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Diel variation of nitrogen fixation in Lake Valencia, Venezuela[1]

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Abstract

During 1981 we examined the diel variations of nitrogen fixation in Lake Valencia, Venezuela. Four species of heterocyst-bearing blue-green algae were common but subdominant in the phytoplankton. In samples taken from and incubated at 0.5 m, the rate of nitrogen fixation per unit volume of water was lowest at night (<6% of the maximum daytime rate), increased from dawn until early afternoon, and then diminished between late afternoon and the first hour of darkness. This pattern was caused partly by diel changes in light intensity and partly by diel migrations of heterocystous blue-green algae. Heterocyst concentrations at 0.5 m increased between midmorning and early afternoon and then decreased from late afternoon until evening. The heterocyst-specific nitrogen fixation rates (nitrogen fixed per heterocyst per unit time) at 0.5 m were much less variable than the nitrogen fixation rates per unit volume of water. Heterocyst-specific rates rose rapidly in early morning and fell slowly in the evening, but were almost constant over much of the day. Heterocyst-specific nitrogen fixation rates were very close to those predicted by a model based on the light dependency of nitrogen fixation. There was no evidence for temporal changes in the light response of nitrogen fixation.

Diel variations of lacustrine nitrogen fixation appear to be common, but the patterns reported are variable: maximum nitrogen fixation rates may occur in the morning (Vanderhoef et al. 1975), at midday (Stewart et al. 1967; Ganf and Horne 1975), or in the afternoon (Paerl 1979; Kellar and Paerl 1980). The inconsistency of its diel pattern indicates that nitrogen fixation is rarely a simple function of light intensity. Acting alone on a cloudless day, the light response of nitrogen fixation should result in midday maxima of nitrogen fixation at subsurface depths (Lewis and Levine 1984). Twin maxima around a midday depression could occur at the surface, where midday light intensities are inhibitingly high. Two phenomena could cause nitrogen fixation to be centered around a morning or afternoon maximum: change in the depth distribution of fixers or change in light response caused by changes in internal or external factors such as oxygen and carbon dioxide concentrations that inhibit or enhance nitrogen fixation. The literature suggests that both phenomena occur. Lake Mendota, Wisconsin, is an example of a lake with diel variations in nitrogen fixation that are strongly influenced by changes in the vertical distribution of heterocystous blue-green algae (Vanderhoef et al. 1975). In Lake Mendota, blue-green algae migrate downward in midmorning and do not return to the surface until late afternoon. Consequently, nitrogen fixation rates usually peak in the morning.

Pearl and Kellar have provided examples of diel variations in nitrogen fixation correlated with certain environmental factors. In studies of three New Zealand lakes (Paerl 1979), one Canadian lake (Kellar and Paerl 1980), and a culture of *Anabaena oscillarioides* (Paerl 1979), these investigators found that most nitrogen fixation occurred in the afternoon (or in the latter half of the culture's light cycle) when oxygen concentrations were maximal. Paerl and Kellar postulated that oxygen evolved by photosynthesis severely inhibited carbon fixation, but scarcely affected nitrogen fixation, which was protected by the heterocyst. While carbon fixation was depressed, the flow of photoreductant and carbon skeletons to nitrogen fixation pathways was enhanced. The mechanism proposed by Paerl and Kellar may not be a general one for lakes, however; it could not be detected in Wintergreen Lake, Michigan (Ward and Wetzel 1980).

The relative importance of physiological change and diel migrations of blue-green al-

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gae in establishing the diel patterns of nitrogen fixation is unclear. To separate the effects of the two processes, one must determine not only nitrogen fixation per unit water volume but also per nitrogen-fixing unit. In recognition of the need to consider biomass change during diel studies, investigators have reported nitrogen fixation as a ratio with cholorophyll *a* (Vanderhoef et al. 1975; Horne 1979), suspended nitrogen (Rusness and Burris 1970), suspended carbon (Paerl 1979), protein (Stewart et al. 1967), blue-green algal filament abundance (Burris and Peterson 1978), and heterocyst abundance (Burris and Peterson 1978). The first four of these ratios can be affected by organisms that do not fix nitrogen. When heterocystous blue-green algae are responsible for nitrogen fixation, as is normally the case in the epilimnia of lakes (Carr and Whitton 1982), the simplest and most meaningful way to express relative nitrogen fixation is in relation to heterocyst number. Cytological and physiological studies have established that the heterocyst is the primary site of nitrogen fixation under aerobic conditions (Carr and Whitton 1982).

