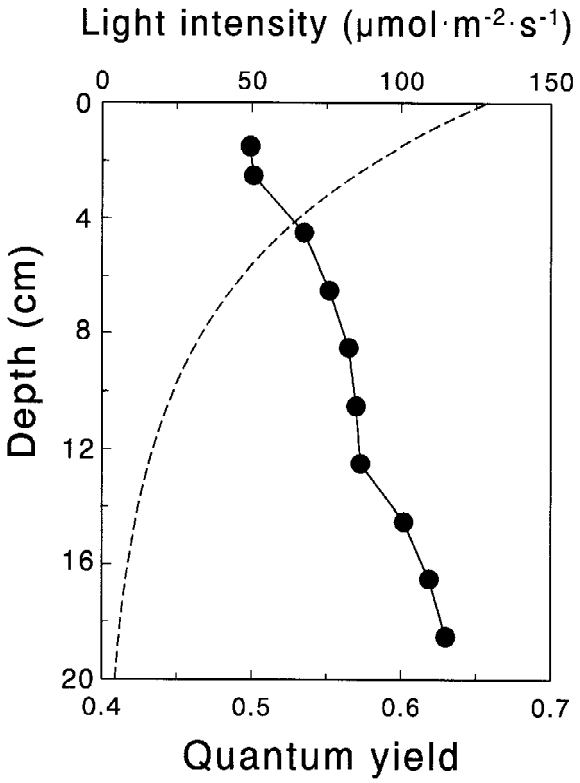

Figure 3. Quantum yields of PhotoSystem-II electron transport (closed circles) of the green alga *Chlorella vulgaris*, measured at various depths within the same culture vessel by means of fluorescence techniques. The dashed line indicates the light gradient in the vessel. The population density of *Chlorella* was 4 million cells/ml.

light penetration through the culture vessel decreased from 35 to 6 μmol photons m^{-2} s^{-1}. In other words, the light gradient became steeper.

Phytoplankton in the light gradient

To develop a proper theory of light-limited growth, it is essential to establish how phytoplankton cells cope with the light gradient. There are two alternative hypotheses:

H0: The null hypothesis is that phytoplankton cells, when vigorously mixed, do not experience the spatial heterogeneity of the light gradient. They respond to the mean light intensity only, and therefore experience the chemostat as a homogeneous environment.

H1: The alternative hypothesis is that phytoplankton, when vigorously mixed, do experience the spatial heterogeneity of the light gradient. They respond to all light intensities in the gradient, and hence experience the chemostat as a heterogeneous environment.

To discriminate between the two hypotheses, we measured the efficiency of photosynthesis *in situ*, at various depths within a 20-cm deep culture vessel containing the green alga *Chlorella vulgaris* Beyerinck. Mixing of the cells through the vessel took only a few seconds (observation by eye). If the phytoplankton cells respond to the mean light intensity only, photosynthetic efficiency should remain constant over depth. Alternatively, if the phytoplankton cells respond to all light intensities within the gradient, photosynthetic efficiency should increase with decreasing light intensity and should thus increase with depth.

The *in situ* photosynthetic efficiency was measured as the quantum yield of PhotoSystem-II electron transport. The quantum yield of PS-II electron transport measures the fraction of photons absorbed by the photosynthetic machinery that is utilized for photosynthesis. The basic idea behind this technique is as follows (e.g., Schreiber et al. 1986; Genty et al. 1989; Kolber & Falkowski 1993). Photons captured by pigment molecules excite an electron. Some of this excitation energy cannot be utilized, however, because the photosynthetic machinery is still processing the energy obtained from previously absorbed photons. In this case, the excitation energy is wasted, either as heat loss or as fluorescence. At low light intensities, only a few photosynthetic units are occupied and most of the absorbed photons are utilized. At high light intensities, many photosynthetic units are occupied and a large fraction of the absorbed photons is wasted. Let F_s denote the fluorescence under normal growth conditions, and let F_m denote the maximal fluorescence under fully saturated light conditions. The quantum yield of PS-II electron transport, ϕ_{PSII}, is then calculated as (Genty et al. 1989):

$$\phi_{PSII} = 1 - \frac{F_s}{F_m} \tag{10}$$

The fluorescence signals were monitored at various depths within the culture vessel using a PAM-Walz 101 fluorometer (Schreiber et al. 1986). The measurements were made by placing the head of the fiber optics directly against the side surface of the culture vessel. Normal fluorescence, F_s, was detected by means of a non-actinic, pulsed measuring beam (peak wavelength 650 nm, half-bandwidth 20 nm, pulse frequency of 100 kHz) delivered by a light-emitting diode. The light intensity from this measuring beam was less than 0.3 μmol photons m^{-2} s^{-1}, which

Table 2. Different mathematical formulations for the specific production rate as a function of light intensity

