Reports

Nitrogen Fixation in Lakes

Abstract. Incorporation of N^{15} into the fixed nitrogen fraction of natural lake waters has been studied for the purpose of estimating rates of primary nitrogen fixation. Experiments in Pymatuning Reservoir, Pennsylvania, in Lake Mendota, Wisconsin, and in two lakes in Alaska indicate that fixation occurs at measurable, sometimes high, rates.

Our understanding of the nature of soil fertility and the availablity of nitrogen under natural conditions expanded greatly upon the discovery and subsequent investigation of symbiotic nitrogen fixation in leguminous plants and nonsymbiotic fixation by soil microorganisms. Whether biological fixation of nitrogen *in situ* is a quantitatively important feature of the nitrogen metabolism also of bodies of water is not yet known, although marine and freshwater biologists have sometimes tacitly assumed that this is the case (*1*). The list of organisms known to be capable of fixing free nitrogen is long; included on it are species of blue-green algae (particularly of the genera *Nostoc* and *Anabaena*, but also of *Tolypothrix*, *Calothrix*, and others), common and often abundant constituents of freshwater plankton, as well as *Azotobacter* and photosynthetic bacteria, including some that have been isolated from the water and mud of Lake Mendota, Wisconsin (*2*). Organisms capable of fixing nitrogen are therefore known to occur in the aquatic environment; it remains only to show that they contribute significantly to the supply of fixed nitrogen there.

We have used uptake of the stable isotope N^{15} as a measure of nitrogen fixation in lake water. Our method employs preliminary removal of dissolved atmospheric nitrogen from water samples contained in special 1000-ml closed vessels by aeration with an 80:20 helium-oxygen mixture at a pressure of 0.8 atm. Sufficient N_2 containing 95 atom percent N^{15} is then added to bring the pressure within the vessel to approximately 1 atm, and the system is equilibrated by shaking. Samples are incubated either in the lake at the depth from which the water was originally collected or under specified laboratory conditions. Subsequent treatment is essentially the same as that applied by Rittenberg, Keston, Roseburg, and Schoenheimer (*3*) and by Burris, Eppling, Wahlin, and Wilson (*4*) in their studies (*5*).

Preliminary measurements were made on the following lakes in 1958 (samples were incubated in the lake): (i) Little Kitoi and Upper Jennifer lakes, Afognak Island (Kodiak region), Alaska; (ii) Pymatuning Reservoir (Sanctuary Lake), Pennsylvania; (iii) Lake Mendota, Wisconsin.

The amount of N^{15} incorporated into the fixed-nitrogen fraction was estimated in all of these instances by subtracting the measured atom percent N^{15} of laboratory air from that of the fixed-nitrogen fractions of the samples after incubation. It has become obvious that this method overestimates the excess of N^{15} for two reasons. (i) Air is not an adequate standard because it gives a value about 0.002 atom percent lower than N_2 from ammonium sulfate which has gone through the conversion procedure. The latter, therefore, provides a standard more comparable to the actual samples. (ii) Lake-water blanks sterilized and carried through the entire experimental procedure appear to be enriched by about 0.004 atom percent N^{15}, as compared with ammonium sulfate. We have, therefore, applied a correction of −0.006 atom percent to these data, not having used adequate blanks at the time the measurements were made. Rates computed in this way are obviously not particularly reliable but may be used as preliminary estimates. Seven measurements made in the Alaskan lakes during July and August yielded rates of fixation ranging from 0 to 0.0047 μg of nitrogen per liter per day. Fixation was not detectable in a single experimental series from Lake Mendota in late October. A single series from Pymatuning Reservoir on 4 October yielded rates of incorporation far too high to be canceled by the most conservative corrections for the blank, of the order of 0.8 μg of nitrogen per liter per day. This figure is based on duplicate samples, the results of which agreed within 1 percent. There is no reason to doubt that here nitrogen was fixed at a high rate. For all experiments in 1958, incubation periods ranged from 4 to 6 days.

In more recent experimental series from Pymatuning Reservoir (beginning 20 April 1959) use has been made of certain technical refinements: (i) reagent-grade ammonium sulfate (instead of laboratory air) was used as a primary standard for isotope ratio; (ii) lake-water blanks were carried through the entire procedure after initial autoclaving within the experimental vessels; and (iii) all experimental vessels were autoclaved prior to the introduction of the sample, sterile procedure being observed subsequently. Water for these experiments was collected at Pymatuning, transported to Pittsburgh, and treated as described above about 8 hours after collection; samples were incubated under constant illumination at 15°C (approximately the temperature of the water when it was collected) for varying lengths of time. The results are given in Fig. 1 as atom percent N^{15} (excess over ammonium sulfate and sterile lake-water blank) plotted against

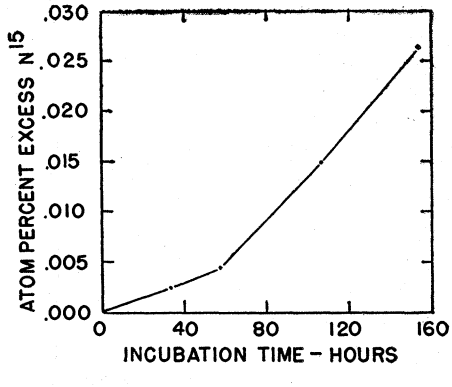

Fig. 1. Incorporation of N^{15} by surface water taken from Sanctuary Lake, Pymatuning Reservoir, Pennsylvania, on 20 Apr. 1959, incubated under constant illumination at 15°C.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

time of incubation. The curve appears to be characterized by two rates of fixation, one of about 0.021 μg of nitrogen per liter per day during the first 58 hours, the other of about 0.068 μg of nitrogen per liter per day during the rest of the experiment (*6*).

