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Nitrogen fixation in Clear Lake, California. 4. Diel studies on *Aphanizomenon* and *Anabaena* blooms[1]

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Abstract

Day and night measurements of N_2 fixation (as acetylene reduction) were made during spring blooms of *Aphanizomenon flos-aquae* and two autumn blooms of *Anabaena* spp. From 9 to 23% of the 24-h fixation occurred between 1100 and 1300 hours. Nitrogen fixation in spring showed complex, physically shallow but optically deep and mobile subsurface peaks of nitrogenase activity, which were totally unrelated to *Aphanizomenon* biomass but may have been due to diel changes in light penetrating the relatively clear water. Nocturnal fixation was uniformly distributed with depth and accounted for ⅕ to ⅓ of daylight fixation. In more turbid autumn waters, the pattern of N_2 fixation for *Anabaena* blooms was simpler, with a surface (or near-surface) peak decreasing with depth. Nocturnal fixation was more uniformly distributed with depth. The difference in fixation patterns between the two species is attributable to the interactions of oxygen with the nitrogenase enzyme system. The diel changes in nitrogenase activity suggest a need to establish whether the precursors of nitrogenase accumulate in an oxygen-stable form.

Nitrogen fixation requires more energy than any other biological process. Thus it should be highly light-dependent in lakes, since only photosynthetic organisms are quantitatively significant in lake N_2 fixation (Horne 1975*a*; Fogg et al. 1973). However, phytoplanktonic N_2 fixation may occur at low light levels and even in total darkness (Horne and Fogg 1970; Duong 1972; Vanderhoef et al. 1975; Burris and Peterson 1978). Quantities fixed are generally but not always low; there are disadvantages to high photosynthesis, particularly the onset of photorespiration which successfully competes with N_2 fixation for available energy (Horne 1975*b*). Photorespiration is enhanced by the afternoon low CO_2 and high O_2 levels, which are possible in planktonic algal colonies and inevitable inside gelatinous *Nostoc* communities in streams, despite the constant O_2 and CO_2 environment provided by the well aerated stream water (Horne and Carmiggelt 1975).

In the more open "flake" association of *Aphanizomenon* or coils of *Anabaena*, photorespiration may not be inevitable since the algae can regulate their position in the water column (Walsby 1972; Reynolds 1972, 1973). Thus there may be less need for nocturnal N_2 fixation as afternoon activity should be less depressed—at least in lakes with several meters of photic zone. The work reported here was designed to test this hypothesis and to provide a quantitative method of calculating daily and hence annual N_2 fixation amounts using incubation normally for only a couple of hours at midday.

A further reason for carrying out five diel studies in both blooms spread over 3 years was to improve the meaningfulness of the data. Generalizations based on any one of the diel studies reported here would not be significant without guidance from the other four.

I thank C. J. W. Carmiggelt and P. Kellar for technical assistance.

Methods

Samples were usually collected at depths of 0, 0.5, 2.0, 3.0, and 4.0 m with an opaque Van Dorn bottle. The photic zone is generally between 1 and 4 m in Clear Lake, shallowest in autumn and winter. Since the main purpose of the ex-

[1] This study was supported by the Clear Lake Algal Research Unit, Lake County, California, and the California Department of Water Resources.

Table 1. Diel variation in nutrients (in $\mu g \cdot liter^{-1}$) during an *Aphanizomenon* bloom, 21–22 June 1972.

Period	Time	PO_4-P	NH_4-N	NO_3-N
3	0930	42	11	14
5	1500	45	45	1
7	2200	43	1	1
9	0600	10	15	1

periments was to document the diel changes in N_2 fixation, relatively few other measurements were made. Collections were taken at intervals of a few hours either from dawn to dusk (3 days) or over a 36-h period (2 days) during spring 1970, 1971, 1972 (*Aphanizomenon* bloom), and autumn 1970, 1971 (*Anabaena* bloom). Details of methods were given by Horne and Goldman (1972). Measurements were made of N_2 fixation as acetylene reduction, carbon fixation, chlorophyll *a*, general chemistry, algal species, biomass and heterocyst counts, water transparency as Secchi depths, solar energy with time using a Belfort pyrheliometer, and estimated wind speed and direction. Algae were counted in spring 1972 at each depth sampled for all nine periods over 36 h, to distinguish the effects of *Aphanizomenon* from those of other less abundant algae that may have contributed disproportionately to photosynthesis and nutrient uptake (Watt 1971; Dozier 1976; Stull et al. 1973). Such influence is unlikely in eutrophic lakes where the most common algae physically can dominate the struggle to gain the most favorable light climate.

Results

All studies were carried out at the height of N_2 fixation activities in Upper Arm, which comprises 70% of the area of the lake. There were many similarities and differences between the three spring, *Aphanizomenon*-dominated blooms and the two autumn, *Anabaena*-dominated blooms. The most convenient way of considering the results is to separate them into those of spring and autumn.

