Nitrogen fixation in Lake Mendota, Madison, Wisconsin[1]

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Abstract

The effects of various environmental conditions, and of cell composition, heterocyst content, and nitrogen content of algal samples, on fixation of N_2 by colonial and filamentous algae in Lake Mendota were investigated.

Heterocyst content and temperature were significantly and positively related to acetylene reduction activity (N_2 fixation); depth of sample collection was negatively related. Available data do not show whether statistical correlations of acetylene reduction activity with dissolved 0_2 and pH represent specific effects of 0_2 and pH on this activity, or simply reflect chemical changes caused by algal photosynthesis.

During summer stratification, when surface-water content of combined inorganic nitrogen was severely depleted, N_2 fixation associated with heterocystous blue-green algae contributed 85% of the total N_2 fixed. Bacterial N_2 fixation was low compared to that of algae. Despite its small overall contribution to the annual nitrogen budget (38,000 kg of nitrogen; ca. 7% of the total input), N_2 fixation is significant in maintaining blue-green algal nuisances in surface water when non-N_2 fixing phytoplankton cannot compete effectively.

In an aquatic environment heterocystous blue-green algae are likely to be quantitatively the most important organisms converting N_2 to combined nitrogen. The contribution of biological nitrogen fixation to the nutrient budget of aquatic systems has been little studied. Lee (1966) estimated, from the data of Goering (1962), that biological N_2 fixation annually supplied ca. 1,000 kg of nitrogen to Lake Mendota, or 0.4% of the total incoming nitrogen. G. P. Fitzgerald (cited in Horne and Viner 1971) later proposed that 5-10% of the combined nitrogen in Lake Mendota is derived from N_2 fixation. Data from 1969 yielded an estimate of 9,456 kg of nitrogen fixed during the 51-day period in which fixation was detected (Stewart et al. 1971). All of these values were based on observations of algae in pelagic areas of Lake Mendota. A study of the extent of nitrogen fixation in various parts of a lake at different times of year enables a more precise estimate of the significance of nitrogen fixation as a source of combined nitrogen in aquatic systems, and we present here an evaluation of the overall significance of biological nitrogen fixation in Lake Mendota and of some of the factors controlling it.

Nitrogen may be fixed in two areas of Lake Mendota not specifically covered in this study. The first is the marshes, where heterotrophic bacteria, algae on the mud surface, and organisms in the rhizosphere of freshwater angiosperms may fix nitrogen. In studying freshwater marshes in the vicinity of Madison, Lonergan (1973) found acetylene reduction rates equivalent to 2-20 kg of N_2 reduced ha^{-1} yr^{-1}, with an average value of 8.8.

We also did not specifically study the littoral zone. Except for *Azorobacter,* the only common planktonic bacterial groups in Lake Mendota which could fix N_2 would do so anaerobically and therefore would not be found in the littoral. Fixation in this area would be due largely to bacterial or algal epiphytes or phytoplankton.

Experimental

Lake Mendota is a eutrophic hard-water lake. Maximum depth is 23 m; average depth is 13 m.

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Total volume of the lake is 487 x 10^6 m^3; surface area is 39.4 x 10^6 m^2. During summer the lake stratifies and temperature and dissolved oxygen are elinograde. The metalimnion is generally at 10-14 m and oxygen is absent below the thermocline after mid-July.

Data were collected from June 1970 through September 1971. Three sites were sampled weekly in ice-free months; one station was occupied monthly from December through March. Samples were taken at 0, 1, 2, 4, 8, 12, 16, and 20 m. The major station was midlake at or near the deep hole, a second station was located beyond the weeds at a depth of about 5 m in University Bay (a weedbed area in the southwest part of the lake), and a third station, also beyond the weeds at a depth of about 5 m, was situated along the south shore of the lake just north of the Water Chemistry Laboratory.

Samples for chemical analysis were collected in acid-washed polyethylene bottles. Soluble reactive phosphate was determined on filtered water (0.45-µm filter) (Strickland and Parsons 1965). Total phosphate was determined on an unfiltered sample by the same method after persulfate digestion under pressure (Am. Public Health Assoc. 1971). Nitrate was determined in the Technicon AutoAnalyzer with a hydrazine reduction or by reaction with brucine; for very low concentrations, a cadmium reduction was used. Ammonia was determined on the AutoAnalyzer by reaction with alkaline phenolhypochlorite, organic nitrogen with the same reaction after Kjeldahl digestion.

Temperature and oxygen concentrations were determined in the field with a Yellow Springs dissolved oxygen meter, or with a Whitney thermistor and the azide modification of the Winkler titration (Am. Public Health Assoc. 1971). Light penetration was measured with a submarine photometer.

Lake water samples, collected from the surface in glass flasks or from depth in a Plexiglas Van Dorn sampler, were filtered if necessary through No. 25 silk plankton net to concentrate plankton before testing for nitrogenase activity. The measured volume was transferred to 5-ml serum bottles and treated by the method of Stewart et al. (1970). Samples from anaerobic waters were gassed with argon before determining N_2 fixation by acetylene reduction. To maintain in situ light and temperature conditions, samples were incubated on the sunny side of the boat at the depth from which the plankton were withdrawn; a glass filter flask filled with water from the depth sampled was used to contain the samples at depth. Algal blanks to which C_2H_2 was not added were subsequently used for analysis of algal composition.

Sediment samples were collected in summer and winter with a Jenkins corer. Water above the mud-water interface—a rather indistinct boundary in Lake Mendota—was siphoned off to about 5 cm above the mud. Samples of 2-3 ml were taken by pipet at intervals in the sediment column and placed in blackened 15-mil serum bottles which were then gassed with argon and treated like plankton samples. Ethylene formed was measured on a gas chromatograph (Varian Aerograph 600-D) set up for hydrogen flame ionization detection. The 1.53-m column, 0.32 cm ID, was packed with Porapak R. Column temperature was 56°C, while the detector was at ambient. Gas flows were 28 mil min^{-1} for N_2 and 48 ml min^{-1} for H_2. The detector was standardized with known dilutions of c.p. ethylene and recorder response was quantitated by measuring peak heights.