We report here measurements of diel variation in the nitrogen fixation of Lake Valencia, Venezuela. Using data from incubations in situ, light measurements, and heterocyst counts, we separate the two major causes of variation, change in abundance of nitrogen fixers at a given depth and change in heterocyst-specific nitrogen fixation. We thank S. Stadler-Morris and D. Morris, Jr., for their field assistance, R. Epp for the gas chromatography, B. Chronic for counting heterocysts, and G. Ganf, A. Horne, and P. Brezonik for their comments on the manuscript.

Lake description and methods

Lake Valencia is a large (351 km^2) lake in the Aragua Valley of northern Venezuela (10°10′N, 6°25′E) with a mean depth of 19 m and a maximum depth of 39 m. For at least 245 years, Lake Valencia has had no outflow (Schubert 1979). Consequently, the specific conductance of the water is about 2,000 $\mu S \cdot cm^{-1}$ and its pH is 8.5–9.5. The lake is unstratified during the dry season (usually December through April) and stratified, with an upper mixed layer averaging 13 m thick, during the rainy season (Lewis 1983). Lake Valencia is eutrophic (mean chlorophyll *a* ca. 40 $\mu g \cdot liter^{-1}$) and sustains large populations of blue-green algae. Heterocystous species are present throughout the year, but are abundant only during the last 6 months of stagnation (June through early December).

Diel studies of nitrogen fixation in Lake Valencia were conducted approximately monthly between January and October 1981. Before each incubation, samples were collected from a marked station in the main basin of the lake. Darkened 250-ml BOD bottles and 125-ml sample bottles were filled at a depth of 0.5 m. The phytoplankton in the sample bottles was preserved with Lugol's solution. The filled BOD bottles were brought to a shaded area near the incubation site in the southeastern bay of the lake. Here sample water was drawn into four 50-ml glass syringes and 5 ml of acetylene were added to each filled syringe. Two syringes served as blanks; the dissolved gas in these syringes was extracted immediately after the acetylene was mixed with the water. Acetylene reduction in the syringes was measured by the method of Flett et al. (1976).

The duplicate sample syringes were incubated in situ, where they were held horizontally by clear plastic holders suspended from a glass float with transparent fishing line. Between January and July, one set of samples was incubated overnight and additional sets were incubated at intervals of about 4 h between dawn and dusk. Daytime incubations were 2 h long during the September and October studies.

During each incubation, total incident solar radiation was measured with a LiCor 510B integrator attached to a LiCor 185B quantum meter and a spherical quantum sensor. These were set up on shore in an unshaded area <100 m from the incubation site. Information on variations in light intensity within an incubation period was obtained from strip charts produced by a bimetal pryheliometer at the meteorological site about 1 km from the incubation site. Light extinction at the incubation site was measured with the quantum sensor and meter. From the data on incident radiation and