Spring Aphanizomenon *blooms*—In spring and early summer clouds are rare and some near-surface thermal stratification re-forms each day. Afternoon winds usually destroyed thermal stratification for at least part of each day on which studies were carried out and the regular stirring mixed such nutrients as were available from the sediments into the photic zone. Nevertheless, biologically important nutrients were scarce, having been depleted by the first nitrate-fueled stages of the massive *Aphanizomenon* bloom. For example, in June 1972 nitrate ranged from 1–14 $\mu g \cdot liter^{-1}$, ammonium from 1–45 $\mu g \cdot liter^{-1}$, and phosphate from 10–45 $\mu g \cdot liter^{-1}$ (Table 1). The imbalance between P and N produced a severe nitrogen stress and was the reason for the N_2 fixation at this time (Horne and Goldman 1972).

An overall view of the diurnal changes in N_2 fixation and chlorophyll *a* in the water column for the three seasons (1970–1972) is given in Fig. 1. In general chlorophyll *a*, which was contained mainly in *Aphanizomenon*, showed no regular pattern but fluctuated considerably due to patchiness (Wrigley and Horne 1975) and vertical migration (Reynolds and Walsby 1975). Nitrogen fixation showed a broadly constant pattern in all 3 years but considerable variation within that general trend. Early sunlight around 0700–0800 hours produced only a small increase in N_2 fixation over nocturnal rates, but by midmorning rates were almost as high as at any other time of day. Early morning activity was definitely dependent on sunlight, since a cloudy start to the day depressed fixation below normal nocturnal levels. Presumably nocturnal fixation after an abnormal (totally cloudy) day would be negligible (Horne 1975*b*). During the major part of the day activity was high and quite constant (200–600 μmol $C_2H_4 \cdot liter^{-1} \cdot h^{-1}$). It decreased at dusk, but not proportionately with illumination. Most unexpected at the time of the experiments was the nocturnal fixation activity which persisted all night, generally at moderately high levels. Relatively

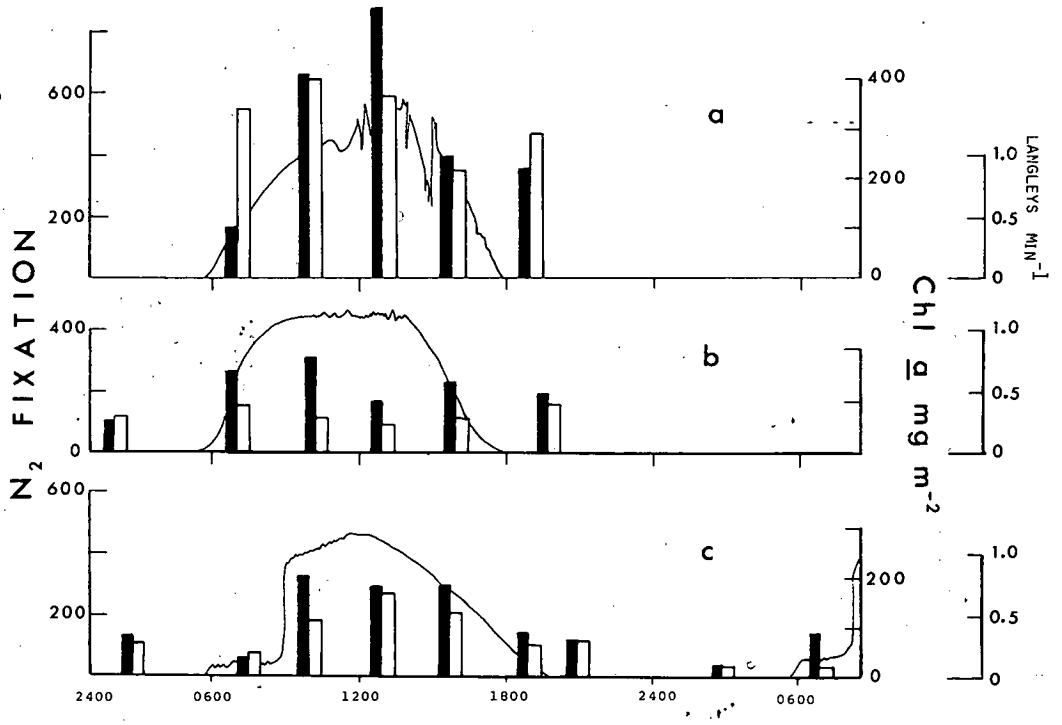

Fig. 1. Diel variations in N_2 fixation (as ethylene produced in μmol·liter^{-1}·h^{-1}), chlorophyll *a*, and sunlight at height of three spring *Aphanizomenon* blooms. a—26 June 1970; b—16 June 1971; c—21–22 June 1972. Solid bars are N_2 fixation and open bars are chlorophyll *a*, both expressed per unit of surface area for mixed water layer. Line represents solar radiation.

few samples (70) were incubated in total darkness, so the actual rates observed are best viewed as preliminary. In 1971 and 1972 fixation from 0100 to 0300 hours was a third to half that found during the 14 h of daylight. There is little possibility that such findings of dark ethylene reduction were due to methodological error, since the technique is quite standardized. Bottles containing algae but no acetylene addition never produced any detectable ethylene (peak height, 0.1). Nocturnal N_2 fixation added considerably to the annual nitrogen income of the lake because this bloom was long, lasting 2–3 months (Horne and Goldman 1972; Horne 1975*a*), and the dark values were consistent.