Several filtration experiments were conducted with 0.45-µm pore size Millipore filters. Water previously put through a No. 25 plankton net was placed on the filter and vacuum applied until only a film of water remained on the filter. After the vacuum was released, the filter was quartered and each segment was incubated like an algal sample in order to measure nitrogenase activity, mostly bacterial, in the filtered water.

Data from all three stations were used in the stepwise multiple regression analysis of the relationships between nitrogenase activity and other

Table 1. Factors considered in analysis of acetylene reduction, together with units and abbreviations used.

Abbrev	Explanation
Depth	Depth below the water surface (m)
Temp	Temperature (^{o}C)
μ-Amp	Light measured operationally in microamps
Time	Hours before or after noon, EST
pH	pH
DO	Dissolved oxygen conc (mg liter^{-1})
Ortho P	Soluble reactive orthophosphate conc (mg PO_4^{3-}-P liter^{-1})
Total P	Total phosphate conc (mg PO_4^{3-}-P liter^{-1})
NH_3	Ammonia plus ammonium conc (mg NH_3-N liter^{-1})
NO_3	Nitrate conc (mg NO_3^--N liter^{-1})
Org N	Total organic nitrogen conc (mg NH_3-N liter^{-1})
Algal N	Nitrogen content of material caught on a No. 25 silk plankton net (mg NH_3-N liter^{-1})
Tot het	Total conc heterocysts per volume of water (heterocysts liter^{-1})
N_2 fixr	Total conc of (potential) N_2-fixing algal cells per volume of water (cells liter^{-1})
Heter %	Percent heterocysts out of all cells counted
N_2 fixr %	Percent (potential) N_2-fixers out of all cells counted
Activ	Acetylene reduction activity (nmoles C_2H_4 formed liter^{-1} h^{-1})

parameters. The variables used in the analysis and their units are listed in Table 1. Stepwise calculations were made, with independent variables entering the regression in order of decreasing importance (forward selection procedure). Computations were done on an IBM 360/195 computer using the BMDO2R Biomedical Computer Program (Dixon 1970). Similar calculations were performed, using a backward selection procedure, on a Univac 1108 computer with the University of Wisconsin Computer Center Program STEPREG1. Results were similar with both. For brevity, results from the forward method only are discussed. All computations were made to a significance level of $P \leq 0.05$. Our intent was to elucidate mechanisms rather than to model these reactions; thus a linear method was used so that our equations could be justified on knowledge of biological mechanisms or compatibility with general biological principles.

Three assumptions were made in calculating total pelagic N_2 fixation (Stewart et al. 1971): 3:1 ratio for the reduction rates of C_2H_2 and N_2; uniform fixation over the lake; 14-h fixation period per day. Nitrogenase activities used in calculations were from the deep hole station. Surface water data (0-10 m) were considered separately from deep water data (10 m to bottom). For each depth, activity was calculated by averaging the activities of two sequential sampling dates. The average activity was multiplied by the number of days between the two samplings, by 14 h a day, by the hypsometric factors describing the volume of water included in each depth interval, by 1/3 (because of the 1:3 N_2: C_2H_2, ratio), and by 28 g N_2 mole^{-1}.

The assumption of complete homogeneity over the lake is not entirely valid. Acetylene reduction was consistently lower at the University Bay station than at the other two, which were essentially the same. For example, using the assumptions and computation methods listed above, the following figures were calculated on a whole-lake basis for the top 6 m in the period 20 May-7 September 1971: deep hole, 29,800 kg of nitrogen fixed; University Bay, 21,000 kg; Water Chemistry, 32,700 kg. The low values in University Bay may be a result of patchy distribution of blue-green algae or of antagonism between the extensive weed growth in the bay and the algae (Hasler and Jones 1949). The values from the deep