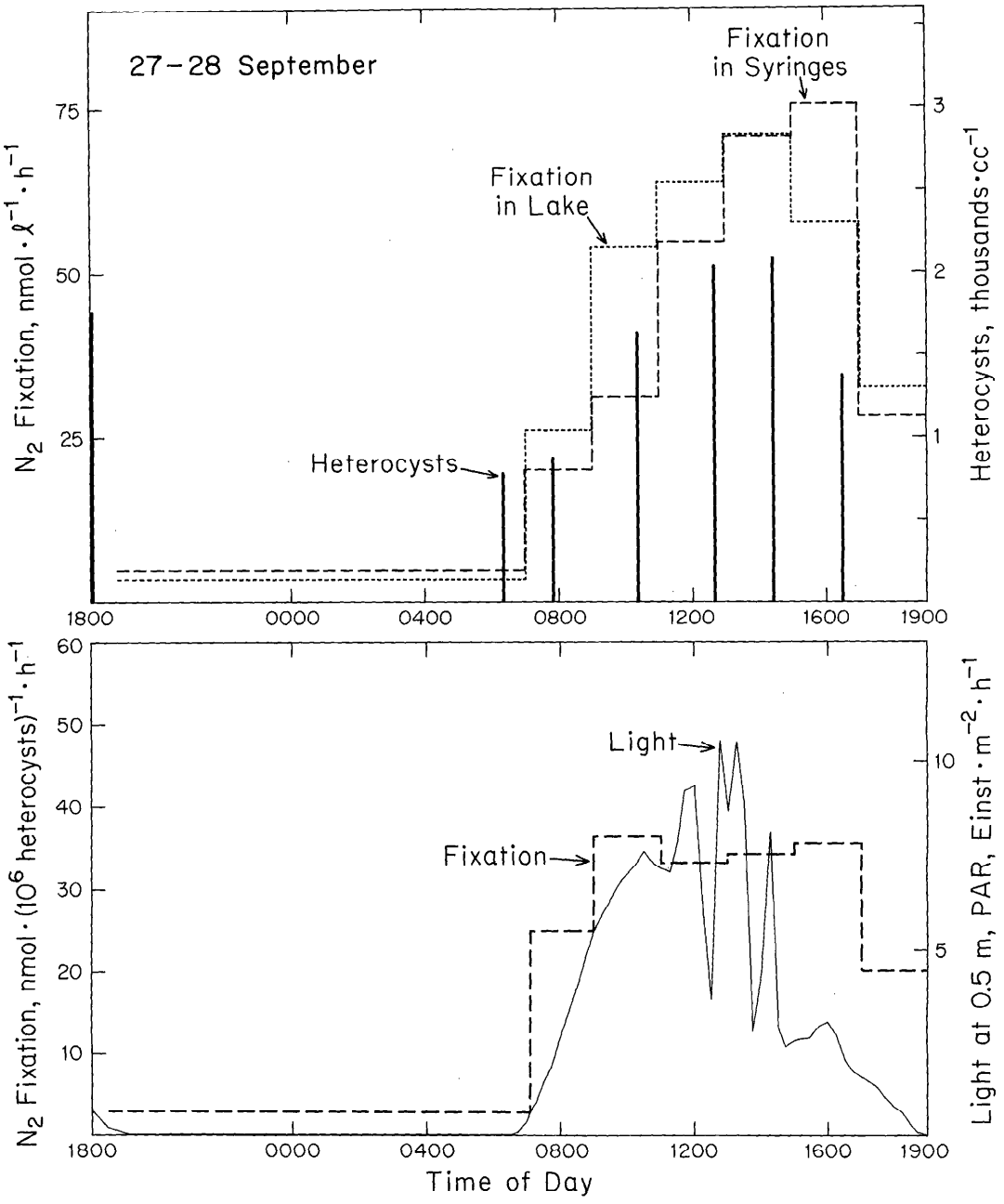

Fig. 1. Diel variations of volume-specific nitrogen fixation in syringes and in the lake, total heterocyst concentration, heterocyst-specific nitrogen fixation, and solar radiation (PAR) at 0.5 m on 27–28 September.

light extinction, light exposure at the incubation depth was calculated.

The gas phase of the air-stripped samples was collected in 5-ml Vacutainer evacuated tubes or serum bottles and sent to Boulder, Colorado, for analysis on a Hewlett-Packard 5840A gas chromatograph equipped with a flame ionization detector and a Dur-