Equation	Reference
$p(I) = \begin{cases} \alpha I & \text{if } I < p_{\max}/\alpha \\ p_{\max} & \text{if } I > p_{\max}/\alpha \end{cases}$	Blackman (1905)
$p(I) = \frac{p_{\max} I}{H+I}$	Baly (1935), Monod (1950)
$p(I) = \frac{p_{\max} \alpha I}{\sqrt{p_{\max}^2 + (\alpha I)^2}}$	Smith (1936)
$p(I) = \frac{\alpha I + p_{\max} - \sqrt{(\alpha I + p_{\max})^2 - 4\alpha I p_{\max} \theta}}{2\theta}$	Rabinowitch (1951)
$p(I) = \alpha I \exp\left(-\frac{bI}{p_{\max}}\right)$	Steele (1962)
$p(I) = p_{\max}\left(1 - \exp\left(-\frac{\alpha I}{p_{\max}}\right)\right)$	Webb et al. (1974)
$p(I) = p_{\max} \tanh\left(\frac{\alpha I}{p_{\max}}\right)$	Jassby and Platt (1976)
$p(I) = p_{\max}\left(\frac{\exp(\alpha I(1+b)/p_{\max}) - 1}{\exp(\alpha I(1+b)/p_{\max}) + b}\right)$	Chalker (1980)

is negligible compared to the light intensities within the culture vessel. Maximal fluorescence, F_m, was induced by a saturating light pulse of 12 000 μmol photons m^{-2} s^{-1} of white light, delivered for 500 ms by a Schott KL-1500 light source.

Figure 3 shows that the quantum yield of PS-II electron transport increased significantly with depth (Spearman rank correlation test: r_s=1, $N = 10$, P<0.001). The quantum yield was highest at the lowest light intensity. This shows that the null hypothesis should be rejected. The phytoplankton cells do experience the spatial heterogeneity of the light gradient. The physiological mechanism behind this result is simple. The efficiency of photon utilization depends on the rate at which absorbed energy is transferred through the electron transport chain of the photosystems. Electron transfer through the photosystems is a very fast process (e.g., Myers & Graham 1971; Falkowski et al. 1985), orders of magnitude faster than the mixing rate in a well-mixed chemostat. Owing to the fast handling of absorbed photons, the efficiency of photon utilization responds to the local light intensities encountered during mixing. In other words, even in a vigorously mixed chemostat, phytoplankton production is a function of all light intensities encountered in the light gradient, not of a single mean light intensity.

Primary production

According to the fluorescence results just described, the total primary production in a light-limited chemostat is the grand total of all local production rates. Let $p(I)$ denote the specific production rate (in mol C per cell per unit time) as a function of light intensity. In the photosynthetic literature, many $p(I)$-relationships have been proposed (Table 2). For mathematical convenience, as explained below, here we chose the rectangular hyperbola (Baly 1935; Monod 1950):

$$p(I) = \frac{p_{\max} I}{H + I} \tag{11}$$

where $p_{\max}$ is the maximal specific production rate, and H is a half-saturation constant. We emphasize, however, that the theory presented in this paper is not restricted to this rectangular hyperbola. In fact, using rigorous mathematical arguments, it can be shown that qualitatively our findings apply equally well to all $p(I)$ functions in Table 2 (Weissing & Huisman 1994). The incorporation of maintenance respiration will be postponed to the next section.

The specific production rate at depth z should be multiplied with the phytoplankton population density to obtain the local production rate at depth z. Subsequent integration of this term over depth yields the total production rate per unit surface area, P (e.g., Platt et al. 1990):

$$P(t) = \int_0^{z_m} p(I(z,t))\omega(t)\, dz \tag{12}$$

We may use Lambert-Beer's law, in Equation (3), to rewrite Equation (12) from an integral over depth into

an integral over light intensity. This yields the following equation for the production rate per unit surface area (Bannister 1974; Huisman & Weissing 1994):

$$P = \frac{k\omega}{k\omega + K_{bg}} \int_{I_{out}}^{I_{in}} \frac{p(I)}{kI}\, dI \tag{13}$$

(Note that, for clarity of presentation, we dropped the time dependence from our notation). Equation (13) has an important interpretation. The first term on the right-hand side of Equation (13) is the fractional light absorption (Equation (8)), which indicates the fraction of the total light absorption that is absorbed by the phytoplankton. The integrand, $p(I)/kI$, is well-known in the photosynthetic literature as the quantum yield of carbon fixation (Kirk 1994). The quantum yield expresses the amount carbon fixed per unit light absorbed (its units are mol C per mol photons). Summarizing, Equation (13) states that the total production rate per unit surface area can be expressed as the product of the fraction of light absorbed by phytoplankton times the efficiency by which this absorbed light is converted into carbon.