The rate of N_2 fixation expressed per unit of N_2-fixing algae gives a measure of efficiency. In this spring bloom, chlorophyll *a* represented *Aphanizomenon* well. Efficiency of N_2 fixation was remarkably consistent day and night and year to year (Table 2). Most values were between 2 and 4 μmol $C_2H_4 \cdot h^{-1} \cdot mg^{-1}$ Chl *a* and efficiency was highest in the afternoon. The constancy of nitrogenase activity was not directly due to the presence of a constant number of heterocysts because, if expressed as a percentage of vegetative cells, heterocysts amounted to 1.5% in 1970 and 1971 and only 0.5% in 1972. This represented photic zone average heterocyst concentrations per milliliter of about 5×10^2 (1970), 10^3 (1971), and 10^2 (1972).

When depth as well as time was considered, the patterns were surprisingly simple. Isopleths of N_2 fixation and chlorophyll are shown in Fig. 2. Thermal stratification occurred on all occasions, lasting from around noon until the onset

Table 2. Diel variations in efficiency of N_2 fixation (as μmol $C_2H_4 \cdot h^{-1} \cdot mg^{-1}$ Chl *a* for whole water column) in spring and autumn blooms 1970–1972. Column numbers represent time periods.

	Dark			0600–0900 hours			1900 hours	Dark 2400 hours		Dawn
	0	1	2	3	4	5	6	7	8	9
Jun '70	—	—	0.6	2.05	2.95	2.15	1.52	—	—	—
Jun '71	—	—	1.92	2.72	4.1	3.14	4.1	2.35	—	—
Jun '72	—	2.1	1.13	2.92	1.94	2.44	2.22	1.4	2.45	8.6
Sep '70	—	—	5.9	11.7	9.15	5.56	4.67	—	—	—
Sep '71	0.39	0.33	1.32	2.05	1.4	1.5	*	—	—	—

* Chl *a* not detected.

of darkness. This partially explains the changes in chlorophyll with depth, but since *Aphanizomenon* can regulate its buoyancy very effectively in Clear Lake the subsurface peaks were due more to gas vacuole collapse and synthesis than to turbulence. Nitrogen fixation exhibited much greater heterogeneity than did chlorophyll (Fig. 2). There was virtually no relationship between layers of chlorophyll and N_2 fixation in the spring bloom, although this was not true in autumn. In 1970, N_2 fixation fell linearly from a maximum of 425 nmol $\cdot$ liter$^{-1} \cdot h^{-1}$ to <100 at 4 m, just below the photic zone (Fig. 2A). The spring seasons of 1971 and 1972 were very different (Fig. 2B, C), illustrating my point that several diurnal studies should be made even if this takes several years to accomplish. In 1971 and 1972 there were distinct surface and subsurface peaks which changed position in the water column and in intensity of nitrogenase activity. Subsurface peaks in activity at 1 m (1971) and 2 m (1972) developed in the early morning, moving downward in the course of the day in 1971, but upward in 1972 when a surface peak was recorded for the first time in late afternoon (Fig. 2B, C). Midsummer in the Coast Range of northern California brings very intense radiation, even at only 400 m above sea level, and on many occasions has been observed to kill surface *Aphanizomenon* blooms, presumably by inducing photo-oxidative cellular destruction. The cells must strike a balance between optimal and lethal light. The ability of a species to be near the surface and thus to gain maximum energy for photosynthesis and the energetically costly N_2-fixing process is dependent on its ability to avoid death from irradiation, and this in turn appears to be dependent on both environmental and intracellular factors (Walsby pers. comm.). This notion is considered further below.

The peaks in N_2 fixation were caused not by a physical accumulation of *Aphanizomenon*, which was quite evenly distributed (Table 3, Fig. 2D–F), but presumably by a physiological condition allowing short-lived high rates. In the longest diurnal observation, in 1972, the subsurface nitrogenase activity peak at 2–3 m was very consistent, forming in midmorning, moving upward, disappearing altogether at night but forming again at the same depth next morning.

Many factors may play a role in determining ephemeral changes in the depth-time distribution of N_2 fixation; the most important are light, mixing, oxygen and carbon dioxide, nutrients, pH, and temperature. Incoming radiation was similar on all occasions (Fig. 1) and only the amount of light-absorbing material influenced the depth for optimal photosynthesis, which was normally between 10 and 30% I_0. The photic zone was much shallower in 1970 (Secchi depth, 85–108 cm) than in 1971 (84–123 cm) or 1972 (100–325 cm), but chlorophyll alone cannot explain the higher transparency of the 1972 season, when chlorophyll *a* was roughly twice that of 1971.