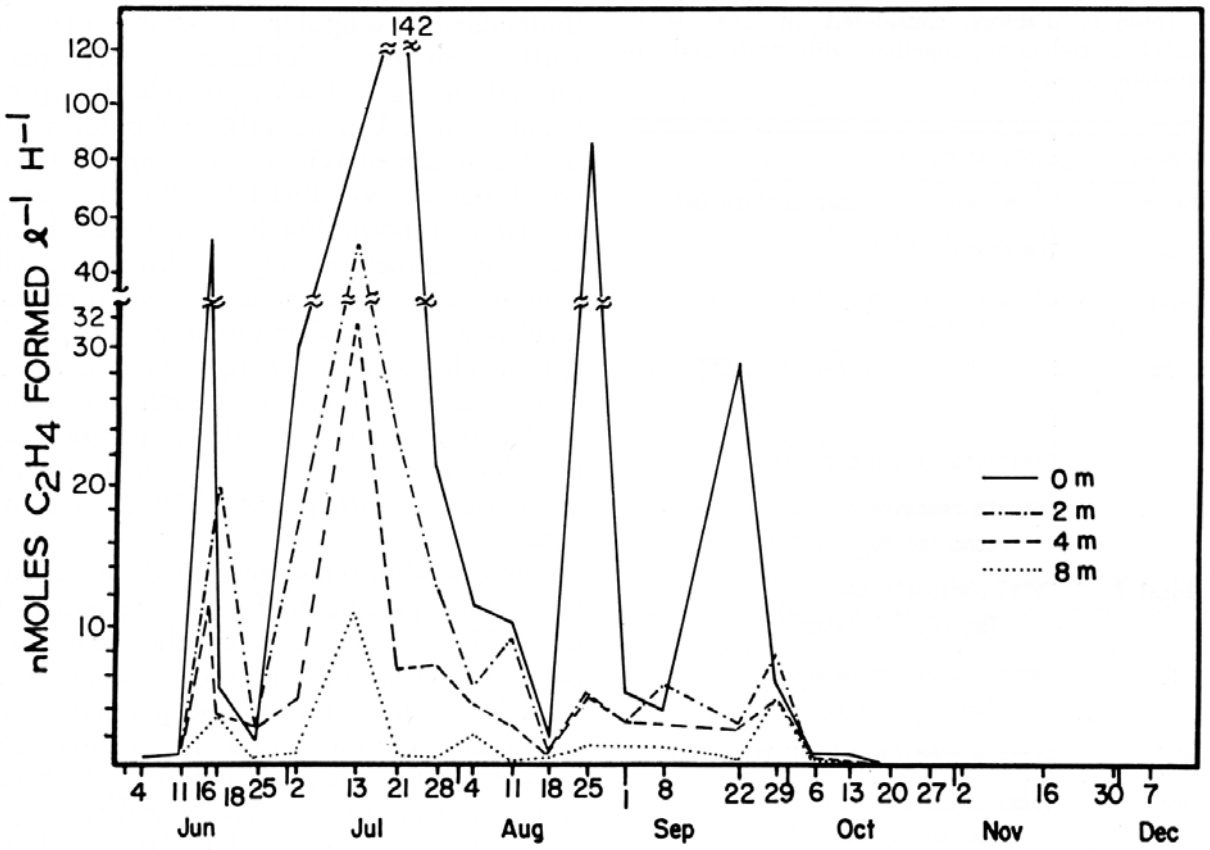

Fig. 1. Acetylene reduction activity at the deep station during June–December 1970.

station and the station just north of the Water Chemistry Laboratory are sufficiently similar so that details for the deep station only are presented below. Additional data are available (Torrey 1972). Considering the relatively small volume of water in University Bay, the data collected at the deep station or at the Water Chemistry station are presumably more representative of the whole lake.

To calculate the amount of acetylene reduction in waters from which the colonial plankton had been removed, we assumed that reduction took place throughout a 24-h day and that activity was uniform within the epilimnion or within the hypolimnion. As discussed below, no activity was detected in oxygenated waters from which colonial algae had been removed. Thus, the final assumption was that activity in anoxic waters from which the algae had been removed persisted for 150 days a year.

Sediment activities were estimated by integrating reduction rates in the top 10 cm of sediment. It was assumed that during the warm half of the year sediments above the 12-m contour were at 24°C; this assumption was made so that data derived from incubations at 24°C could be used. Sediments below 12 m were assumed to be at 14°C. The sediments of the entire lake were assumed to be at 4°C during the cold half of the year.

Results

Physicochemical data—Lake Mendota exhibits the classic characteristics of a dimictic, eutrophic lake in the temperate zone. During 1970-1971 the summer thermocline formed at about 12 m, while inverse thermal stratification developed a few weeks after the lake froze over. Very little light penetrated below 4 m during summer, when the high planktonic biomass in the epilimnion absorbed and scattered incident light. Profiles of pH were clinograde during summer and winter stratification and vertically uniform during mixing.

