

The horizontal heterogeneity of nitrogen fixation in Lake Valencia, Venezuela¹

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Abstract

Spatial and temporal variability of nitrogen fixation in Lake Valencia, Venezuela, were quantified on the basis of duplicate water samples collected from a depth of 0.5 m at 16 sites on 10 dates. The concentration of heterocysts in samples was determined and the samples were incubated with acetylene in situ. Two-way ANOVA was used to separate the variance associated with site (fixed spatial patchiness), date (temporal variation), the interaction between site and date (ephemeral spatial patchiness), and sampling error. The nitrogen fixers in Lake Valencia are arranged in large (40–200 km²), ephemeral patches with distinctive fixation rates per heterocyst. Both variability in fixation per heterocyst and variability in heterocyst concentration contribute significantly to variation in fixation per unit volume of lake water, but the variability attributable to heterocyst abundance is greater. Spatial variation in fixation and heterocyst concentration exceeds temporal variation in these parameters, and the ephemeral component of patchiness is much greater than the fixed component.

Although a boat ride on a calm lake during a blue-green algal bloom clearly demonstrates that the surface distribution of blue-green algae is patchy, limnologists rarely measure the horizontal variability of nitrogen fixation. The synoptic studies of the autumnal bloom of *Anabaena circinalis* and the spring bloom of *Aphanizomenon* in Clear Lake, California, by Horne et al. (1972, 1979) are exceptions to this generalization. Horne et al. measured nitrogen fixation on three occasions at 10–32 sites in the fall and on eight occasions at 31 sites in late spring. They found that fixation on any sampling date varied over two orders of magnitude along a horizontal plane and that fixation rate per unit volume of lake water was positively correlated with heterocyst concentration and negatively correlated with nitrate concentration.

Between December 1980 and October 1981, we studied the horizontal heterogeneity of nitrogen fixation in Lake Valencia, Venezuela. Four species of heterocystous blue-green algae were present. Our study differed from that of Horne and others in two

respects. First, we examined not only the volume-specific nitrogen fixation (fixation per liter of water), but also each of the two components of heterogeneity in nitrogen fixation: variability in heterocyst abundance and variability in nitrogen fixation rate per heterocyst (the heterocyst is the primary site for nitrogen fixation in aerobic environments: Carr and Whitton 1982). Second, we sampled monthly at 16 widely spaced sites through most of an annual cycle; this allowed us to separate the contributions of temporal and spatial variance to the overall variation of nitrogen fixation in Lake Valencia (Lewis 1978). This approach also allowed us to distinguish between fixed spatial patchiness, which is attributable to differences in station averages, and ephemeral spatial patchiness, which is due to temporally unstable differences between stations caused by such factors as shifting water currents and nutrient upwelling. Due to time constraints and to avoid confounding horizontal heterogeneity with vertical heterogeneity, we sampled at a single depth, 0.5 m. However, given the three-dimensionality of patches, we expect horizontal pattern to be unique for any depth.

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Methods and study site

Lake Valencia has been described extensively elsewhere (Lewis and Weibezahn 1976; Lewis 1983a; Levine and Lewis 1984). It is a large (351 km²), eutrophic lake in the Aragua Valley of northern Venezuela (10°12'N, 67°44'W) with a mean depth of 19 m and a maximum depth of 39 m. It is unstratified during the windy dry season (usually December to April) and stratified with an upper mixed layer averaging 12 m thick during the calmer rainy season (Lewis 1983a). Winds blow predominantly from the northeast during the dry season and from the southeast during the wet season, but their trajectories are complicated by the effects of mountains that surround the valley. Blue-green algae are predominant in Lake Valencia during all but 1 or 2 months of the year (Lewis in press).

Samples were collected from 16 sites on Lake Valencia at approximately monthly intervals ($n = 10$) between December 1980 and October 1981. The sites were chosen to represent all regions of the lake (Fig. 1). At each site, darkened 250-ml BOD bottles and 125-ml sample bottles were filled at a depth of 0.5 m and the phytoplankton in the sample bottles was preserved with Lugol's solution. Sample collection required 2.5–4 h. The filled BOD bottles were returned to a darkened building near the incubation site where 30 ml of the sample water from each collection site were drawn into each of four 50-ml glass syringes along with 5 ml of acetylene. Two syringes of the four from each collection site served as blanks; the dissolved gas in these syringes was extracted immediately after the acetylene was mixed with the water. The other two syringes were incubated in situ. Acetylene reduction in the syringes was measured by the method of Flctt et al. (1976), as modified by Levine and Lewis (1984).