One advantage of Monod's rectangular hyperbola, instead of one of the other $p(I)$ functions in Table 2, is that the integral in Equation (13) can be solved. This yields a relatively simple equation for the production rate per unit surface area (Huisman and Weissing 1994):

$$P = \frac{k\omega}{k\omega + K_{bg}} \frac{p_{max}}{k}\, ln\left(\frac{H + I_{in}}{H + I_{out}}\right) \tag{14}$$

We note a few properties of the production rate, using Equation (14). First and quite obviously, in the limit $\omega \to 0$ we have $P(t) \to 0$. That is, if a phytoplankton population is nearly absent, then there is nearly no production. Second, Equation (14) states that the production rate per unit surface area is an increasing but saturating function of the phytoplankton population density. Saturation occurs because at increasing population densities the phytoplankton cells start shading each other, thereby hampering a further increase in total production. Third, in the limit $\omega \to \infty$, fractional light absorption approaches the value 1 and light penetration approaches 0. Hence, in the limit $\omega \to \infty$, the total production rate approaches an upper asymptote:

$$\lim_{\omega \to \infty} P = \frac{p_{max}}{k} \ln\left(\frac{H + I_{in}}{H}\right) \tag{15}$$

That is, a limited supply of light energy per unit area (I_{in}) results in an upper limit on the total production rate per unit area.

Population dynamics

The population dynamics in a light-limited chemostat depends on population growth by primary production and population decline by dilution and maintenance respiration. Accordingly, the change of phytoplankton population density in time can be expressed as:

$$\begin{aligned}\frac{d\omega}{dt} &= \text{population growth} - \text{population loss} \\ &= \frac{Y}{z_m} P - D\omega - \mu_e \omega\end{aligned} \tag{16}$$

where Y is the yield on carbon (which expresses the number of cells produced per unit of carbon fixed), D is the dilution rate, and μ_e is the specific maintenance rate (sensu Pirt 1973; Gons & Mur 1980). We note that division by mixing depth, in the first term on the right-hand side of Equation (16), converts the production rate per unit surface area into a production rate per unit volume.

The maximal specific growth rate of the phytoplankton population, μ_{max}, can be written as the product of the maximal specific production rate times the yield on carbon:

$$\mu_{max} = Y p_{max} \tag{17}$$

Substituting Equations (14) and (17) into Equation (16), combined with Equation (5), defines our full dynamical system for phytoplankton growth in light-limited chemostats:

$$\begin{aligned}\frac{d\omega}{dt} =& \frac{k\omega}{(k\omega + K_{bg})} \frac{\mu_{max}}{k z_m} \ln\left(\frac{H + I_{in}}{H + I_{out}}\right) \\ &- D\omega - \mu_e \omega\end{aligned} \tag{18a}$$

$$I_{out} = I_{in} e^{-(k\omega + K_{bg}) z_m} \tag{18b}$$

This dynamical system clearly illustrates the tight coupling between the phytoplankton population dynamics and the light environment. Equation (18a) describes the changes in population density, which depend on the growth parameters of the phytoplankton species concerned and on the light conditions. Equation (18b) describes the accompanying light gradient, which depends on the phytoplankton population density.

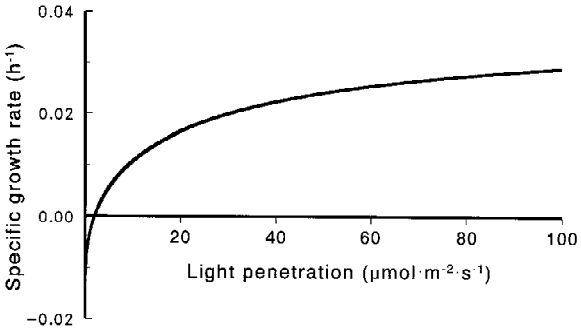

Figure 4. Specific growth rate as a function of light penetration, assuming an incident light intensity of $I_{\text{in}} = 100$ μmol photons $\text{m}^{-2}\ \text{s}^{-1}$. Species parameters: $\mu_{\max} = 0.07\ \text{h}^{-1}$; $\mu_{\text{e}} = 0.025\ \text{h}^{-1}$; $H = 30$ μmol photons $\text{m}^{-2}\ \text{s}^{-1}$.

Equation (18a) can be used to derive a simple equation for the specific growth rate obtained in a light-limited chemostat. This reveals that the specific growth rate for cells mixed through a light gradient can be interpreted as a function, $\mu(I_{\text{in}}, I_{\text{out}})$, of incident light intensity and light penetration. More precisely, combining Equation (18a) with Lambert-Beer's Law, the specific growth rate in a light-limited chemostat is given by:

$$\mu(I_{\text{in}}, I_{\text{out}}) = \mu_{\max}\left(\frac{\ln(H + I_{\text{in}}) - \ln(H + I_{\text{out}})}{\ln(I_{\text{in}}) - \ln(I_{\text{out}})}\right) - \mu_{\text{e}} \tag{19}$$

Figure 4 plots this equation as a function of light penetration. We note the following properties: The specific growth rate becomes negative if light penetration approaches zero. The specific growth approaches an upper asymptote, given by the difference $\mu_{\max} - \mu_{\text{e}}$, if both incident light intensity and light penetration greatly exceed the half-saturation constant H.

The steady state

The dynamics of our light-limited chemostat lead to a steady state. Unfortunately, the complexity of Equation (18a,b) prevents an explicit solution of the dynamical system. We may obtain some graphical understanding of the dynamics, however. This can best be seen if we graph the total population growth rate and total population loss rate as functions of population density (Figure 5). Equation (16) states that the total population growth rate is proportional to the production rate per unit surface area. Hence, in view of Equation (14), the total population growth rate is an increasing but saturating function of population density. Equation (16) also states that the total population loss rate is a linear function of population density, with slope $D + \mu_{\text{e}}$. Intersection of the total population growth rate and the total population loss rate corresponds to a steady state.