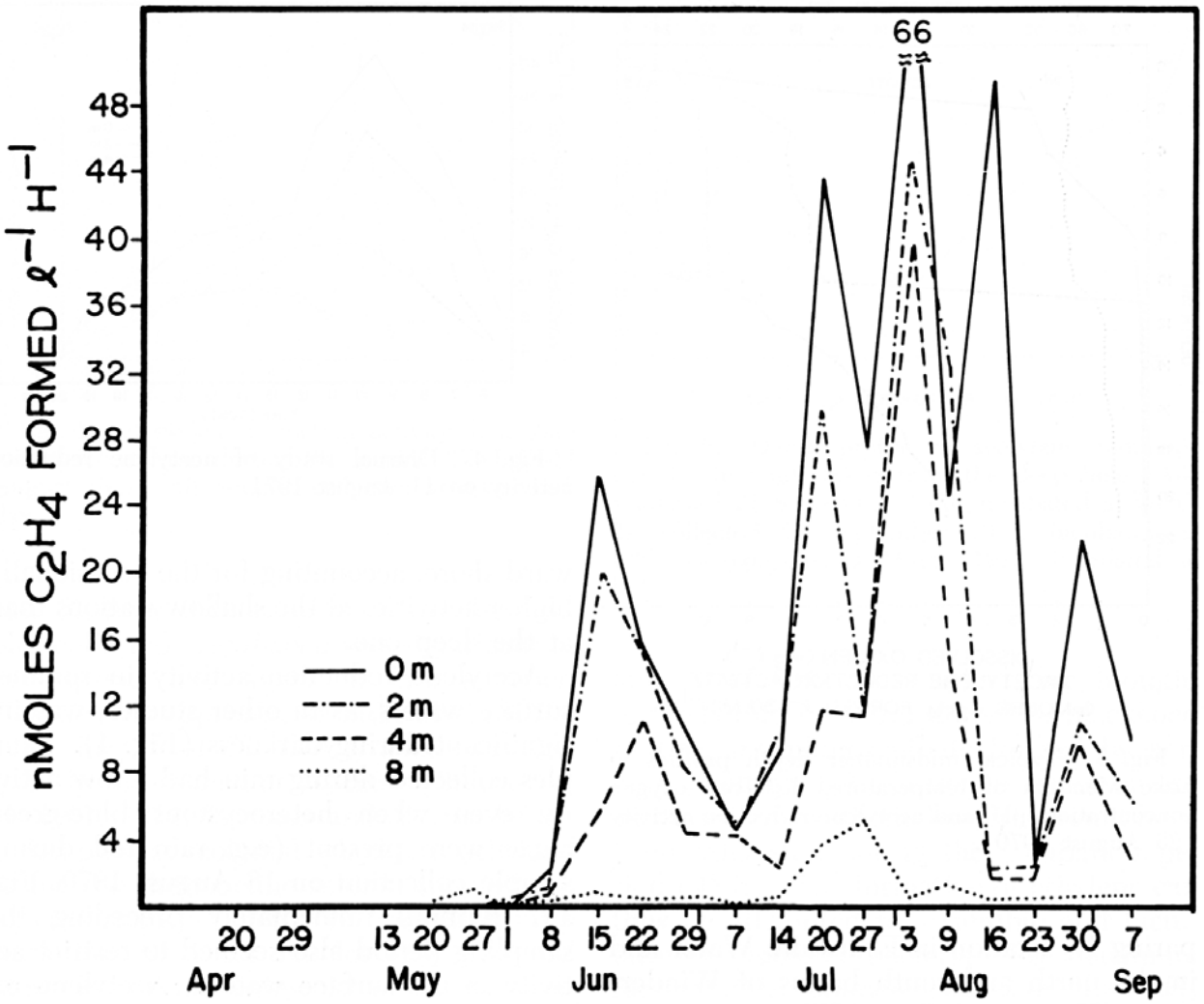

Fig. 2. Acetylene reduction activity at the deep station during April–September 1971.

Soluble reactive and total phosphate exhibited inverse clinograde profiles during stratification and uniformity during circulation; ammonia profiles paralleled phosphorus distributions. The nitrate profile was positively heterograde during early summer stratification, reaching a maximum immediately below the thermocline; during winter stagnation, nitrate concentrations were inversely clinograde. Organic nitrogen concentrations were fairly uniform at 0.6-0.7 mg N except during phytoplankton blooms. Dissolved oxygen concentrations were clinograde during stratification and relatively uniform during mixing. Dissolved oxygen supersaturations coincided with peaks of algal activity.

Acetylene reduction activity—Once thermal stratification was established in late spring, nutrient concentrations dropped and heterocystous blue-green algae appeared. From this time till fall turnover, acetylene reduction was consistently detected (Figs. 1 and 2). During fall, winter, and spring, when nutrients were more abundant, there was no acetylene reduction activity, even though blue-green algae without heterocysts, mainly *Aphanizomenon*, were present.

Peaks in acetylene reduction activity at the deep station (Figs. 1 and 2) were generally paralleled by maxima at the two shallow stations. However, absolute values were seldom the same. Multiple regression analysis demonstrated no dependence of C_2H_2 reduction activity on sampling site in Lake Mendota. Sampling site verged on statistical significance in a study comparing N_2 fixation in

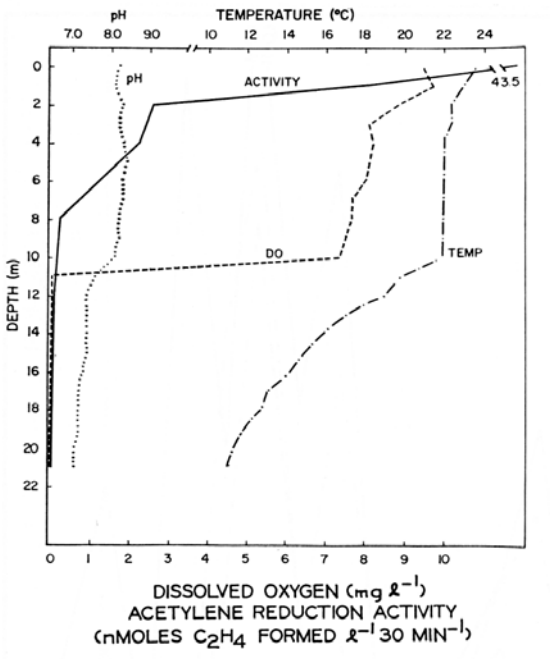

Fig. 3. Typical midsummer depth profiles in Lake Mendota of temperature, dissolved oxygen concentration, pH, and acetylene reduction activity (25 August 1970).

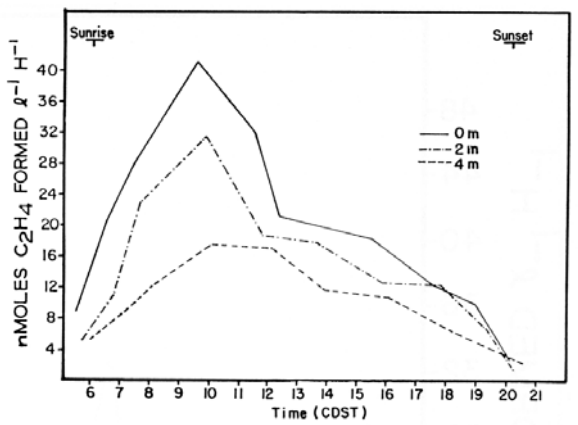

Fig. 4. Diurnal study of acetylene reduction activity on 11 August 1971.

Esthwaite Water and in the north and south basins of Windermere (Horne and Fogg 1970).

Activity was usually highest at the surface; the maximum was sometimes at 1 or 2 m, especially at the shallow stations. Within a water column, maximal acetylene reduction activities coincided with temperature, dissolved oxygen, and pH maxima (Fig. 3). Of these four variables, acetylene reduction decreased most rapidly with increasing depth and was virtually zero by 8 m. The other three parameters were fairly uniform with depth above the thermocline and then fell off sharply at the metalimnion.

Differences in activities between deep and shallow stations appeared occasionally. For example, relative maxima in the subsurface waters at the deep station on 13 July 1970 (Fig. 1) were not evident until 21 July at the shallow stations. The maximum on 16 August 1971 (Fig. 2) was a midlake phenomenon only. Wind-driven currents at times carried algal blooms toward shore, accounting for the occasionally higher activities at the shallow stations than at the deep one.