The two sample syringes from each of the 16 sites were incubated at a depth of 0.5 m

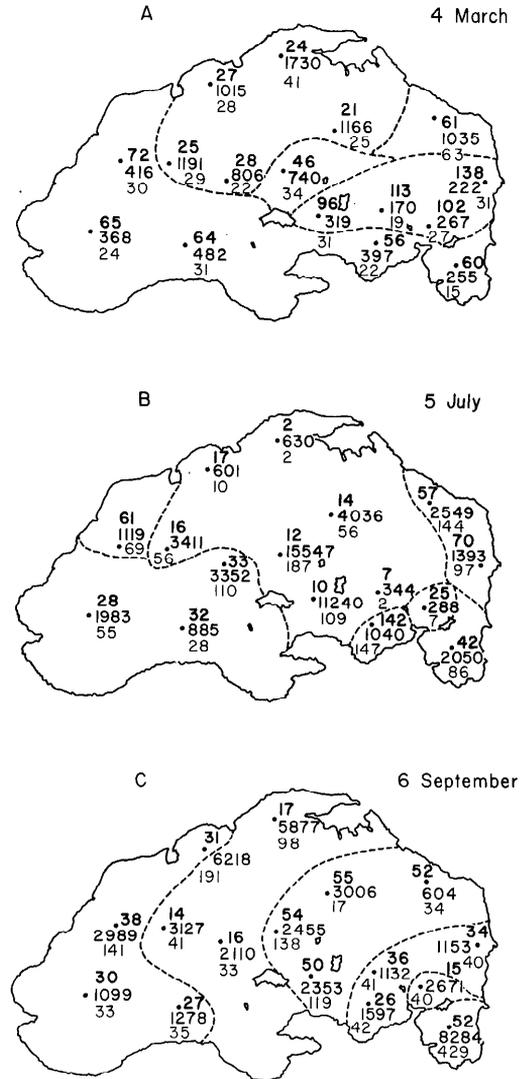


Fig. 1. Maps of Lake Valencia showing the horizontal distribution of heterocyst-specific nitrogen fixation [$\text{nmol} (10^6 \text{ heterocyst}^{-1} \text{ h}^{-1}$; top number shown], heterocyst concentration (No. ml^{-1} ; middle number shown), and volume-specific nitrogen fixation ($\text{nmol liter}^{-1} \text{ h}^{-1}$; bottom number shown) at 0.5-m depth on three representative dates. Dashed lines enclose patches of nitrogen fixers with similar heterocyst-specific nitrogen fixation rates.

in the lake, held by surgical tubing attached to a Plexiglas sheet suspended from a floating rectangular frame. At the end of each incubation, the samples were transported to shore in a light-tight box and their gases stripped (Levine and Lewis 1984). The gas phase was collected in 5-ml Vacutainer

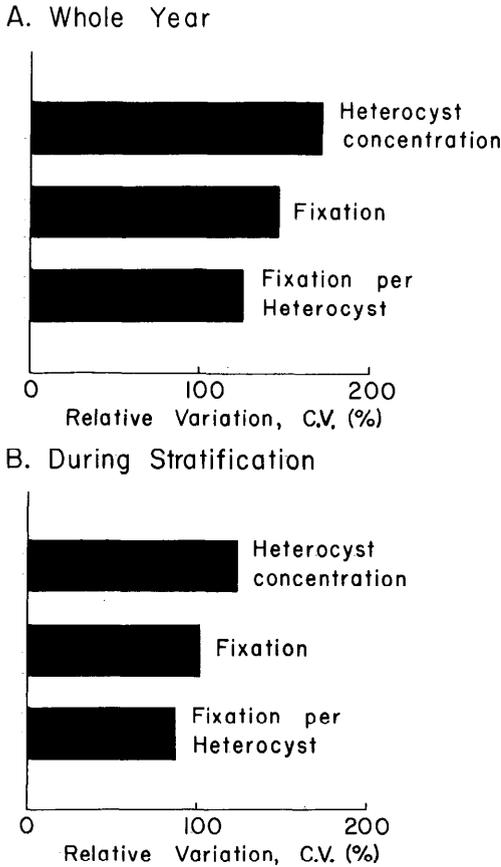


Fig. 2. The relative variation of heterocyst concentration, volume-specific nitrogen fixation rate, and heterocyst-specific nitrogen fixation rate at 0.5-m depth in Lake Valencia over the entire sampling period (A) and during lake stratification (B).