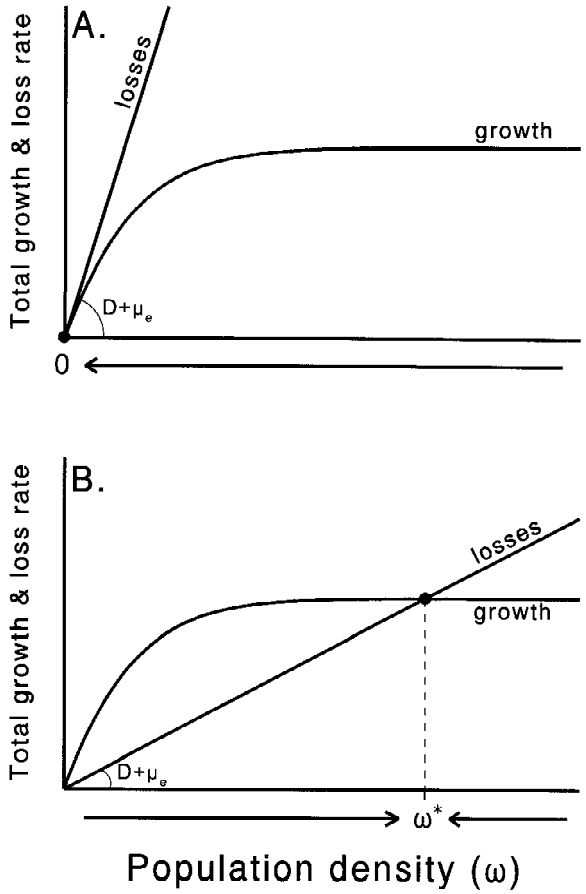

Figure 5. Total growth rate and total loss rate as a function of population density. (A) Losses exceed growth. The dilution rate is too high, and the population is washed out. (B) Growth exceeds losses at low population density, whereas losses exceed growth at high population density. The population density settles at the steady state ω^*.

This reveals two possible scenarios. Either the dilution rate is too high, so that the population losses always exceed the population growth rate irrespective of population density (Figure 5A). In this case, the phytoplankton population is washed out, and will disappear. Or the dilution rate is sufficiently low, so that population growth exceeds population losses at low population densities, whereas population losses exceed population growth at high population densities. In this case, a stable steady-state population density