The extent of mixing in the water column was controlled by the depth of the

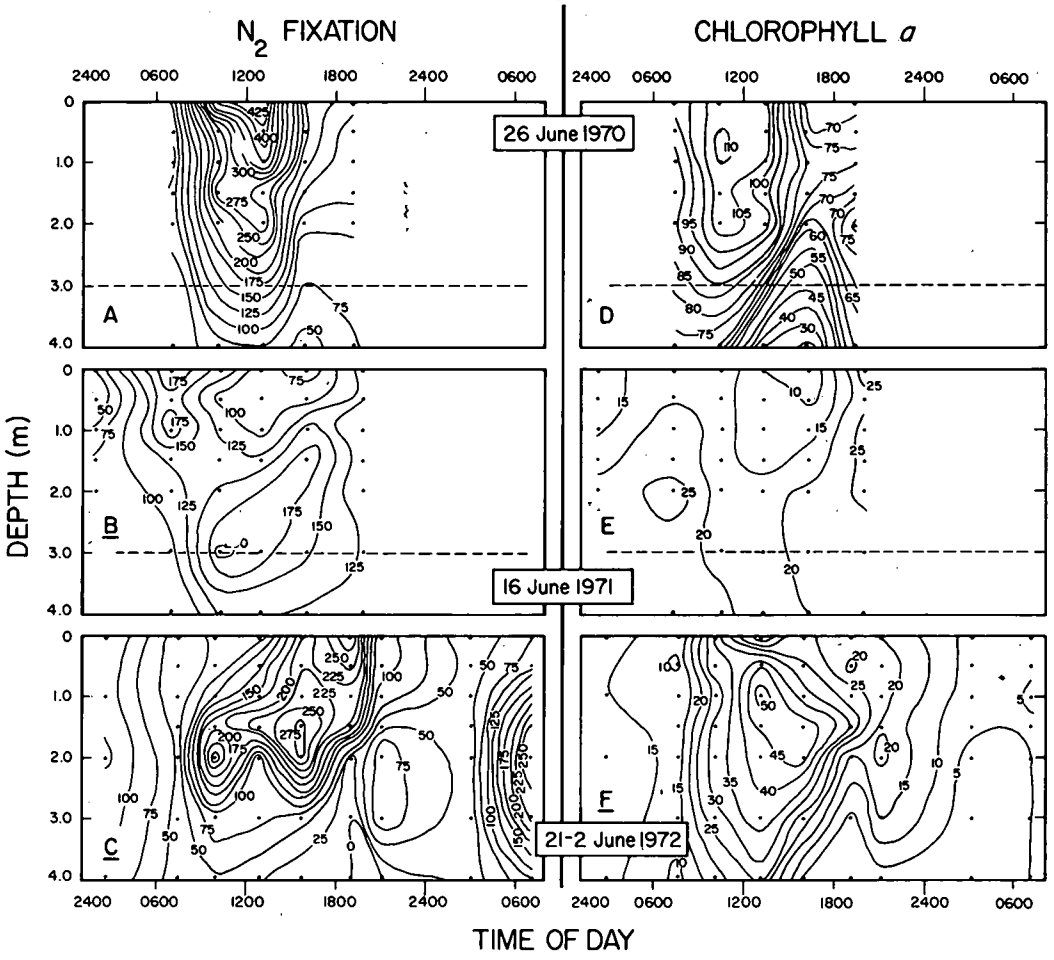

Fig. 2. Isopleths of N_2 fixation and chlorophyll *a* for three spring *Aphanizomenon* blooms, 1970–1972. Units same as Fig. 1. N_2 fixation assayed as ethylene produced; isopleths are for every 25 nmol ethylene·liter^{-1}·h^{-1}. Chlorophyll *a* isopleths every 5 μg·liter^{-1}. Horizontal line(s) approximates photosynthetic compensation point in 1970 and 1971, but is below 4 m in 1972.

temporary thermocline produced in midmorning while the lake was calm. The exact depth of mixing changes with time and is difficult to establish, since small relative increases at these high absolute temperatures may have produced strong density gradients. Stratification lasted longer in the afternoon in 1972 than in the previous years and the consequent lack of mixing in the upper meter probably inhibited photosynthesis and N_2 fixation. Certainly self-shading was not involved, since the surface maxima for the highest and lowest chlorophyll values varied by a factor of 5 and did not correspond to the intermediate chlorophyll levels. Oxygen levels exceeded saturation in all diurnal observations (Table 4) but were not particularly high in spring. Significant CO_2 depletion is not often evident in the moderately hard water of Clear Lake and has never been detected in our frequent routine analyses. Since spring 1972 pH values have hovered between 8 and 9 (Table 5), and CO_2 limitation has been transient or more likely

Table 3. Diel variations in *Aphanizomenon* biomass ($\mu^3 \times 10^6$) and heterocyst numbers (•), with depth at height of spring bloom 1972; underlined values are maxima for each time period. The samples from 0145 hours had 90 *Anabaena* heterocysts at 0 m and 68 at 4 m.

Depth (m)	1 0145	2 0630	3 0930	4 1230	5 1500	6 1800	7 2200	8 0200	9 0600
0	0.16*	15.2	43.9	39.7	34.6	17.0*	22*	21.8	9.3
	(0)	(678)	(2,373)	(2,508)	(2,102)	(1,536)	(1,040)	(1,220)	(678)
0.5	0.94	8.4	34.1	79	43	17.5*	21.3*	8.5*	8.6
	(23)	(429)	(1,627)	(4,882)	(2,634)	(1,964)	(1,107)	(610)	(542)
1.0	0.6	9.2	9.1	118.4*	50	27.8	29	11.3	10.8*
	(23)	(407)	542	(9,040)	(3,028)	(1,650)	(1,874)	(723)	(587)
1.5	0.5	12.1	44.3*	50.8*	73.5*	42.4	24.6	3.6*	11.4*
	(0)	(329)	(2,400?)	(3,118)	(4,700)	(2,645)	(1,401)	(226)	(497)
2.0	24.6*	12.0*	34.2*	54.3	67.5*	12.0	39.3	6.6*	8.8*
	(971)	(814)	(1,604)	(2,892)	(4,113)	(723)	(2,818)	(339)	(429)
3.0	—	11.8	35.6	28.4	13.5	2.4	14.4	10.6*	9.8
		(768)	(2,056)	(1,762)	(949)	(136)	(520)	(610)	(633)
4.0	0.22	9.24*	22.9*	25.5	2.8	1.0	0.68	11.7	11.3
	(0)	(542)	(1,174)	(1,717)	(136)	(23)	(45)	(678)	(723)
Σ Vol	27.02	77.9	224.1	396.1	284.9	120.1	151.28	74.1	70
Σ Het	(1,017)	(4,067)	(9,376) (11,776?)	(25,519)	(17,662)	(8,677)	(8,805)	(4,406)	(4,099)
max het at depth of max N_2 fix	yes?	yes	yes?	yes	yes	no	no	no	no