evacuated tubes or serum bottles and sent to Boulder, Colorado, for analysis on a gas chromatograph. The handling of samples in Boulder and the calibration of acetylene reduction with ^{15}N tracer techniques are described by Levine and Lewis (1984). Duplicate counts of the heterocysts in each sample were made with a Wild M-40 inverted microscope.

The method of Lewis (1978) was used to estimate the contributions of temporal variation, ephemeral spatial variation, and fixed spatial variation to total variation. This method is based on a two-way ANOVA (random effects, model 2). Its application requires that samples be spaced over the entire lake and span the full range of sea-

sonal conditions; the two-way ANOVA is then used to estimate variance components. Variance associated with the main effect of sampling date is taken as an estimate of temporal variation, variance associated with the station main effect is taken as an estimate of fixed spatial variation, and the interaction of date and station is taken as an estimate of ephemeral spatial variation.

Results

Synoptic study of nitrogen fixation in Lake Valencia revealed substantial horizontal heterogeneity in nitrogen fixation rates. On many sampling dates, the volume-specific nitrogen fixation rates [$\text{nmol N fixed (liter water)}^{-1} \text{ h}^{-1}$] at our 16 sites had ranges of an order of magnitude or more (Fig. 1). The heterocyst concentrations on these dates also ranged over 1–2 orders of magnitude, but variation in volume-specific fixation could not be attributed solely to variability in heterocyst abundance because several-fold variability in nitrogen fixation rate per heterocyst was normal. Coefficients of variation ($s \times 100/\bar{x}$), shown in Fig. 2, provide a quantitative estimate of the relative variability of volume-specific nitrogen fixation rate, heterocyst concentration, and heterocyst-specific fixation rate. Heterocyst concentration was slightly more variable than fixation per heterocyst, but both factors varied substantially.

It is possible by use of multiple regression to separate the contributions of heterocyst abundance from those of fixation per heterocyst to the overall variation in fixation per unit volume. Heterocyst abundance and fixation per heterocyst did not interact significantly ($P > 0.05$), hence their effects on fixation per unit volume were essentially independent. In the multiple regression, most variables were highly significant ($P < 0.001$) and 57% of the variance was explained; 53% of this was due to abundance of heterocysts and 4% to fixation per heterocyst.

Maps of heterocyst abundance and volume-specific nitrogen fixation rate like those in Fig. 1 showed either no pattern or a complex pattern, while maps of heterocyst-specific nitrogen fixation rate consistently revealed distinct large-scale patches over the lake. These patches probably were com-

posed of fixers that had a similar environmental history. The number of patches of algae with similar heterocyst-specific fixation rates and the boundaries of these patches changed from sampling to sampling, i.e. they were ephemeral. Typically the patches covered 40–200 km² of lake surface and spanned 6–15 km along their longest axes.

Separation of the components of variance for total heterocyst concentration, volume-specific nitrogen fixation rate, and heterocyst-specific nitrogen fixation rate showed that ephemeral spatial heterogeneity was the major component of variability in all three parameters over our 10-month study (Fig. 3A). Fixed spatial variation accounted for no more than 9% of the explained variance in any of the parameters. The importance of temporal variation was also secondary to that of ephemeral patchiness: the ratios of total spatial (fixed plus ephemeral) variation to temporal variation for heterocyst concentration, volume-specific nitrogen fixation, and heterocyst-specific nitrogen fixation for the 10-month study were 5:1, 2:1, and 2:1. Variance due to sampling error was <8% of total variances for all variables except heterocyst-specific nitrogen fixation rate, for which error was 29% of total variance.