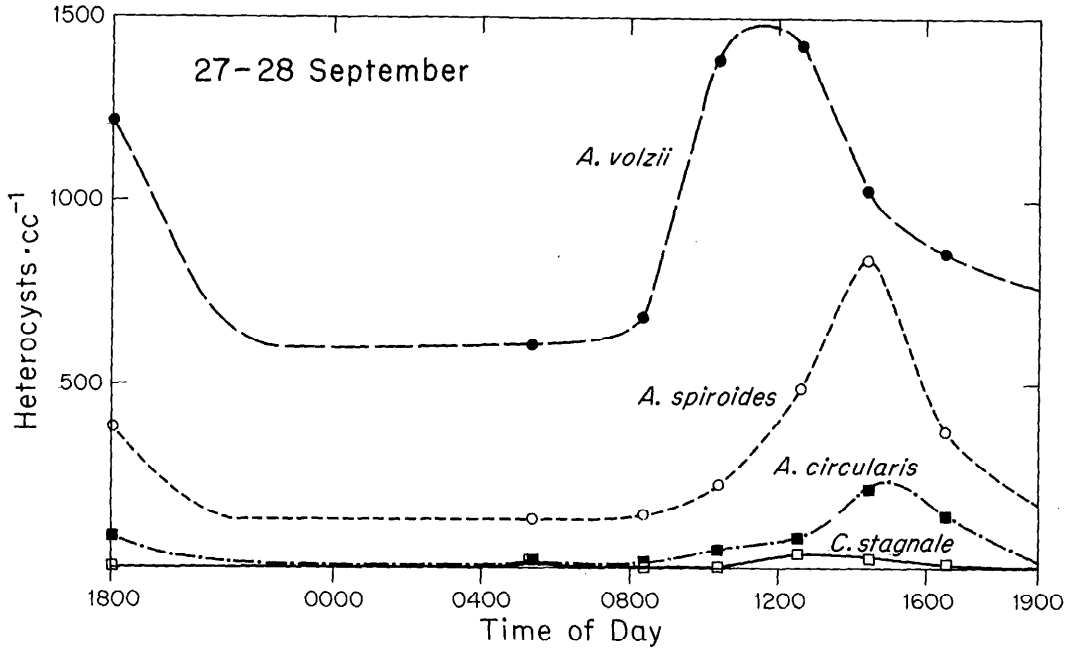

Fig. 2. Diel variations of the heterocyst concentrations of *Anabaena volzii*, *Anabaena spiroides*, *Anabaenopsis circularis*, and *Cylindrospermopsis stagnale* at 0.5 m on 27–28 September.

apak column heated to 60°C. Curves of ethylene loss from storage containers vs. time were used to correct for leakage during the transport period. These corrections were small (<7%) when evacuated tubes were used. The serum bottles, however, lost about 1% of their ethylene content per day. Nitrogen fixation was calculated from acetylene reduction with calibration factors obtained through simultaneous measurements of acetylene reduction and ^{15}N fixation. Heterocysts were counted with a M-40 Wild inverted microscope. All counts were replicated two or more times.

Results

During the months when Lake Valencia was circulating and during the early weeks of stagnation, when dissolved inorganic nitrogen was still relatively abundant, the nitrogen fixation rates during diel studies were rarely significantly different from zero. Studies conducted between June and October, while the nitrogen fixers in Lake Valencia were abundant and active, showed fairly regular diel patterns. The pattern for September is illustrated in Fig. 1. The September data are shown because the 2-h incubations on this data provide better definition of trends than the 4-h incubations of earlier studies. The amplitude of diel changes during September and October was less than during early stagnation, however.

The rate of nitrogen fixation at 0.5 m was strongly dependent on the concentration of heterocystous blue-green algae, which changed with time of day (Fig. 1). The heterocyst concentration was lowest at daybreak. As the morning proceeded, heterocyst concentration at 0.5 m increased, usually gradually at first and then rapidly. Maximum heterocyst concentrations at 0.5 m were reached in early to midafternoon. Sunset collections showed that heterocyst concentrations at 0.5 m decreased during the last 2 or 3 h of daylight, sometimes dramatically. The sunset heterocyst concentrations were still greater than those at dawn, however, suggesting that the movement of heterocystous blue-green algae away from 0.5 m was completed after nightfall. The exact timing of the evening decrease in het-

erocyst numbers apparently varies somewhat from day to day; the heterocyst concentration at 1800 on 27 September was greater than that at 1630 on 28 September. Student-Newman-Keuls multiple range tests showed that the change in heterocyst concentration at 0.5 m was usually significant at a 0.05 level when 4-h intervals were considered (but not when samples taken 2 h apart were compared).