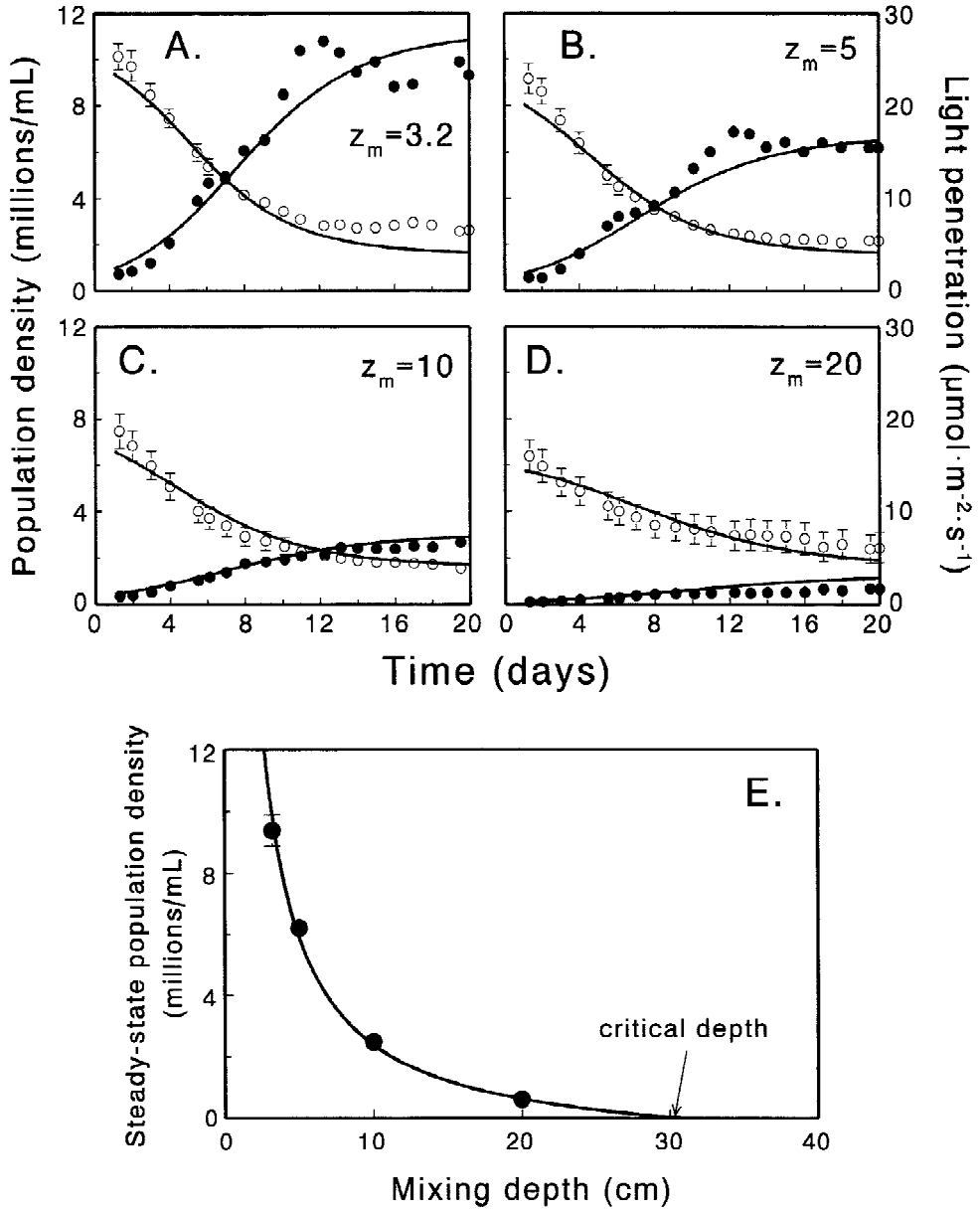

Figure 6. Effects of mixing depth. (A–D) Time courses of the population density of *Chlorella vulgaris* (closed circles) and light penetration through the cultures (open circles) in four light-limited chemostats with different mixing depths: (A) $z_m = 3.2$ cm, (B) $z_m = 5$ cm, (C) $z_m = 10$ cm, (D) $z_m = 20$ cm. Error bars around the open circles indicate the spatial standard deviation of I_{out} ($n = 10$). When not visible, the spatial standard deviation did not exceed the size of the circle. Solid lines show the time courses predicted by Equations (18a,b). (E) The steady-state population densities obtained in the experiments (A–D), plotted against mixing depth. The solid line shows the steady-state population density predicted by Equation (22). Beyond a critical mixing depth of 30 cm, the *Chlorella* population would be washed out. System parameters: $I_{in} = 34.5$ μmol photons m^{-2} s^{-1}; $K_{bg} = 0.057$ cm^{-1}; $D = 0.020$ h^{-1}. Species parameters: $\mu_{max} = 0.076$ h^{-1}; $H = 36$ μmol photons m^{-2} s^{-1}; $k = 5.1 \cdot 10^{-8}$ cm^2 cell^{-1}. (Redrawn from Huisman (1999), with permission from the Ecological Society of America.)

will be reached, at which the total population growth rate equals the total population loss rate (Figure 5B). We shall indicate the steady state with a superscript *.

One of the major advantages of studying steady-state chemostats, including light-limited chemostats, is that cells can be maintained at a constant specific

growth rate for a prolonged time. The specific growth rate at steady state equals the dilution rate.

The critical light intensity

The steady state in a light-limited chemostat is characterized by a corresponding steady-state light penetration. In fact, one can derive from Equations (18a,b) that the dynamics will always equilibrate at the same steady-state light penetration, irrespective of mixing depth and background turbidity. Hence, the steady-state light penetration acts as a 'critical light intensity' for phytoplankton growth. That is, if we denote the critical light intensity by the symbol I^*_{out}, the following rules hold:

$$\begin{aligned} &\text{If } I_{\text{out}} > I^*_{\text{out}}, \text{ then } \mathrm{d}\omega/\mathrm{d}t > 0. \\ &\text{If } I_{\text{out}} = I^*_{\text{out}}, \text{ then } \mathrm{d}\omega/\mathrm{d}t = 0. \\ &\text{If } I_{\text{out}} < I^*_{\text{out}}, \text{ then } \mathrm{d}\omega/\mathrm{d}t < 0. \end{aligned} \tag{20}$$

The critical light intensity is a species-specific parameter. Its value depends on the maximal specific growth rate, the half-saturation constant for light-limited growth, and the specific maintenance rate of a species. Also, the critical light intensity is an increasing function of the dilution rate and a decreasing function of the incident light intensity. It can be shown that the existence of a critical light intensity is not restricted to Monod's equation but holds for all $p(I)$ relationships of Table 2. The interested reader is referred to Huisman & Weissing (1994) and Weissing & Huisman (1994) for a mathematical derivation of this key result. Experimental evidence for the existence of a species-specific critical light intensity is provided by Huisman (1999) and Huisman et al. (1999a).

Owing to the complexity of Equations (18a,b), a simple equation for the critical light intensity is lacking. However, the value of the critical light intensity can be estimated in two different ways. First, the value of the critical light intensity can be measured directly from a light-limited chemostat culture. It is simply the light penetration through the culture measured at steady state. Second, the value of the critical light intensity can be calculated numerically, by estimating all model parameters in Equations (18a,b), and subsequent numerical simulation of Equations (18a,b) until a steady state is reached. The critical light intensity is then the calculated steady-state light penetration. Laboratory experiments show that, generally speaking, these two different methods yield similar estimates of the critical light intensity (Huisman et al. 1999a).

