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The light response of nitrogen fixation in Lake Valencia, Venezuela[1]

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Abstract

Water samples from six depths in the upper water column of Lake Valencia, Venezuela, were incubated in situ with acetylene at several depths corresponding to different light exposures. On five dates there was sufficient nitrogen fixation to define the light-response curve for samples taken at 2 and 100 cm. The light response of nitrogen fixation was modeled successfully with an equation previously developed for the light response of photosynthesis. All parameter values of the response curves for samples originating at a given depth were relatively stable across dates, but differed between depths. Rates of increase of N fixation with irradiance in the subsaturation range (α) were much lower than is typical of photosynthesis. While the general shape of nitrogen fixation curves was similar to that of photosynthesis curves, critical parameters were more sensitive to the light history (depth of origin) of cells than would be expected for photosynthesis.

Light-response curves for phytoplankton photosynthesis have been useful as a basis for modeling primary production under natural conditions (e.g. Fee 1969; Bannister 1974). The parameters that describe the shapes of such curves can be interpreted physiologically and thus facilitate comparison of field populations between lakes or between seasons in a given lake (Harris 1978). Light-response curves for nitrogen fixation should offer the same advantages but have not yet been well defined for natural populations. It has been generally established that there is a correlation between light and nitrogen fixation, and that inhibition can occur at high irradiances (e.g. Dugdale and Dugdale 1962; Goering and Neess 1964; Horne and Fogg 1970; Flett et al. 1980). A more detailed and extensive knowledge of the shapes of light response curves for N fixers in situ would be useful, however. We provide here information on the light response of nitrogen fixation in situ by heterocystous blue-green algae in Lake Valencia, Venezuela. We thank S. Stadler-Morris and M. Benson for field assistance, B. Chronic for heterocyst counts, and P. Brezonik, A. Horne, and T. Platt for their comments on the manuscript.

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Methods and study site

Lake Valencia is described briefly by Levine and Lewis (1984) and more extensively by Lewis and Weibezahn (1976) and Lewis (1983). Light response determinations were done on samples originating at six depths on 29 different dates between December 1980 and December 1981. Until 13 October 1981, samples to be used in studies of nitrogen fixation were collected at the index station near the center of the lake. After this they were collected over deep water near Isla Otama, a large island midway between the index station and the south shore (map reference: Lewis 1983). Because nitrogen fixation was too low to meet minimum requirements for a study of light response except for samples originating near the surface between September and December, our analysis is limited to the data taken on five dates between September and December 1981 for samples originating at depths of 2 and 100 cm. These dates coincide with the last portion of the seasonal stratification, when nutrient depletion is most pronounced.

At the beginning of each study, water was pumped from depths of 2 and 100 cm into dark bottles. Since the pump would draw water from an area of several centimeters, the samples should be considered to have originated only in the close vicinity of the nominal depth. Samples were returned to shore and held in a dark box except when

being handled. Glass syringes were filled with this water, inoculated with acetylene, and suspended in situ. For each depth of origin (2 cm, 100 cm), duplicate syringes from each sample were prepared for incubation at 0, 0.5, 1, 2, and 5 m. All syringes were returned to the lake for incubation. The surface incubations were floated in a Plexiglas box so that they were just covered with water. Other aspects of the handling and inoculation procedures were as described by Levine and Lewis (1984). Two blanks were also prepared from each sample as described by Levine and Lewis (1984). A sample for phytoplankton counting was preserved with Lugol's solution.

The incubations lasted for 4 h (±30 min) and typically began between 1100 and 1200 hours. Over the course of the incubation period, the surface radiation was recorded as described by Levine and Lewis (1984). The vertical extinction of PAR (300–700 nm) was measured with a submersible quantum sensor at the incubation site just before the incubation. The combined data on extinction and total surface radiation over the incubation period were later used to compute the amount of light reaching each of the sets of syringes at the five different depths of incubation.

At the end of each incubation, the samples were transported to shore in a light-tight box and stripped (Levine and Lewis 1984). Samples awaiting stripping were held in the dark. The amount of ethylene stripped from the samples was determined by gas chromatography with appropriate adjustments for sample volume, headspace, and other variables affecting recovery of ethylene. The blank was subtracted from each of the measurements, thus correcting for any background ethylene that might have been in the lake water or in the acetylene inoculum. Acetylene reduction was converted to nitrogen fixation by use of depth-specific conversion factors obtained from ^{15}N calibration of the acetylene procedure.