Acetylene reduction activity in summer surface waters, as in other studies, was insignificant during darkness (Fig. 4). Samples collected during rain had a low activity even when heterocystous blue-green algae were present (e.g. rain fell during sample collection on 18 August 1970, Fig. 1). Rainfall immediately preceding the sampling period also seemed to restrict activity in the surface waters. Acetylene reduction was high around noon on 30 and 31 July 1971 (Fig. 5). Rain on the morning of 1 August was followed by low activities at midday. Precipitation and runoff may contribute enough nitrogen to the lake to alleviate temporarily the dearth of algal nutrients and to decrease the need to reduce N_2, for a combined nitrogen source.

There are several similarities in the data from 1970 and 1971 (Figs. 1 and 2). In both years, within a week after summer stratification began there was a sharp rise in planktonic activity until about 16 June, then activity fell to the end of June only to rise again in the first 2 weeks of July. Values were maximal each summer in July, followed by a minimum in mid-August and a final spurt of activity at the end of August. These July and August patterns were also observed in 1968 and 1969 (Rusness and Burris 1970; Stewart et al., 1971). *Aphanizomenon* was

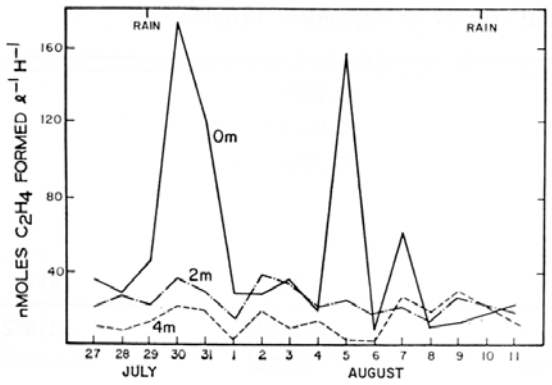

Fig. 5. Acetylene reduction activity at a shallow-water station during the period 27 July–11 August 1971. All samples collected at noon each day.

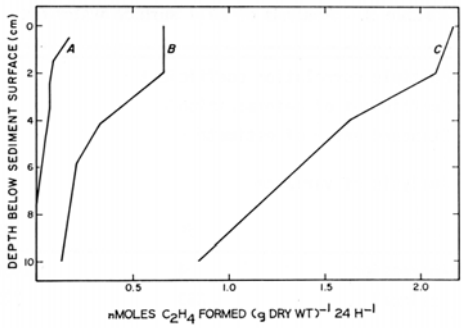

Fig. 6. Depth profile of acetylene reduction activity in muds taken from the deep hole. A—Collected 21 September 1971, incubated at 24°C; B—collected 21 September 1971, incubated at 4°C; C—collected 4 March 1971, incubated at 4°C.

usually the predominant N_2-fixer, if there were any, in the samples collected. *Anabaena* was significant in late spring and again in late summer. *Gloeotrichia* appeared sporadically in 1970-1971; in 1967-1969, *Gloeotrichia* was frequently the dominant N_2-fixer (Stewart et al. 1967, 1971; Rusness and Burris 1970).

Samples of colonial plankton collected below the thermocline seldom showed significant reduction activity. Reduction rates were less than 0.4 nmoles C_2H_4 formed liter^{-1} h^{-1} at and below 12 m during summer stratification in both 1970 and 1971. For a fleeting period in late September and early October 1970, reduction rates were measurable below 10 m at levels where O_2 had penetrated, but activity quickly fell to zero as mixing was completed, even though blue-green algal blooms of species able to fix nitrogen were present through November. It is noteworthy that the *Aphanizomenon* in these autumnal blooms lacked heterocysts.

Incubating sediment samples for 1-2 days produced consistent detectable C_2H_2 reduction activities. Rice and Paul (1971) found acetylene reduction occurring at a constant rate between 10 and 48 h in continuously wetted waterlogged soils, as did Bristow (1974) in unamended pond sediments. Activity, on a per gram dry weight basis, was highest in Mendota at the sediment surface and decreased with depth (Fig. 6). While the muds were exposed to a minimal amount of light as samples were brought to the surface and transferred to blackened bottles, any photosynthate formed by aerobic photosynthetic nitrogen fixers or anaerobic photosynthetic bacteria would have been exhausted during the incubation period (48 h). The formation of ethylene can thus be attributed to anaerobic and facultatively anaerobic bacteria. The maximum rate noted in sediments was 2 nmoles C_2H_4 formed (g dry wt)$^{-1}$ 24 h^{-1}. Although this is much lower than maximum rates measured in the phytoplankton during summer, such activities may be significant over an annual cycle. Rice and Paul (1971) found that the amount of acetylene reduced in soil systems was a measure of potential fixation activity while only ^{15}N-N_2 reduction represented true activity.

During summer stratification surface water from which the colonial algae had been removed showed no activity; fixation was associated with the algae retained by the plankton net. Similar samples collected below the thermocline and incubated in the dark achieved a maximum activity of 0.35 nmoles C_2H_4 formed per liter filtered water per hour after removal of colonial algae.

Regression analyses—Many factors have been shown to influence nitrogen fixation rates directly under laboratory conditions, but there have been few

Table 2. Analysis of 1971 surface water data using activity as dependent variable.

Multiple correlation coefficient	0.7697
Coefficient of determination	0.5824
Standard error of estimate	5.3446

Analysis of variance

	df	SS	MS	F ratio
Regression	4	10502.770	2625.692	91.922
Residual	253	7226.809	28.564	

Variables in equation

Variable	Regression coefficient	SE	F ratio*
Constant	0.6250		
Depth	- 0.4619	0.1539	9.0080
Algal N	21.8609	3.2318	45.7552
Tot het	0.00008	0.00002	15.3248
N_2 fixr	7.0×10^{-7}	1.0×10^{-8}	29.5738

*All numbers significant to the 1% level.

studies of the factors in the natural environment related to N_2 fixation; notable exceptions include the work of Horne and Fogg (1970) and Horne and Goldman (1972). Our data were analyzed by stepwise multiple regression analysis to identify relationships between environmental factors and acetylene reduction activities in Lake Mendota.