Most temporal variation of blue-green algal abundance in Lake Valencia stems from the annual alternation of lake stratification and lake circulation that accompanies the shift from wet to dry season. Nitrogen-fixing blue-green algae are most abundant during lake stratification. When we restricted our analysis of the variance in nitrogen fixation and heterocyst abundance to the stratification period, the relative importance of time as a source of variation greatly diminished (Fig. 3B).

Four planktonic nitrogen fixers were common in Lake Valencia during 1981: *Anabaena spiroides* Kleb., *Anabaena volzii* Lemm., *Cylindrospermopsis stagnale* (Wolosz.) Seenayya et Subba Raju, and *Anabaenopsis circinalis* (G. S. West) Wol. and Miller. Patches of nitrogen fixers with distinct heterocyst-specific fixation rates sometimes differed in species composition. Species differences were not entirely re-

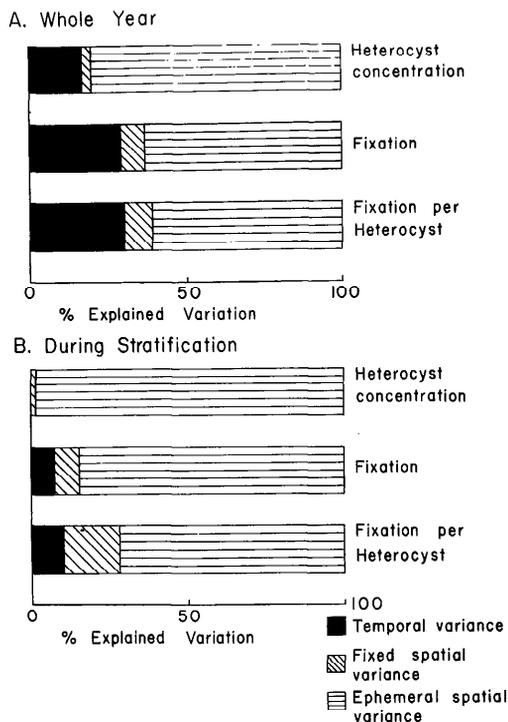


Fig. 3. Components of variance for heterocyst concentration, volume-specific nitrogen fixation, and heterocyst-specific nitrogen fixation.

sponsible for the variation in heterocyst-specific nitrogen fixation rate, however, because there was patchiness even under monospecific conditions. For example, on 4 March (Fig. 1A), five major patches of fixers could be distinguished in Lake Valencia on the basis of heterocyst-specific fixation rates, although 90% or more of the heterocysts at 14 of 16 sites belonged to one species, *A. volzii*. We could not measure species-specific nitrogen fixation rates, but our species-specific heterocyst counts show that the importance of spatial variation relative to temporal variation was not strictly a community-level phenomenon; it extended to the species as well. For three of the four nitrogen fixers, the ratio of spatial variance to temporal variance on an annual basis was 5:1 or greater. *Anabaena volzii*, which almost disappeared from the surface waters during early stratification, was the only nitrogen fixer in Lake Valencia whose variance over time exceeded its spatial vari-

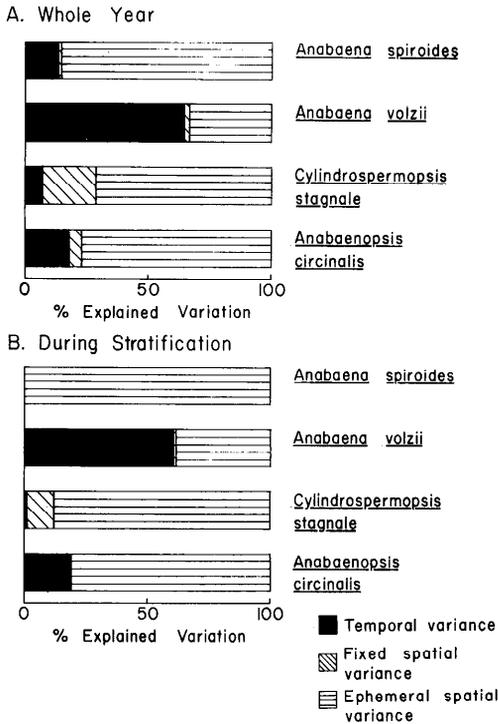


Fig. 4. Components of variance for heterocyst concentration for the four species of nitrogen fixers.

ation (Fig. 4). Fixed spatial variation in heterocyst concentration was low for all taxa. The relative importance of fixed spatial variation was greatest for *C. stagnale*, whose fixed spatial variation accounts for 22% of the explained variance.