Four species of heterocystous blue-green algae were common in Lake Valencia during 1981: *Anabaena volzii* Lemm., *Anabaena spiroides* Kleb., *Cylindrospermopsis stagnale* (Wolosz.) Seenayya et Subba Raju, and *Anabaenopsis circularis* (G. S. West) Wol. and Miller. Detailed descriptions of the Valencia populations of these species are given by Lewis and Riehl (1982). Species-specific heterocyst counts indicate that the vertical distribution of all four heterocystous blue-green algal species changed with time of day and that these diel patterns were species-specific (Fig. 2). *Anabaena volzii* was the first species to move to 0.5 m during the course of a day. It reached maximum concentrations at this depth shortly before noon. Maximum concentrations of *C. stagnale, A. spiroides,* and *A. circularis* at 0.5 m were attained at about 1300, 1430, and 1500.

The volume-specific nitrogen fixation rates (nitrogen fixed·liter^{-1}·h^{-1}) at 0.5 m were lowest at night (<6% of the maximum daytime rates). At dawn the rate of nitrogen fixation began to increase from this nocturnal minimum. The nitrogen fixation in syringes continued to increase with each incubation between dawn and late afternoon so that maximum rates were attained sometime between 1500 and dusk. Fixation then dropped abruptly to nighttime rates. Student-Newman-Keuls multiple range tests indicated that the fixation rates measured during adjacent 2- or 4-h periods usually differed at a 0.05 significance level. For the September data shown here, nitrogen fixation rate was not significantly different during the 1300–1500 and 1500–1700 incubations, but it did differ significantly at all other periods.

While the syringes were incubating, the vertical distribution of heterocysts in the lake changed. Therefore, the volume-specific nitrogen fixation rates in the lake and syringes differed: only instantaneous measurements of nitrogen fixation would have yielded similar rates for the two systems. To correct for changes in heterocyst concentration during incubations, we drew a curve through the plot of heterocyst concentration vs. time and calculated the mean heterocyst concentration at 0.5 m during each incubation. The volume-specific nitrogen fixation rate in the lake was estimated as the product of heterocyst-specific nitrogen fixation rate and mean heterocyst concentration. These corrections indicate that the ambient nitrogen fixation rate at 0.5 m was maximal in midafternoon (at about 1400) rather than in late afternoon, as the measurements in syringes suggest (Fig. 1).

Heterocyst-specific nitrogen fixation did not follow the same diel trends as volume-specific nitrogen fixation. During most of the day, the heterocyst-specific nitrogen fixation rates were fairly uniform, between 31 and 37 nmol·(10^6 heterocysts)$^{-1}$·h^{-1} in September. At night, heterocyst-specific nitrogen fixation was an order of magnitude lower, 3.0 nmol·(10^6 heterocysts)$^{-1}$·h^{-1} in September. Incubations that started at dawn or ended at dusk yielded heterocyst-specific nitrogen fixation rates below the midday rates, but still several times as high as the night rates.

Discussion

The diel pattern for volume-specific nitrogen fixation that we found for Lake Valencia was skewed similarly to the pattern that Paerl and Kellar described for New Zealand and Canadian lakes, which they attributed to the stimulation of nitrogen fixation by afternoon depression of carbon fixation (Paerl 1979; Kellar and Paerl 1980). For Lake Valencia, however, there was no evidence for diel change inthe light response of nitrogen fixation; diel variation in heterocyst-specific nitrogen fixation rate appeared to be due to change in light intensity alone. This is illustrated by the data for 28 September. We used a model of the light response of nitrogen fixation (Lewis and Levine 1984) with parameters set from the data of a midday incubation on 27 September to predict nitrogen fixation through the

Table 1. Measured and predicted heterocyst-specific nitrogen fixation rates [nmol $N_2 \cdot (10^6$ heterocysts$)^{-1} \cdot h^{-1}$] during the diel study on 27–28 September. The predicted rates are from the light-response model described by Lewis and Levine (1984) with model parameters based on data gathered on 27 September.