Steady-state population density

The steady-state population density can be expressed as a function of the critical light intensity. That is, according to Equation (18b), the steady-state population density reads (Huisman 1999):

$$\omega^* = \frac{\ln(I_{\text{in}}) - \ln(I^*_{\text{out}})}{k z_{\text{m}}} - \frac{K_{\text{bg}}}{k} \tag{21}$$

Equation (21) provides two major insights. It shows (i) that the steady-state population density is a linearly decreasing function of background turbidity and (ii) that the steady-state population density is inversely proportional to mixing depth. To test the latter prediction, we ran light-limited chemostats with *Chlorella vulgaris* using four different mixing depths. Figures 6A–D show the time courses of these experiments. This shows that, despite large differences in mixing depth and population density, light penetration in all four chemostat experiments equilibrates at more or less the same steady-state value (ca. 5 μmol photons $\text{m}^{-2}\ \text{s}^{-1}$). Hence, our earlier prediction that the critical light intensity is independent of mixing depth is confirmed. Figure 6E plots the corresponding four steady-state population densities as a function of mixing depth. This clearly demonstrates that population density becomes much higher in shallow chemostats than in deep chemostats, fully in line with the prediction of Equation (21). The explanation is straightforward. Light is supplied as a flux, and a given photon flux can sustain only a given production per unit surface area (Equation (15)). In deep systems this production per unit surface area is divided over a much larger volume than in shallow systems. Hence, under light-limited conditions, the steady-state population density is inversely related to mixing depth.

Extrapolating the curve in Figure 6E reveals that the curve will intersect the x-axis. Hence, there is a maximal mixing depth beyond which a steady-state phytoplankton population cannot be sustained. Following Sverdrup's terminology, we call this point the 'critical depth' (Sverdrup 1953; Platt et al. 1991; Huisman 1999). Equation (21), solved for $\omega^* = 0$, shows that the critical depth, z_{cr}, is inversely proportional to the background turbidity:

$$z_{\text{cr}} = \frac{\ln(I_{\text{in}}) - \ln(I^*_{\text{out}})}{K_{\text{bg}}} \tag{22}$$

When phytoplankton is mixed to depths beyond this critical depth, the background turbidity of the water column imposes depth-averaged light conditions

too dark to support a phytoplankton population. Accordingly, turbid waters have a shallow critical depth, whereas clear waters have a deep critical depth. Furthermore, remember that the critical light intensity is a function of the dilution rate. Thus, via Equation (22), the critical depth is a function of the dilution rate as well. In fact, the critical depth increases with decreasing dilution rate.

Turbidostat

An alternative method to culture phytoplankton under light-limited conditions is the turbidostat. In a chemostat, the dilution rate is kept constant, and the population density adjusts until a steady state is reached. In a turbidostat, the population density ('turbidity') is kept constant at a desired value, by adjusting the dilution rate until the desired population density of the phytoplankton is reached. Figure 5 clarifies the mechanism. If the dilution rate is increased, the slope of the line labeled 'losses' increases and consequently the steady-state population density (ω^*) decreases. Conversely, if the dilution rate is decreased, losses decrease, and the steady-state population density increases.

An advantage of the turbidostat is that the experimenter can impose a desired light environment. Turbidostats are therefore well suited for studies on the effects of different light regimes on phytoplankton physiology (Post et al. 1984; Ibelings et al. 1994; Kroon 1994; Visser et al. 1997). Moreover, the population density in a turbidostat can be maintained at a very low value, which might be advantageous for a variety of applications. For instance, a low population density does not require extra carbon dioxide or a high nutrient dosage, avoids the development of a steep light gradient (thus providing a more homogeneous light environment), and may help to avoid direct interference between phytoplankton cells within the vessel.

Discussion

Ecological relevance of light limitation

In many freshwater and marine ecosystems, phytoplankton growth is primarily limited by the availability of nutrients like phosphorus, nitrogen, and iron (Vollenweider 1976; Martin et al. 1990; Platt et al. 1992; De Baar et al. 1995). There is growing consensus among phytoplankton ecologists, however, that light limitation of phytoplankton growth may be more important than previously thought. For instance, Colijn & Cadée (2002) investigated light and nutrient limitation of phytoplankton growth in the Wadden Sea, a shallow coastal sea with a large input of nutrients from several European rivers. An emphasis on eutrophication-related research has led to a focus on nutrient-oriented investigations in the Wadden Sea. Yet, using an index developed by Cloern (1999) to assess the importance of light limitation and nutrient limitation in the field, Colijn and Cadée found that the importance of light limitation far exceeded the effects of nutrient limitation. Colijn & Cadée (2002, p.1) conclude: 'The results of this analysis show how one-sided research may develop when policy makers start to direct research priorities.

Recent studies show that, in general, light limitation is important in a wide variety of aquatic ecosystems. These include (1) eutrophic waters where all nutrients are in ample supply (Mur & Schreurs 1995; Visser et al. 1996; Phlips et al. 2000), (2) turbid waters like estuaries, shallow coastal areas, and turbid lakes (Kromkamp & Peene 1995; Cloern 1999; Colijn & Cadée 2002), (3) nutrient-poor waters where phytoplankton are exposed to low light conditions encountered during deep mixing (Mitchell et al. 1991; Boyd et al. 2001), and (4) during winter when incident light intensities are low (Maldonado et al. 1999).