Only 46% of the total variation in acetylene reduction activity during 1971 in Lake Mendota surface waters (0-10 m) could be explained by the independent variables suggested by Horne and Fogg (1970): sampling site, depth, temperature, nitrate, and total organic-N. We expanded this list of independent variables to include dissolved oxygen, soluble reactive phosphate, total phosphate, ammonia, algal nitrogen (that caught on a No. 25 silk net), pH, light, time of day, and cell concentration. Preliminary computations showed that removal of total phosphate from the set of independent variables did not alter the final regression equation; therefore, soluble reactive phosphate was used because it probably more nearly represents the phosphorus immediately available to the phytoplankton. Nitrate yielded a regression equation with a lower coefficient of determination than did ammonia; NH_3 concentrations were used in the final computations.

With acetylene reduction activity as dependent variable, 58% of the variation in the surface waters in 1971 was explained by depth and by parameters related to algae (Table 2). With the $\log_{10}$ of activity as dependent variable, 83% of the variation was accounted for (Table 3), which shows the advantage of using a logarithmic expression.

Bottom water (10-23 m) data from 1971 yielded no satisfactory regression equation: only a third of the variation was explained by the variables listed. Statistical errors caused by operating near detection limits, as well as differences in the previous history of bottom water organisms potentially able to fix N_2, probably contributed to the fact that patterns were not readily distinguishable. Combination of 1970 and 1971 data also yielded an equation in which no more than a third of the bottom water variation was explained.

Table 3. Analysis of 1971 surface water data using the logarithm of activity as dependent variable.

Multiple correlation coefficient	0.9086
Coefficient of determination	0.8256
Standard error of estimate	0.4360

Analysis of variance

	df	SS	MS	F ratio
Regression	6	219.446	36.574	192.429
Residual	244	46.376	0.190	

Variables in equation

Variable	Regression coefficient	SE	F ratio*
Constant	- 8.4648		
Temp	0.1005	0.0076	174.2585
DO	0.0426	0.0162	6.8879
Algal N	0.7508	0.2594	8.3815
pH	0.6359	0.1498	18.0190
Org N	0.4973	0.1298	14.6711
Tot het	0.00001	0.000002	37.5749

*All numbers significant to the 1% level.

To see whether further relationships would emerge from a longer period of record, we combined acetylene reduction data in the surface 10 m for 1970 with those from 1971. The independent variables remained the same, with one exception: the total percent N_2-fixers and percent heterocysts in the total population were substituted for cells per liter total N_2-fixers and heterocysts. Again, the logarithmic expression proved superior, for 79% of the variation was accounted for when the logarithm of activity was dependent variable (Table 4) compared with 48% with untransformed values for acetylene reduction activity.

The negative regression coefficient of depth with activity noted in Table 2 was anticipated not only from the field data of Horne and Fogg (1970) but also from the fact that light decreases with depth. Although algal nitrogen was correlated positively with acetylene reduction activity, we cannot infer from this mathematical relation that algal nitrogen regulates C_2H_2 reduction. However, since algal N is one measure of biomass, we would expect some sort of direct correspondence between biomass and N_2 fixation when N_2-fixers constitute a large portion of the organic matter. The total counts of heterocysts per liter and the total number of (potential) N_2-fixers were also positively related to reduction activity, as noted previously by Horne and Goldman (1972) and Horne et al. (1972). The signs of regression coefficients related to algae (algal N, heterocysts, N_2-fixers) are expected to be positive if N_2-fixers such as *Aphanizomenon, Gloeotrichia,* and *Anabaena* are responsible for nitrogen fixation in the samples and if heterocysts are the site of fixation in aerobic blue-green algae.

After the dependent variable was expressed as the logarithm of activity (Table 3), several more independent variables appeared in the regression equation and the coefficient of determination increased to 83%. Temperature had a positive relationship, as demonstrated by Stewart (1966), for within a biological temperature range, N_2 fixation increases with increasing temperature. As with the

Table 4. Analysis of all surface water data, collected in both 1970 and 1971, using the logarithm of activity as dependent variable.

Multiple correlation coefficient	0.8914
Coefficient of determination	0.7946
Standard error of estimate	0.4201

Analysis of variance

	df	SS	MS	F ratio
Regression	9	314.869	34.985	198.235
Residual	461	81.360	0.176	

Variables in equation

Variable	Regression coefficient	SE	F ratio*
Constant	- 7.3317		
Depth	- 0.0721	0.0116	38.3346
Temp	0.0712	0.0058	152.7741
Heter %	0.2657	0.0585	20.6297
Ortho P	- 2.5662	1.0204	6.3249
Algal N	1.6185	0.1530	111.9018
pH	0.6611	0.1030	41.1866
μ-Amp	- 0.00003	0.00001	16.4218
Time	- 0.0304	0.0096	9.9971
N_2 fixr %	0.0053	0.0009	35.3263

*All numbers significant to the 1% level.

nonlogarithrnic dependent variable of Table 2, algal nitrogen and heterocysts were related positively to activity. Organic nitrogen had a positive relationship to reduction activity, as noted in previous ^{15}N-N_2 field studies (Horne and Fogg 1970; Dugdale and Dugdale 1962). Laboratory studies (Stewart 1966) have related N_2 fixation to pH; in our study C_2H_2 reduction and pH had a positive association, not entirely anticipated for a system as undefined as Lake Mendota. Certainly some relation to pH was apparent in the patterns, exemplified by Fig. 3, which were repeated week after week. On the basis of the work of Stewart and Pearson (1970), the positive regression coefficient with dissolved oxygen concentrations was unexpected. A mathematical association of this sort does not identify a biological cause-and-effect relationship. However, the parallels between reduction activity and dissolved oxygen are like those between activity and pH (Fig. 3).