In determining the shape of the light-response curve, we used the equation proposed by Platt et al. (1980) for modeling the light response of photosynthesis. The equation, with appropriate modification, is as follows:

$$N = N_s(1 - e^{-a})e^{-b} + D \qquad (1)$$

where $a = \alpha I N_s^{-1}$ and $b = \beta I N_s^{-2}$. N, the dependent variable, is the amount of N fixation per unit time expressed in relation to the number of heterocysts [nmol $N \cdot (10^6$ heterocysts$)^{-1} \cdot h^{-1}$]. N_s, a parameter representing maximum fixation in the absence of inhibition, is expressed in the same units as N, and D is dark fixation, also with the same units as N. α is the slope of the rising limb of the light-response curve and has units nmol $N \cdot (10^6$ heterocysts$)^{-1} \cdot (\text{Einst} \cdot m^{-2})^{-1}$. β is a parameter for inhibition, and has the same units as α. I, the independent variable, is given as PAR and, since we are dealing with a quantum phenomenon, we express it in terms of Einsteins per unit area per unit time. To make the light conform dimensionally to the measurements of N fixation, we use Einst $\cdot m^{-2} \cdot h^{-1}$ for all values of I (Einst $\cdot m^{-2} \cdot h^{-1} \times 60 =$ Watts $\cdot m^{-2}$). This causes the time dimension to drop from parameters α and β.

The equation used here differs from the one proposed by Platt et al. only in minor ways. N fixation per unit time takes the place of carbon fixation per unit time, and the number of heterocysts takes the place of chlorophyll weight. The last term of the equation (D) is not given by Platt et al., who were able to assume that dark fixation for practical purposes was equal to zero. We have added this term to the equation because the comparable assumption is not valid for nitrogen fixation in Lake Valencia.

For present purposes, the equation of Platt et al. offers the special advantage that light inhibition receives specific treatment with the parameter β. Nitrogen fixation can be strongly inhibited at high irradiances and the inhibition is not necessarily a straightforward function of α, as might be assumed by certain other types of curves. Although a greater number of parameters reduces degrees of freedom in curve fitting, an equation with fewer parameters than the one suggested by Platt et al. would be physiologically unrealistic for the N fixation data.

The parameter values of the equation were estimated by nonlinear regression with Marquardt's algorithm. The Marquardt method converges more rapidly than the

Table 1. Heterocysts per cubic centimeter for the dates and depths sampled as part of the light response study.

1981	Depth (cm)	*Anabaena volzii*	*Anabaena spiroides*	*Anabaenopsis circinalis*	*Cylindrospermopsis stagnale*
13 Sep	2	1,100	1,300	10	70
	100	2,000	1,100	20	90
27 Sep	2	1,800	340	80	140
	100	1,700	300	100	70
4 Oct	2	1,900	1,100	140	20
	100	2,000	740	320	60
18 Oct	2	3,800	3,000	180	160
	100	5,100	210	330	160
22 Nov	2	2,400	510	190	210
	100	2,500	70	250	290

Gauss method used by Platt et al., but either would probably be satisfactory.

Results

Table 1 summarizes the total number of heterocysts on each of the five dates and shows how the total numbers of heterocysts are distributed according to species. Taxonomy follows Lewis and Riehl (1982). Several points are worth noting. First, the total number of heterocysts was determined primarily by the contributions of two *Anabaena* species. The two secondary species contributed on all occasions $<10\%$ of the heterocysts. Second, on any given date the difference in heterocyst abundances between the two depths used as source water for the incubations was relatively small at the time the collection was made. Finally, the abundance of heterocysts on the five different dates differed by at most a factor of 3. Except for the notably higher concentrations on 18 October, heterocyst concentrations were very similar on the five dates. Conversions are useful for comparison with other studies that have expressed fixation in relation to cell volume or chlorophyll *a*: for Lake Valencia, one heterocyst represents about 16 vegetative cells, a total cell volume of about 600 μm^3, or Chl *a* of 3×10^{-6} μg.