Ecological applications of the light-limited chemostat

One advantage of chemostats is that they may help to bridge the gap between physiological studies and ecosystem research. This is relevant, for instance, in the context of research on harmful algal blooms. A variety of phytoplankton species produce toxins, thereby providing a threat for other aquatic species, for fisheries, and for recreation (Anderson & Garrison 1997; Chorus & Bartram 1999). A well-studied example is the cyanobacterium *Microcystis*, which is widespread in eutrophic lakes and produces a toxic compound called microcystin. Research at the gene level has revealed that transcription rates of the gene complex responsible for the production of microcystin synthetase depend on threshold light intensities (Kaebernick et al. 2000; Dittmann et al. 2001). Using light-limited continuous cultures, Utkilen & Gjølme (1992) showed that microcystin production of *Microcystis* increases with light intensity up to an intensity of about 40 μmol photons m^{-2} s^{-1}, and then slightly decreases with a further increase in light intensity. These results were

supported by field incubations, which showed that the microcystin content of *Microcystis* near the surface was considerably higher than the microcystin content of *Microcystis* deep in the water column. According to Utkilen & Gjølme (1992; see also Aanesen et al. 1998; Rapala & Sivonen 1998), manipulation of light availability should be one of the key parameters in further studies on the toxin production of cyanobacteria.

A major challenge for future work on the edge between physiology and ecology is to combine the substrate-limited and light-limited approaches (Huisman & Weissing 1995; Cloern 1999; Diehl et al. 2002). Nutrients provide building blocks for the photosynthetic machinery, whereas light provides the energy for nutrient uptake. As a consequence, nutrient limitation and light limitation frequently interact (Healey 1985; Riegman & Mur 1985; De Nobel et al. 1998). Consider, for example, iron and light. Iron is a major limiting factor in several oceanographic regions (Martin et al. 1990; De Baar et al. 1995; Maldonado et al. 1999). The majority of iron incorporated in phytoplankton cells is needed in the photosynthetic electron transport chain (Raven 1990). Accordingly, Sunda & Huntsman (1997) and Timmermans et al. (2001) showed that co-limitation of marine phytoplankton by iron and light can be easily observed in culture experiments. Co-limitation by iron and light is species specific, however. Small phytoplankton species have a relatively higher iron uptake rate than large species. Hence, small phytoplankton species can thrive under low iron and low light conditions, whereas large phytoplankton species require conditions with a high iron and light availability (Sunda & Huntsman 1997; Timmermans et al. 2001). At the ecosystem level, these species-specific differences may in turn have major implications for aquatic foodwebs and the carbon and silicon cycles in the oceans.

Other applications of the light-limited chemostat focus on species interactions, like competition for light (Huisman & Weissing 1994, 1995; Huisman et al. 1999a). Figure 7 shows an example with the small green alga *Chlorella vulgaris* and the large green alga *Scenedesmus protuberans*. Monoculture experiments had revealed that *Chlorella* has a lower critical light intensity than *Scenedesmus*. In a competition experiment, both species initially increased, but once *Chlorella* had 'depleted' light penetration below the critical light intensity of *Scenedesmus* (at ca. 6 μmol photons m^{-2} s^{-1}), *Scenedesmus* started to decline. Thus, *Chlorella* outcompeted *Scenedesmus*. That is, under constant light conditions, the species with low-

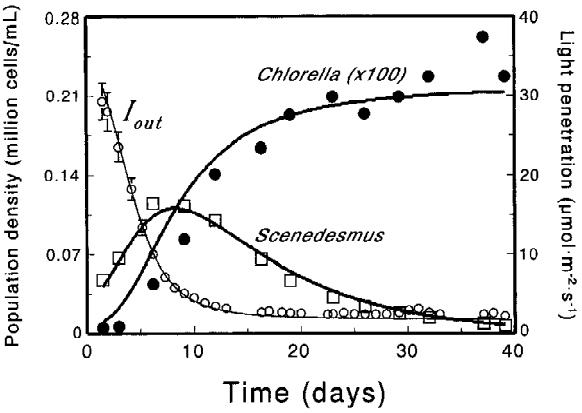

Figure 7. Competition for light. The green alga *Chlorella vulgaris* (closed circles) displaces the green alga *Scenedesmus protuberans* (open squares). Open circles indicate the corresponding light penetration. Error bars around the open circles indicate the spatial standard deviation of I_{out} ($n = 10$). When not visible, the spatial standard deviation did not exceed the size of the circle. Solid lines show the time course predicted by a multispecies version of Equations (18a,b). Note: *Chlorella* has much smaller cells than *Scenedesmus*, and these tiny cells occur in much higher numbers; hence, the scale along the y-axis should be multiplied with 100 to obtain the population density of *Chlorella*. (Redrawn from Huisman et al. (1999a), with permission from the Ecological Society of America.)

est critical light intensity is the better competitor for light (Huisman et al. 1999a). In natural ecosystems, light fluctuates on a wide range of timescales. Recent work extended the competition theory to phytoplankton under fluctuating light (Litchman & Klausmeier 2001). This shows that fast light fluctuations still lead to competitive exclusion, but will favor opportunistic species with a high maximal growth rate at high irradiances. In contrast, slow light fluctuations can promote species coexistence. These theoretical predictions are confirmed by competition studies under light-limited conditions, which report competitive exclusion under fast light fluctuations but a high species diversity at slow light fluctuations (Flöder et al. 2002). Such competition studies provide information on the competitive abilities of phototrophic species, and will contribute to a better understanding of the biodiversity and species composition of phytoplankton communities.