Several of the independent variables singled out in the regression equations of 1971 data (Tables 2 and 3) were also apparent when the combined data of 1970-1971 were analyzed with the logarithm of activity as dependent variable (Table 4). The inclusion in these equations of algal N, heterocysts, nitrogen-fixers, pH, temperature, and depth, as well as the signs of the regression coefficients, have been discussed above. Table 4 shows additionally the appearance of light, soluble reactive phosphate, and time. Because photosynthesis provides energy for N_2-reducing activity, acetylene reduction might be expected to vary directly with light; however, the regression coefficient in Table 4 is negative.

Probably light takes the sign associated with depth since the amount of light is a direct function of water depth. This example of substitutability of variables shows that when a portion of the variation of the dependent variable can be accounted for by either of two variables (light or depth), then the contribution of either one to the regression is smaller when the other variable is in the equation than when it is not.

The rationale for the negative regression coefficient for soluble phosphorus is also not obvious. Stewart et al. (1970) found that when new phosphorus was added to phosphorus-starved laboratory cultures of N_2 fixers, acetylene reduction activity increased. The negative relation in Table 4 is not proof that in nature higher phosphate levels inhibit fixation, but may reflect the fact that all the N_2-fixers may be near the surface due to light requirements. In eutrophic Lake Mendota, phosphorus increases with depth: the statistical relationship between C_2H_2 reduction and phosphorus may he fortuitous, since algal light requirements are only indirectly related to phosphorus concentrations. Time is the final variable of interest in Table 4. Since time is linked with various diurnal meteorological changes, the negative regression coefficient simply indicates that acetylene reduction activity is highest near midday.

Discussion

Although the variables selected by Horne and Fogg (1970) were insufficient for Lake Mendota, Horne and Fogg did point out the value of using the logarithm of nitrogen fixation as dependent variable. The logarithmic function is especially pertinent where the range of fixation values is large (A. J. Horne personal communication), as in an annual cycle of Lake Mendota. When additional variables were included, 58% of the variation in the surface waters during 1971 was explained by depth, algal nitrogen, cell composition and heterocysts Table 2). The algal N concentration was the most significant independent variable in the equation. Population parameters—total number of N fixers, total concentration of heterocysts—were next in order of significance, as Horne and Goldman (1972) found in Clear Lake, California. Depth, also significant in Table 2, may act as a summary term to encompass a number of depth-dependent variables in a stratified eutrophic lake. In Table 2 only 58% of the variation is explained: a logarithmic dependent variable (Table 3) accounts for 83% of the variation. Two groups of variables appear in Table 3—those that vary with depth (temperature, dissolved oxygen, pH) and those that are not closely connected with depth but do correlate with the plankton (algal N, organic N, and heterocysts). The greater fraction of the variation explained by using a logarithmic dependent variable emphasizes the influence of parameters exponentially related to temperature and depth (such as light) and linearly related to cell populations and the oxygen accompanying them.

In the regression equations developed above, the relation between heterocysts and acetylene reduction activity was significant and positive. Higher temperatures accompanied higher acetylene reduction activities. The depth at which the sample was collected was negatively correlated with acetylene reduction. Dissolved oxygen had a positive regression coefficient. This is the first environmental study in which pH has appeared to be related to acetylene reduction. Dissolved phosphorus and nitrogen species were generally excluded from the regression equations.

We cannot unequivocally assign causes and effects to the independent variables included in these regression equations. For example, dissolved oxygen and pH may be indications only of the photosynthetic activity that provides carbon skeletons and energy to the nitrogenase. Elevated pH and oxygen concentrations may be the effect of the presence of a photosynthesizing population rather than the cause of the increased acetylene reduction.

One weakness of the entire computer analysis of the data in these studies is that historical effects cannot be considered. Reynolds (1972) concluded that a knowledge of the previous history of the algae was

Table 5. Summary of measured acetylene reduction and calculated N_2 fixation in the pelagic zone.

Period	Depth Interval (m)	Ethylene formed (kmoles)	Nitrogen fixed (kg)
4 Jun-22 Sep 1970	0-10	3,538	33,000
4 Jun-7 Dec 1970	0-10	3,747	34,900
4 Jun-22 Sep 1970	10-23	37	350
4 Jun-7 Dec 1970	10-23	67	630
14 Apr-1 Jun 1971	0-10	19	170
14 Apr-14 Sep 1971	0-10	3,466	32,500
14 Apr-1 Jun 1971	10-23	8	74
14 Apr-14 Sep 1971	10-23	35	330

Table 6. Estimated annual contributions of nitrogen fixation in Lake Mendota during 1970–1971.

Source	1970	1971
Surface water algae	34,900 kg	32,400 kg
Bottom water algae	600	300
Surface water filtrate	0	0
Bottom water filtrate	1,700	1,700
Anoxic sediments	1,300	1,300
Sum	38,500 kg	37,700 kg

needed to describe blue-green algal blooms. Each sample collected represents only the acetylene reduction by that sample at point P and at time T: there is no way to ascertain what influences on the algae at some time T – *t* may have determined the activity at T. Nor is it known what influences on the N–fixers at some point *p* have gone to determine the observed acetylene reduction by the same fixers at point P.

If historical effects could be included in this analysis by incorporating some kind of lag function, then we might see the influence of changes in nutrient concentrations on acetylene reduction. Detailed data would be necessary to define a lag function. An additional consideration is that blue-green algae can take up more phosphorus than they can immediately metabolize (luxury consumption). We would need to develop a mathematical expression that includes luxury consumption as well as defining lag functions of the historical influence of nutrient concentrations.