* Maximal values of nitrogenase activity.

Table 4. Diel temperature (°C) and dissolved oxygen (mg·liter^{-1}) profiles for fall and spring.

Depth (m)	Fall 1971				Spring 1972			
	2030		1130		1230		2300	
	Temp	DO	Temp	DO	Temp	DO	Temp	DO
0	29	20	26	18	26	9.6	24.2	9.9
0.5	28	20	26	16	25	9.9	24.2	10.3
1.0	27	8.9	25	6.9	24	12	24.2	10.4
1.5	25.5	—	23.5	2.8	23	9.7	24.1	10.4
2.0	24	—	23	1.1	22.5	8.9	24	10.2
3.0	23	—	22.5	0.4	22.5	8.8	23.7	9.7
4.0								
		Bottom			22	8.5	23.1	9.1
6.0					22	4.8	22.1	5.7
8.0					20	2.5	21.3	2.6

absent in spring. Lake temperatures during the spring diurnals varied from 20°C at the deeper points to 26°C at the surface near noon. Nitrogen fixation does not have a very large Q_{10} over this range (Fogg and Stewart 1968; Horne unpubl.), so the subsurface patches are unlikely to be due to simple temperature effects. Variations in major nutrients were measured during one diel study only, since N_2 fixation itself is not affected rapidly by small changes (Horne et al. 1979). Nevertheless it was surprising to find no overnight accumulation of scarce nutrients such as nitrate or ammonium, as apparently occurs in Lake George, Uganda (Ganf and Viner 1973). The only conclusions to be drawn are that all inorganic nitrogen species were always in short supply and phosphate was not (Table 1).

The most fascinating feature of the spring diel studies was the occurrence of nocturnal N_2 fixation (Fig. 1). Figure 2B and C illustrate the rapid breakdown of the surface and subsurface peaks of N_2 fixation with the onset of dusk to produce a uniform distribution of activity with depth. This was to be expected, since all the N_2 fixation would be accomplished by use of stored energy evenly spread

Table 5. Diel variations in major physical variables at surface of lake in spring.

	Variable*	1700	2030	0600–0800	0930–1030	1230–1330	1500–1700	1800–2000	2200–2400	0200–0400	0600–1800
Spring 1970	Secchi	—	—	90	85	85	108	—	—	—	—
Spring 1971	Temp	—	—	21	21.5	23	25	22	—	—	—
	Secchi	—	—	104	103	123	120	84	—	—	—
	$1\%I_0$	—	—	>300	275	275	250				
Spring 1972	Temp	—	—	23	26	26	23	24.4	24.2	23.8	23.8
	DO	—	—	9.4	8.6	9.6	11.2	11.9	9.9	9.7	9.0
	pH	—	—	—	8.4	—	8.5	—	8.8	—	—
	Secchi	—	—	325	175	100	138	150	0	0	—
	Turb	—	—	—	5.2	—	3.7	—	3.0	—	—
Fall 1970	Temp	—	—	19.5	20	21	24.5	21.5			
	Secchi	—	—	20	69	62	68	67	—	—	—
Fall 1971	Temp	29	28	24.5	23	26	30	32	—	—	—
	DO	>20	>20	13	16.2	17.7	18.3	>20			
	pH	10	9.4	9.4	9.3	9.3	9.7	9.7	—	—	—
	Secchi	40	0	45	34	39	50	45	—	—	—
	Turb	42	17	22	27	26	18	20	—	—	—

* Secchi depth, cm; temperature, °C; 1% I_0, cm; DO, mg·liter^{-1}; turbidity, JTU.