Table 2 summarizes the results of the parameter estimations and Fig. 1 shows the curve shapes corresponding to the parameters. The goodness-of-fit is represented by the root mean square (rms, square root of error variance), which is shown in the table. Figure 2 gives a visual impression of goodness-of-fit for a data set yielding median rms. In addition to the parameter valucs, N_m, a derived value, is shown in the table.

Table 2. Parameter estimates for the 10 datc/depth combinations of the study, and the root mean square (rms) for the fit of the curve to the data points.

1981	Depth (cm)	N_s*	α†	β†	D*	rms*	N_m*
13 Sep	2	4.5	135	0	0	3.4	4.5
	100	1.8	14	0.3	0.6	0.3	2.3
27 Sep	2	1,000.0	72	700.0	0.7	3.3	38.0
	100	7.6	11	0	0.7	1.0	8.2
4 Oct	2	690.0	54	460.0	9.3	7.0	38.0
	100	13.0	26	2.4	1.7	0.8	11.0
18 Oct	2	690.0	54	610.0	0.8	3.1	22.0
	100	7,000.0	14	3,700.0	0.4	0.6	10.0
22 Nov	2	50.0	57	10.0	3.8	6.8	34.0
	100	2,000.0	8	470.0	1.4	1.0	14.0

* nmol N·(10^6 heterocysts)$^{-1}$·h^{-1}.
† nmol N·(10^6 heterocysts)$^{-1}$·(Einst·m^{-2})$^{-1}$.

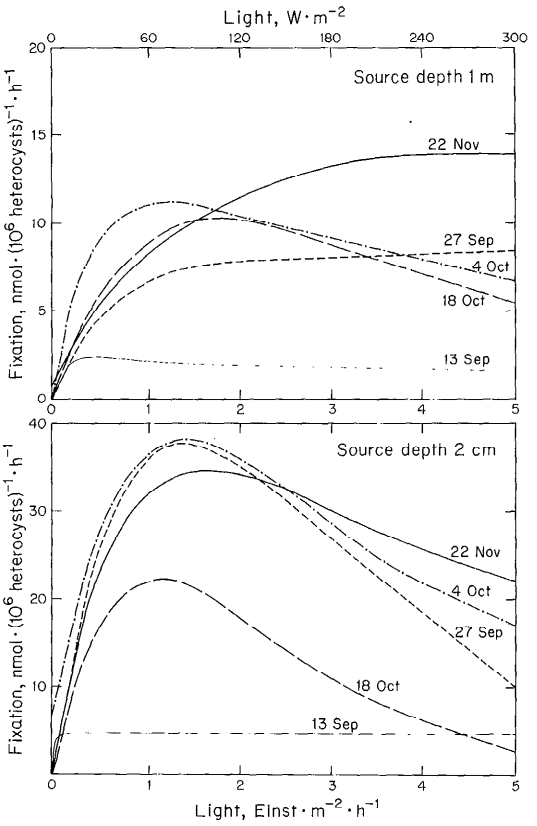

Fig. 1. Light response curves for each of the five dates for samples taken at 2 cm and 1 m.

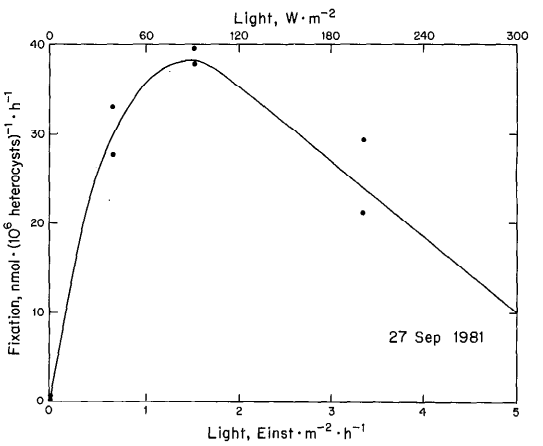

Fig. 2. Illustration of the fit of points to the curve for a sample of median rms.