Light-limited chemostats may also be very helpful in studies of other species interactions, including research on aquatic foodwebs. Supply rates of light and phosphorus have a major effect on the carbon:phosphorus ratio of phytoplankton. The C:P ratio of phytoplankton, in turn, is an important determinant of the food quality of phytoplankton for zo-

oplankton (Sterner & Hessen 1994). That is, a high light but low phosphorus supply leads to phytoplankton with a high C:P ratio, which is poor food for zooplankton like *Daphnia*. Thus, using laboratory experiments, Urabe & Sterner (1996) showed that *Daphnia* growth increased with light intensity at low values of the light/phosphorus supply due to increases in phytoplankton biomass, but *Daphnia* growth decreased with a further increase of the light supply due to decreases in algal quality. Field experiments corroborated these findings, showing that reduced light in a phosphorus-limited lake may actually enhance zooplankton production by improving the food quality of the algae (Urabe et al. 2002). Studies on the effect of the light-nutrient balance on algal food quality may therefore greatly improve our understanding of aquatic foodwebs (Elser et al. 2000).

Limitations of the light-limited chemostat

Despite many advantages and a wide variety of potential applications, there are also limitations in using chemostats. These limitations are related to the classic problem of microcosm experiments: the problem of scale (Daehler & Strong 1996; Petersen 1997). The laboratory scale of the light-limited chemostat leads to:

- high population densities,
- a high nutrient dosage,
- fast mixing rates through the light gradient.

For instance, theory and experiments show that the steady-state population density is inversely proportional to mixing depth (Figure 6). Our experiments were performed in light-limited chemostats with mixing depths ranging from 3 to 20 cm. At a larger scale, in mesocosms, similar scaling arguments were found by Petersen et al. (1997). They observed that primary production per unit volume was inversely proportional to mixing depth in light-limited mesocosms with mixing depths of 45–215 cm. At again a larger scale, in field experiments, Diehl et al. (2002) report the same inverse relation between primary production per unit volume and mixing depth, using mixing depths of 2–15 m. Hence, this scaling rule seems to work over several orders of magnitude. As a result, all else being equal, population densities are predicted to be 100 times higher in a chemostat vessel of 5 cm depth than in a lake of 5 m depth, and 2000 times higher than in a surface-mixed layer of 100 m depth in the ocean. Accordingly, laboratory chemostats may have extremely high population densities. High population densities may have several side effects. For instance, allelopathic interactions are potentially much more intense in densely populated chemostats than in dilute seawater. Also, to sustain the unnaturally high population densities of light-limited chemostats, light limitation in the chemostat requires a much higher nutrient and carbon dosage than light limitation in the field. Turbidostats offer a suitable alternative, when working with low population densities is desirable.

To translate results from the chemostat to the field, the development of proper scaling rules is essential. Similar scaling problems are encountered in industrial applications when small-scale chemostats are scaled up to large-scale cultivation of algae (e.g., Janssen et al. 2000). Derivation of new scaling rules, preferably supported by laboratory and field experiments, may provide further insight in the dynamics of light-limited ecosystems. Dimensional analysis, a frequently used technique by engineers, might be very helpful in this context (Huisman et al. 1999b; Petersen & Hastings 2001; Ebert et al. 2002).

Conclusions

In this paper, we have described the theory and potential applications of a chemostat system specifically designed for the study of phototrophic microorganisms under light-limited conditions. We have shown that many principles of the light-limited chemostat differ fundamentally from principles of the usual chemostat system. This stems from a variety of differences between substrate limitation and light limitation. For instance, whereas nutrients can be vigorously mixed, light forms a steep gradient over depth. Also, whereas the nutrient supply rate is directly coupled to the dilution rate, light is supplied independently of the dilution rate. One common feature of substrate-limited and light-limited chemostats is that in both systems a steady state will be reached. Under substrate-limited conditions, the steady state is reached through depletion of the limiting substrate. Under light-limited conditions, the steady state is reached through mutual shading within the light gradient. In a sense, one might say that the photon flux is depleted. A major advantage of steady-state growth in chemostat systems is that organisms can be investigated under well-controlled conditions, at a constant specific growth rate (equal to the dilution rate), for a prolonged time. It is our hope that the theory, techniques, and potential applications outlined in this paper will contribute to a

better understanding of the ecology of phototrophic microorganisms and of light limitation in general.

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