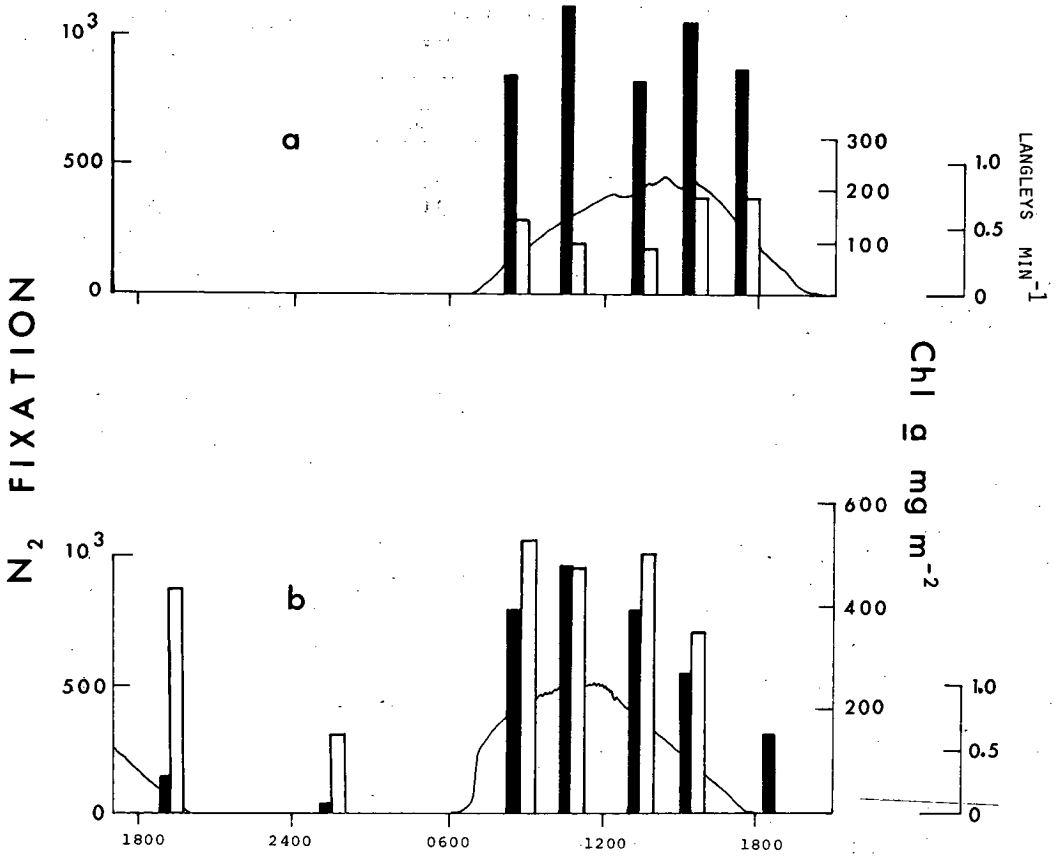

Fig. 3. As Fig 1, but for two autumn *Anabaena* blooms. a—30 September 1970; b—14 September 1971.

through the population following thermal destratification by late afternoon winds.

The spring 1972 diel study was carried out in slightly deeper water than in previous springs or in any autumn when the lake was shallower: the algae had between 6 and 8 m in which to move. The diel movements of active heterocyst-bearing *Aphanizomenon* populations, illustrated in Table 3, did not follow the generalized pattern proposed for blue-green algae in lakes of this type by Reynolds and Walsby (1975), in that photic zone biomass increased during the day and decreased at night. This finding is examined in further detail below. In general the diel movement of heterocysts followed N_2 fixation patterns for the first day of the experiment, but on the next day the pattern was not repeated. The relationship between maximal nitrogenase activity, heterocysts, and vegetative cells is shown in Table 3. During the day the number of heterocysts corresponded well with the depth of maximum nitrogenase activity. By contrast nocturnal fixation showed no relationship of heterocysts with maximum activity, implying that the vegetative cells were contributing some fixed nitrogen.

Autumn Anabaena *blooms*—Expressed per unit area, there was little difference between the diel patterns of N_2 fixation of *Anabaena* and those of *Aphanizomenon*. Fixation rose rapidly in mid-morning and remained relatively constant until dusk (Fig. 3). Quantities of N_2 fixed were also similar in both spring and

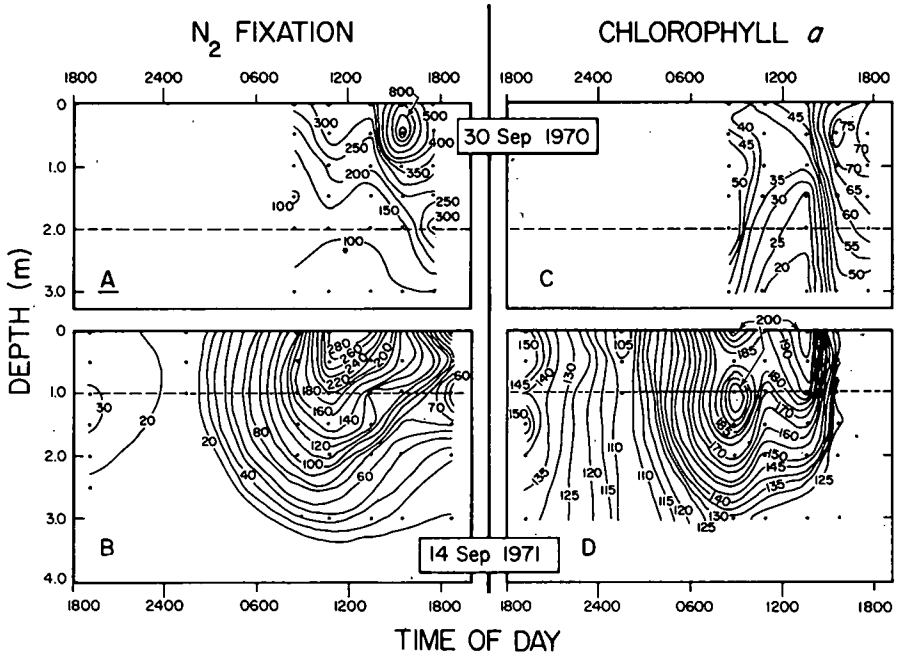

Fig. 4. As Fig. 2, but for two autumn *Anabaena* blooms of 1970 and 1971; N_2 fixation isopleths every 100 nmol·liter^{-1}·h^{-1} and chlorophyll *a* isopleths at 50 μg·liter^{-1} intervals.

fall, although the efficiency of fixation per unit biomass by *Anabaena* was considerably greater than by *Aphanizomenon* in 1970 and somewhat less in 1971.