N_m is the maximum fixation. As shown by Platt et al. (1980), it is determined as follows:

$$N_m = N_s\left(\frac{\alpha}{\alpha+\beta}\right)\left(\frac{\beta}{\alpha+\beta}\right)^{\beta/\alpha} + D.$$

Among the values given in Table 2, those for α, D, and N_m are easiest to relate to observed fixation. α, the slope of the initial linear rising limb of the light-response curve, is familiar from photosynthesis response curves. The values of α in Table 2 show certain consistencies. α for samples taken at 2 cm was higher on all dates than it was for samples taken at 100 cm. The values for α for samples taken at 2 cm fell within a relatively narrow range, as did the values of α for the 100-cm sample. Dark fixation, which averaged about 10% of N_m, was always a minor but not entirely trivial proportion of fixation. N_m was always considerably higher for the 2-cm sample than for the 100-cm sample. Except for the first sampling date, when fixation rates were well below those of the other dates, the values for N_m at 2 cm fell within a relatively narrow range, as did the values of N_m at 100 cm.

Interpretation of the parameters N_s and β is a little less straightforward than of the other parameters. N_s is the maximum fixation that would be observed in excess of D if β were 0. The direct ecological meaning of N_s is not very great in this case, since β is typically not 0. β gives an indication of the degree of light inhibition, but the effect of β is dependent on other parameters. A better indicator of light inhibition is a derived value, I_b, which will be described below.

The response curve defined by a set of data points for a given date and depth of origin defines certain critical irradiance values. These are tabulated in Table 3 (symbols follow Platt et al. 1980). I_m is the irradiance at which maximum fixation occurs. When there is measurable photoinhibition ($\beta > 0$), curves of the form used here give a unique value for I_m. In two of the Lake Valencia incubations, there was no evidence of inhibition ($\beta = 0$). In such a case, there is no unique value for I_m. In fact, I_m is undefined when $\beta = 0$ because of division by 0 when the equation is solved for I_m. In biological

Table 3. Incident light (PAR) over the incubation period (I_o) and values related to light derived from the light response curves.

1981	Depth (cm)	I_m	I_k	I_s	I_b	I_o
		(Einst·m^{-2}·h^{-1})				
13 Sep	2	(0.15)*	0.033	0.033	—	4.2
	100	0.5	0.16	0.13	6.1	4.2
27 Sep	2	1.4	0.53	14.0	1.5	3.2
	100	(3.5)*	0.75	0.69	—	3.2
4 Oct	2	1.4	0.70	13.0	1.5	4.9
	100	1.2	0.42	0.50	5.4	4.9
18 Oct	2	1.1	0.41	13.0	1.1	1.6
	100	1.9	0.71	500.0	1.9	1.6
22 Nov	2	1.7	0.60	0.88	5.0	3.3
	100	4.3	1.75	250.0	4.3	3.3

* I_m undefined, value in parentheses is light required to reach 0.99 ($N_s + D$).

terms, however, it is just as meaningful to report in place of I_m the minimum irradiance required to produce the maximum fixation response, which is $N_s + D$ when $\beta = 0$. Maximum fixation is actually approached asymptotically, so for present purposes fixation is considered to have reached $N_s + D$ when it equals 99% of $N_s + D$. This can be determined either graphically or algebraically.

There is a cluster of I_m values between 1 and 2 Einst·m^{-2}·h^{-1}. Values of I_m in this range are equal to about ¼ of I_o in the middle third of a sunny day, about half of midday I_o on a partly cloudy day, and are roughly equal to midday I_o on a very cloudy day such as 18 October. The values of I_m are very similar to those expected from similar studies of photosynthesis (e.g. Platt et al. 1980). On a couple of occasions the values were exceptionally high or low. On 13 September, I_m at both depths was lower than on other dates. High values occurred for the 100-cm sample on 27 September and 22 November.

Table 3 also reports values for I_k, analogous to the familiar descriptor of photosynthesis response curves originating with Talling (1957*a,b*). In this instance I_k is defined by the intersection of N_m and the linear extension of the initial rising portion of the response curve. Algebraically, $I_k = N_m \cdot \alpha^{-1}$. A similar index is I_s, which is defined on the basis of N_s rather than N_m and must therefore always be equal to or greater than I_k. Algebraically, $I_s = N_s \cdot \alpha^{-1}$.