Period	Time	Predicted	Measured
1	1850–0700	0.7	3.0
2	0700–0900	30	25
3	0900–1100	35	36
4	1100–1300	31	34
5	1300–1500	37	35
6	1500–1700	34	36
7	1700–1900	12	20

day on 28 September, assuming no diel change in the response. The results support the conclusion that the light response is fixed on a given day: for every incubation between the hours of 0900 and 1700, the measured and predicted heterocyst-specific nitrogen fixation rates differed by no more than 10% (Table 1). Predictions of nitrogen fixation during the night and during incubations that included dawn or dusk exposures were less accurate, but the lower accuracy is easily explained without invoking hypotheses about competition between nitrogen fixation and carbon fixation. The nitrogen fixation rate at night was so low that measurement errors alone could have accounted for the discrepancy between the predicted and measured rates. That nitrogen fixation rates were slightly below the predicted rates at dawn and substantially above the predicted rates at dusk was probably due to the lag time between the production of photogenerated material and its use in nitrogen fixation (Fay 1976).

The difference between the diel patterns of volume-specific and heterocyst-specific nitrogen fixation at 0.5 m in Lake Valencia was due to diel changes in heterocyst concentration. These changes were almost certainly produced by vertical migrations of algae rather than by the horizontal movement of water masses. Although the distribution of nitrogen fixers in Lake Valencia is patchy, the weather patterns during our study were too variable to produce regular diel patterns of water mass circulation. Also the patches of nitrogen fixers in Lake Valencia are normally several kilometers across (Levine and Lewis unpubl. data) so that only on those days when an interface between patches passes a sampling site will the total biomass in the water below a collection site change greatly with time of day. The possibility that random horizontal movements of water masses repeatedly yielded similar diel patterns in heterocyst abundance seems remote.

The directions of vertical migration were not established in our study. Although buoyancy regulation by heterocystous blue-green algae is well documented (Carr and Whitton 1982), few vertical migrations of heterocystous blue-green algae have been described in the literature. Exceptions include the studies of migrations by *Aphanizomenon* sp. and *Anabaena* sp. in Lake Mendota already cited and observations of migrations (or cycles of sinking and resuspension) by *Anabaenopsis* spp. in Lake George, Uganda (Ganf 1974). Vertical migrations by *Anabaena* sp. in large tanks have also been described (Sirenko et al. 1968). Significantly, each of these migration studies has yielded the same results: algae move down from the surface at or before midday and up in late afternoon or evening.

Algae probably move away from the surface in response to midday light intensities, which are sometimes great enough to photooxidize plant pigments (Eloff et al. 1976). Many blue-green algae are positively phototactic at low light intensities and negatively phototactic at high light intensities (Carr and Whitton 1982). It therefore seems likely that the heterocystous blue-green algae in the surface waters of Lake Valencia migrated in the same directions as those in Lakes Mendota and George. The order of descent of blue-green algal species to 0.5 m may have been determined by the sensitivity of each species to photo-oxidation. That four species of heterocystous blue-green algae migrated suggests that diel migrations may be a common feature of the ecology of this group of organisms.

As the first report of a diel pattern for nitrogen fixation in a stratified tropical lake, our findings strengthen the contention that diel patterns are characteristic of lacustrine nitrogen fixation. The lack of evidence for afternoon enhancement of nitrogen fixation

through depression of carbon fixation in Lake Valencia and Wintergreen Lake (Ward and Wetzel 1980) suggests that this phenomenon may be less universal than vertical migration of fixers. Vertical distribution of nitrogen fixers may be of such importance in nitrogen fixation that differences in diel migration patterns at the species level could account for some of the variability reported in the diel patterns of nitrogen fixation. Without data on the concentration of nitrogen fixers in samples, the prevalence of environmentally controlled physiological processes that enhance or inhibit nitrogen fixation may be overestimated.

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