Isopleths of N_2 fixation with depth and time showed that it was confined to the upper layers, i.e. the shallow photic zone, except at night (Fig. 4A, B). Another dissimilarity from the spring blooms was a reasonably good correlation between algal biomass and N_2 fixation (Fig. 4). Surface fixation (0–0.5 m) dominated quantitatively, in contrast to the situation in spring. Once again this was probably because autumn sunlight was less lethal.

As with *Aphanizomenon* blooms, the variations in efficiency were not due to differences in heterocysts. *Anabaena* populations normally had 5 times as many heterocysts as *Aphanizomenon* (Horne and Goldman 1972; Horne 1975*b*). In 1970 there were about 4% heterocysts in *Anabaena* populations, in 1971 about 6%.

Anabaena and *Aphanizomenon* showed similar patterns of biomass, both producing distinct surface and subsurface maxima which broke down overnight (cf. Figs. 2 and 4). *Anabaena* was generally nearer the surface—a position best explained by the turbidity of the water. Secchi disk depths were 20–70 cm in 1970 and 34–45 cm in 1971 when turbidity was measured by nephelometry at 17–30 JTU. The photosynthetic compensation point in September was at about 2 m in 1970 and 1 m in 1971, and all *Anabaena* biomass peaks were above these depths (Fig. 4C, D). For the spring *Aphanizomenon* bloom the compensation depth was 3 to 4 m, with subsurface chlorophyll maxima correspondingly deeper. The diminished clarity in fall was not wholly due to algae, the biomass of which reached similar levels in fall and spring, but to sediments stirred up by winds at the edges of the lake. It is not evident why late summer sediments should have been more prone to suspension than those of spring. Suspended sediment is

transported clockwise round the lake in autumn and mixes with both deep and edge waters (Wrigley and Horne 1974). Another possible reason for reduced water clarity is the presence of a substantial population of small flagellates and cryptomonads amongst the *Anabaena* bloom. *Aphanizomenon* blooms were almost unialgal. Small phytoplankton contribute more to light absorption than do large clumps of blue-green algae (Horne and Blank unpubl.), so although chlorophyll concentrations were similar in spring and autumn biotic light absorption was not.

Nocturnal N_2 fixation by *Anabaena* was apparently less important than that by *Aphanizomenon*, although there were indications of some quite high *Anabaena* activity after dusk.

Lake temperatures, higher in autumn than in spring and showing more diel variation, had the net effect of producing stronger thermal stratification (Tables 4 and 5), unless clouds prevented the daily replenishment of surface heat. During these diel experiments there were no clouds (Fig. 3); algae were redistributed apparently either by buoyancy regulation (Walsby 1972) or by wind breakup of loose *Anabaena* aggregations up to 5 cm across. Winds did not affect thermal or algal stratification even at 1700 hours (Fig. 4B, D).

Discussion

Although indisputable positive dark N_2 fixation by aquatic algae was unknown in 1970, it has since been shown in lakes (Duong 1972; Vanderhoef et al. 1975; Burris and Peterson 1978), in a stream (Horne 1975*b*), and in wet soils (Henriksson 1971; Jones 1974). Some previous 24-h dark bottle $[^{15}N]N_2$ studies may show dark N_2 fixation, since dark values were sometimes almost as high as light ones (Horne and Fogg 1970). Nocturnal fixation in the spring *Aphanizomenon* bloom accounts for about a third of the 24-h total of N_2 fixed, and since the spring bloom contributes half of the lake's annual nitrogen income (Horne and Goldman 1972), nocturnal N_2 fixation is important for the whole lake's ecology. *Anabaena* seems less able to make use of darkness for N_2 fixation, although the results are not totally conclusive. Three questions spring to mind concerning nocturnal N_2 fixation in lakes: Why does it occur at all? Why does it occur at night, but not in the aphotic zone during the day or in the sediments? How does the dark fixation stragegy of *Aphanizomenon* and *Anabaena* relate to that used by stream *Nostoc*?

Blue-green algae are an ancient group and lack many complex enzymatic induction and repressor systems found in other microorganisms (Carr 1973). Even though N_2 fixation is energy demanding, it is not always directly harnessed to photosynthesis (Horne and Fogg 1970; Horne 1975*b*). Unlike photosynthesis, N_2 fixation should fall slowly to insignificance after sunset—the length of time for which it continues being related by the amount of energy stored in the glycogen pool produced during the preceding day. This seems to be the case for terrestrial algae, lichens, and attached stream *Nostoc* where daily light regimes can be measured (Henriksson 1971; Horne 1975*b*). In lakes the vertical mixing process prevents knowledge of the exact prior light history of the algae. However, most planktonic blue-green algae will have energy available for N_2 fixation at sunset. Three factors will determine the amounts fixed: the stored energy available, the need for nitrogen, and the dissolved oxygen levels during the night.