Discussion

In concurrence with the findings of Horne et al. (1972, 1979) for Clear Lake, our study of Lake Valencia indicated that both volume-specific nitrogen fixation rate and heterocyst concentration are distributed heterogeneously along a horizontal plane. The spatial variability of heterocyst concentration in Lake Valencia is so substantial that for three of the four species of fixers it overshadows seasonal variability. Although Lake Valencia is tropical, and thus lacks some extremes of temporal variation typical of temperate lakes, the conditions for plankton are far from constant. The alternation of a stratification season with a mixing season induces considerable variation in the nu-

trient concentrations and light regime (Lewis in press).

The large-scale patches of fixers with distinct fixation rates typical of Lake Valencia have not been demonstrated for other lakes, but this is probably because fixation per heterocyst has seldom been estimated. These patches in Lake Valencia are reminiscent of the patchiness of phytoplankton in the sea (Steele 1976). Oceanographers attribute phytoplankton patchiness to the opposing forces of growth at "points" of upwelling or "points" of low grazing pressure and physical dispersion. That the patch size in Lake Valencia (6–15 km along the longest axis) is at the lower end of the range of patch sizes in the ocean (2–50 km) is easily explained by the inverse relationship between turbulence and wind fetch. Patch radius is proportional to the ratio of phytoplankton growth rate (a function of patch area) to the rate of dispersion (a function of patch circumference) (Okubo 1978). Patches of algae with similar chlorophyll *a* content or similar primary productivity have been observed in lakes even smaller than Lake Valencia (Richerson et al. 1978).

Because we did not include nutrient chemistry or measurements of mortality factors in our synoptic study, we cannot pinpoint the cause of patch formation in Lake Valencia. A few generalizations are possible, however. First, we can rule out the possibility that the patches were produced exclusively by wind piling of surface scums since the physiology of nitrogen fixers differed between patches. Similarly, sources of mortality such as grazing and sedimentation that do not affect physiology directly cannot be the primary causes of patchiness in heterocyst-specific nitrogen fixation. Therefore, patchiness probably arises from spatial differences in nutrient availability. Spatial heterogeneity in nutrient availability can be generated through the upwelling of metalimnetic waters, uneven watershed runoff, or even spotty atmospheric precipitation (cf. Lewis 1983b). The introduction of nitrate or ammonium inhibits nitrogen fixation, whereas the introduction of iron or molybdenum often stimulates fixation rates (Carr and Whitton 1982). The ephemeral nature of fixer patchiness in Lake Valencia suggests

either random nutrient inputs or random variation in mixing.

The results of the Lake Valencia study have some implications for the design of sampling programs. Clearly, one cannot sample at one site on Lake Valencia on a given date and obtain a reliable estimate of nitrogen fixation in the entire lake on that date. On the other hand, because of the small component of fixed spatial variation, repeated observations at any given site provide a good unbiased estimator of the lake mean through time. What one measures by sampling weekly at one site are temporal changes in overall heterocyst concentration and fixation rate superimposed on large local changes due to the random passage of patches.

Horne and Wrigley (1975) have suggested remote sensing as an alternative to time-consuming synoptic studies in estimating the horizontal heterogeneity of blue-green algal biomass in lakes. This approach would be inadequate for estimating nitrogen fixation in Lake Valencia for two reasons. First, it assumes that all surface chlorophyll is incorporated in nitrogen fixers, whereas in Lake Valencia a large fraction of the surface algal scum is composed of *Microcystis*, which does not fix nitrogen. Second, it assumes homogeneous nitrogen fixation rates per heterocyst, whereas in Lake Valencia spatial variation in fixation per heterocyst is substantial.

Spatial patchiness is not simply a sampling problem; it is also important to the understanding of population dynamics. Exploring the causes and effects of spatial patchiness is a necessary adjunct to studies of seasonal changes in lakes.

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