Calculations of the significance of nitrogen fixation are difficult because of vertical, horizontal, and temporal inhomogeneities of species composition, biomass, and activity. The calculations in Table 5 are approximate. In 1970, 95% of the nitrogen fixation in the surface waters (0-10 m) occurred in 110 days during summer stratification (Tables 5 and 6). Activities in the bottom waters were much lower than in the surface waters. Less than two-thirds of the total fixation by net plankton in deep waters took place during summer, with 32% in the period 22 September-6 October 1970, at the time of fall turnover, when oxygen reached greater depths and the bottom waters attained their maximum annual temperatures of 14-16°C. Although the relative fraction of reduction in the deep waters was greatest during turnover, it was relatively unimportant to the lake since large concentrations of NH_3 from the bottom waters were being mixed into the entire lake at the same time. Just before fall overturn in 1970, hypolimnetic concentrations of NH_3-N ranged from 0.3 mg liter^{-1} at the top of the hypolimnion to 1.0 at the bottom, while comparable figures for 1971 were 0.8-2.5.

In 1971, fixation before onset of stratification and blue-green algal blooms was negligible (Table 5). Again, more than 95% of the nitrogen fixation in the surface waters occurred during stratification. Acetylene reduction activity in the bottom waters was about 1% of that in the top 10 m. In both 1970 and 1971, nitrogen fixation in the surface waters during stratification comprised 85% of the total estimated N_2 fixation.

Stewart et al. (1971) calculated that, in the 51 days in 1969 in which activity was detectable in Lake Mendota, 9,456 kg of N_2 (or 2.4 kg ha^{-1} yr^{-1}) were reduced in the top 5 m. In the top 6 m in 1970, 30,300 kg of N_2 (7.5 kg ha^{-1} yr $^{-1}$) were reduced from 4 June-11 September, while in 1971, from 1 June-14 September, 30,700 kg of N_2 (7.4 kg ha^{-1} yr^{-1}) were

Table 7. Estimated sources of nitrogen for Lake Mendota.

Source	Annual Contribution (kg)	Reference
Waste water	313	Lee 1966
Urban runoff	33,000	Sonzogni and Lee 1974
Rural runoff	236,300	Sonzogni and Lee 1974
Rain and snow on lake surface	31,300	Sonzogni and Lee 1974
Dry fallout on lake surface	61,300	Sonzogni and Lee 1974
Groundwater	77,700	Sonzogni and Lee 1974
Base flow	61,400*	Sonzogni and Lee 1974
Marsh and littoral areas	0	Sonzogni and Lee 1974
Woodland	0	Sonzogni and Lee 1974
Nitrogen fixation	38,100	This study

*Insufficient information was available to estimate the potential significance of nitrogen fixation in marshes and the littoral area of Lake Mendota. Lonergan (1973) has shown that nitrogen fixation in marshes may be significant. However, for the purposes of these calculations it was assumed to be zero since data are unavailable to show whether nitrogen fixed in the marsh can be released to the lake.

reduced in these surface waters. The reason for the difference between 1969 and 1970-1971 is not obvious from published data. *Microcystis*, a nonnitrogen-fixing blue-green alga, predominated for most of summer 1969 (G. P. Fitzgerald personal communication); during 1970 and 1971, *Aphanizomenon* and *Anabaena* usually predominated in our samples. The disparity probably lies in the difference in algal dominants.

Nitrogen fixation by bacteria in the surface waters was not detected in either 1970 or 1971. Bacterial nitrogen fixation in the bottom waters amounted to less than 5% of the surface water activity (Table 6). The amount of N_2 fixed in the bottom sediments was about 4% of the N_2 fixed in the surface waters. These additions to the combined nitrogen pool of the bottom waters may be relatively unimportant on a whole-lake basis. Since most of this fixation occurs during periods of the year when circulation is minimal, the newly formed combined nitrogen has little chance to reach the nitrogen-depleted surface waters. (For example, NH_3-N and NO_3-N each run ≤ 0.06 mg liter^{-1} in the summer epilimnion.) In addition, denitrification occurs contemporaneously in the anaerobic waters (Brezonik and Lee 1968) and the sediments (Keeney et al. 1971) of the hypolimnion. Thus the summer bottom waters act as a sink for nitrogen from N_2 fixation and denitrification. Newly formed combined nitrogen cannot be mixed to a significant extent into the epilimnion, and combined nitrogen is converted to N_2–available to very few microorganisms and plants.

Excluding the marsh and littoral values, the amount of combined nitrogen furnished by nitrogen fixation (Table 6) forms a relatively small portion of the total nitrogen entering Mendota each year (Table 7). For the purpose of computation, the values developed by Sonzogni and Lee (1974) were used to calculate the significance of N_2 fixation in the nitrogen budget. Rural runoff, groundwater, and dry fallout constitute the major sources of nitrogen to Lake Mendota. The contribution of groundwater is uncertain due to the difficulty in estimating groundwater influx to the lake and the extent of denitrification as groundwater enters the lake. The data in Table 7 show that nitrogen fixation contributes about 7% of the total combined nitrogen entering Lake Mendota each year.

Thus the contribution of nitrogen fixation to the nitrogen budget of Lake Mendota is much higher than the 0.5% of Windermere (Horne and Fogg 1970), much lower than the 30-40% of Lake George, Uganda (Horne and Viner 1971), Clear Lake, California (Horne and Goldman 1972), and Lake Erken, Sweden (Granhall and Lundgren 1971), and similar to that for Lake Tschornoje, Russia (Fogg and Horne 1967).

One previous calculation of fixation in Mendota (Lee 1966) based on scant data (Goering 1962) was 0.4%. Our study supports the estimate of 5-10% by G. P. Fitzgerald (*cited in* Horne and Viner 1971).

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