Nitrogen stresses are severe in Clear Lake in both spring and autumn, but due to release of ammonium from the sediments in summer (Lallatin 1972; Horne 1975*a*), which alleviates the nitrogen stress in autumn, the spring depletion is most extreme (Table 1). This may account for greater nocturnal fixation in spring.

Two factors involving oxygen favor nocturnal N_2 fixation in the spring *Aphanizomenon* bloom over that of the autumn *Anabaena* population: the lower

nocturnal oxygen concentrations in spring, and the colony shape and size of the two genera. The spring bloom does not raise dissolved oxygen levels as high as does the dense fall bloom (Tables 4 and 5). High afternoon oxygen levels persist through much of the night in the fall bloom, even though there is some decrease. Oxygen, even at normal concentrations, inhibits N_2 fixation in blue-green algae partially by inactivating the O_2-labile nitrogenase enzyme (Stewart and Pearson 1970). *Aphanizomenon* in Clear Lake grows in two colony forms, one transient, the other relatively permanent. The permanent form is the "flake" (O'Flaherty and Phinney 1970) consisting of upward of several hundred trichomes, each of a hundred or more cells (Horne 1975*a*). Only very vigorous shaking in a closed vessel will destroy this form. The ephemeral form consists of thousands of flakes in a ball often several centimeters in diameter, which is easily broken by agitation of the surrounding water. *Anabaena* forms much smaller, durable colonies, often of coiled filaments, and produces much smaller, temporary balls less frequently. At night the center of a large colony of respiring cells may be depleted in oxygen, which would both enhance N_2 fixation in the heterocysts and enable N_2 fixation in the vegetative cells. This was postulated for the similarly shaped colonies of the marine cyanophyte *Trichodesmium* (Carpenter and Price 1976). By analogy with *Trichodesmium*, vegetative cells of *Aphanizomenon* could fix measurable amounts of N_2 overnight. A further indication of vegetative fixation is the lack of relationship between heterocyst number and maximum nitrogenase activity at night but close correspondence by day (Table 3). Levels of fixation by night were low but significant.

Algae in darkness (below the photic zone) should fix N_2 during the day, using both heterocyst and vegetative nitrogenase; this was not observed in Clear Lake because the mixed depth normally exceeds that of the photic zone. Algae collected below the photic zone may have been illuminated shortly before and will have quantities of photosynthetic oxygen nearby. It takes several hours to synthesize very much nitrogenase, so the aphotic zone N_2 fixation is akin to the early postdusk N_2 fixation, i.e. a tailing off of the daytime processes.

A conceptual problem with the interpretation of diel curves of N_2 fixation is variation in rates of nitrogenase synthesis. If oxygen-stable precursors of nitrogenase could be stored, many of the variations in diel curves of N_2 fixation by phytoplankton and periphyton could be explained. This biochemical problem is in need of study.

Nostoc growing in balls up to several centimeters in diameter firmly attached to rocks in streams shows a strong afternoon depression in N_2 fixation (Horne 1975*b*), similar to that found for both N_2 fixation and photosynthesis in some lakes (Ganf and Horne 1975; Horne and Viner 1971). This depression is strikingly absent from the daylight curves of both *Aphanizomenon* and *Anabaena* blooms. In most cases N_2 fixation for these species quickly reaches high values after daybreak and rates of activity fluctuate around this level until after sunset (Figs. 1, 3). Photorespiration was proposed as a major mechanism of the afternoon depression for *Nostoc* (Horne 1975*b*) and for very dense equatorial phytoplanktonic blue-green algae (in Lake George, Uganda: Ganf and Horne 1975). In Clear Lake the relatively lower populations (200–500 $mg \cdot m^{-2}$ Chl *a* as against 800 in Lake George) and moderate chemical hardness apparently prevented photorespiration from robbing the nitrogenase system of photosynthetically generated energy. The absence of depressed afternoon N_2 fixation would decrease nitrogen stress and is also the most probable reason for the lack of a nocturnal peak, along with the general blurring of the overall picture by nocturnal water mixing.

A generally favorable climate for other biological processes, especially photosynthesis, may explain why the algae in

Clear Lake do not follow the classic pattern of pronounced sinking during the day and rising overnight (Reynolds and Walsby 1975). There were more *Aphanizomenon* cells in the photic zone at noon than at any other time (Table 3). However, the data on surface accumulations of blue-green algae on which Reynolds' (1972) pattern was based were taken on very calm days. Under more normal conditions of afternoon winds, or on larger lakes, the pattern may shift in time. The speeds of rising and sinking of the algae in Clear Lake relative to lake wind mixing, together with the need for energy for N_2 fixation, can account for a midday algal maximum. Alternatively, recruitment of buoyant algae to the photic zone following nocturnal deep mixing continued well into the morning, whereas the average tendency to sink out was not evident until later in the day. It has been suggested, however, that nitrogen stress would interfere with buoyancy regulation (Reynolds and Walsby 1975).

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