Platt et al. (1980) suggested that I_b, defined as $N_s \cdot \beta^{-1}$, is a good indicator of photoinhibition. Increasing values of I_b indicate weakening inhibition, and the value of I_b for $\beta = 0$ is infinite. The values of I_b in Table 3 overlap with those obtained by Platt et al. for photosynthesis, but the values of Platt et al. are typically higher.

The values of I_m, I_k, and I_s were lower at 2 cm than at 100 cm on all dates except one (4 October). No pattern can be discerned in the relative values of I_b in samples originating at 2 and 100 cm.

Discussion

Some general comparisons are possible between the light response of N fixation as determined for Lake Valencia and the much more extensively studied light response of carbon fixation. One approach is to express both carbon and nitrogen fixation in relation to morphological units (for carbon fixation, the vegetative cell; for N fixation, the heterocyst) and then compare the values of α. The internal volume of a heterocyst is about the same as the volume of a vegetative cell in the two *Anabaena* species of major interest for nitrogen fixation in Lake Valencia. For the sake of computation, we use the cell volume of *Anabaena spiroides*, which is the bigger of the two species by a factor of about 2. The cell volume of this species averages 46 μm^3 in Lake Valencia and is relatively stable (Lewis and Riehl 1982). The Chl *a* per unit cell volume is variable in Lake Valencia, as it is in other lakes, but a

median value is about 5 $\mu g \cdot mm^{-3}$. This implies that 1 mg of chlorophyll is equivalent to about 4.4×10^9 cells. The literature survey of Platt and Jassby (1976) shows that α for photosynthesis most often falls in the range 2–4 mg $C \cdot mg^{-1}$ Chl $a \cdot mEinst^{-1} \cdot m^{-2}$. After conversion to nmol and cell number for cells the size of *A. spiroides* in Lake Valencia, the range would be 38,000–77,000 $nmol \cdot (10^6 \text{ cells})^{-1} \cdot (Einst \cdot m^{-2})^{-1}$. This number can be compared with the values of α in Table 2, which shows that α for N fixation is about three orders of magnitude lower than for C fixation. Nitrogen need be fixed only a tenth as fast as carbon in order to meet the needs of growth, but this effect is essentially cancelled by the maximum ratio of heterocysts to vegetative cells, which is seldom more than 1:10. Thus there is a very wide gap between the amount of carbon and the amount of nitrogen that can be fixed, relative to the needs of a growing cell, at light intensities in the subsaturation range.

An unexpected feature of the light response curves for Lake Valencia is the consistently higher α for samples taken just at the surface (2 cm) than for samples taken at 100 cm on a given date. By analogy with carbon fixation, this is surprising, since cells deeper in the water column are expected to be more responsive to light if significant physiological light adaptation has occurred or to be equally responsive if no adaptation has occurred. When nitrogen and carbon fixation are both possible, however, any such predictions must take into account the possibility that nitrogen and carbon fixation compete for photogenerated reductant. Although our diel studies show no evidence of temporal changes in priorities of reductant use (Levine and Lewis 1984), the persistent difference in α between depths is suggestive of spatial partitioning in priorities of reductant use. Ward and Wetzel (1980) also failed to find evidence for temporal differences but did find evidence for spatial differences in reductant partitioning. Their data suggest that the priority for N fixation is higher at low light intensities than at higher ones. Our α values suggest the opposite: samples taken from the surface show a stronger light response for nitrogen fixation. Clearly spatial or temporal changes in reductant use can be important, but it is equally clear that no generalization is warranted yet.

Inhibition is the rule for N fixers in Lake Valencia at midday surface light intensities. Since inhibition is known to be a time-dependent phenomenon for carbon fixation, it is possible that the inhibition parameters overestimate the effective inhibition in a water column, just as ^{14}C bottle incubations are thought to do for carbon fixation (Harris 1978, 1980). The determination of inhibition in bottles is perhaps more realistic for nitrogen fixation than for carbon fixation, however, simply because the fixing taxa have a pronounced degree of bouyancy control and are thus able to remain near the surface for extended periods when there is little wind.

The close parallel between the shapes of nitrogen fixation response curves to light in situ in Lake Valencia suggests that such response curves might indeed be quite useful as an aid in predicting the total nitrogen fixation of lakes. Sensitivity of α to depth, which is not so characteristic of carbon fixation, may result in some complications that are not encountered for carbon fixation models based on the same principles, however.

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