RICHARD DUGDALE
VERA DUGDALE
Department of Biological Sciences, Pymatuning Laboratory of Field Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

JOHN NEESS
JOHN GOERING
Department of Zoology, University of Wisconsin, Madison

References and Notes

1. C. E. ZoBell, *Marine Microbiology* (Chronica Botanica, Waltham, Mass., 1946); F. Ruttner, *Fundamentals of Limnology* (Univ. of Toronto Press, Toronto, 1953).
2. E. S. Lindstrom *et al., J. Bacteriol.* **61**, 481 (1951).
3. D. Rittenberg, A. S. Keston, F. Rosebury, R. Schoenheimer, *J. Biol. Chem.* **127**, 285, 291 (1939).
4. R. H. Burris, F. J. Eppling, H. B. Wahlin, P. W. Wilson, *Soil Sci. Soc. Am. Proc.* **7**, 258 (1942).
5. A detailed description of our entire procedure is in preparation.
6. We wish to express our appreciation to R. H. Burris of the department of biochemistry, University of Wisconsin, and Richard Abrams of the research laboratory, Montefiore Hospital, Pittsburgh, for the advice they have generously given and for the use of the mass spectrometers in their laboratories. Both spectrometers are Consolidated-Nier isotope ratio machines. This research was supported by the University of Kentucky Faculty Research Fund and by the National Institutes of Health. The Alaska Department of Fish and Game provided laboratory facilities at the Kitoi Bay Research Station on Afognak Island.

13 July 1959

Sensory Deprivation and Visual Speed: An Analysis

Abstract. Speeds of moving objects were markedly underestimated by human observers after prolonged patternless visual stimulation. Even greater underestimation followed exposure to a "noisy" visual field; on the other hand, exposure to a hyperstable field caused overestimation. The effects of external visual noise simulate those of deprivation; this finding suggests that similarly disordered but spontaneous neural discharge dominates the visual nervous system in deprivation.

Experiments in sensory deprivation have been made to explore the consequences of prolonged exposure to visual fields in which normal spatiotemporal variation is sharply reduced. Results of experiments performed at McGill University (*1*) suggest that the apparent speed of a moving line markedly changes as one consequence of long-term exposure to an illuminated but patternless visual field. After such deprivation, observers reported that a straight black line slowly rotating against a dimly lit screen looked S-shaped. This distortion was attributed to an induced "perceptual lag": the ends of the line lag behind the center.

In preliminary investigations we found no evidence for a perceived distortion of the line. Instead, the apparent speed of the entire line was clearly reduced. Consequently, in further investigations we have used an apparatus (Fig. 1) designed to measure and thus quantify changes in apparent speed (*2*). The observer was instructed to fixate a black dot 1 m distant at eye level. He then saw a black bar (Fig. 1, *A*) sweep, like the second hand of a clock, through 90 deg from a horizontal to a vertical position, at a rate of 60 deg/sec. At the vertical, the bar disappeared from the observer's sight behind a screen. His task was to judge when the now-hidden bar would reach a fixed marker (Fig. 1, *B*) 10 deg beyond. This judgment was based on the observer's estimate of the speed of the bar during its visible 90 deg of travel. When he judged that the hidden bar had traversed the 10 deg to reach the marker, he stopped the coupled time clock (Fig. 1) which gave a measure of the apparent speed of the bar. The average of the initial judgments made by practiced observers was very close to the actual travel time. Twenty trials per observer were made before and after each experimental exposure.

In a first test to evaluate our interpretation of the McGill results, seven observers were deprived of patterned vision for 8 hours under conditions similar to those of the McGill study (*3*). The observers, lying on cots, were subjected to a masking acoustic noise at the maximum tolerable level; they wore tubes over their arms and hands and translucent goggles over their eyes. The goggles provided only a homogeneously illuminated, nonpatterned field of vision. After deprivation, each of the seven observers judged the speed of the sweep to be less (by an average of 16 percent) than he had initially judged it to be. The observers showed no accompanying change in simple motor reaction time. Having demonstrated and measured this consequence of generalized deprivation, we proceeded to further analyses.

Each of 12 observers was instructed to fix his gaze on the center of a patternless but illuminated circular field (intensity 0.2 ml) subtending 43 visual degrees in an otherwise dark field (patternless exposure field). Although only visual input was controlled, a significant reduction in apparent speed was produced with only ½ hour of exposure (Table 1). This test demonstrates that control of extravisual stimulation is not essential for obtaining the change in apparent speed.

Table 1. Changes in apparent speed after ½ hour of exposure. Exposure fields: H, hyperstable; D, dark; P, patternless; N, noisy.

Exposure field	Change in speed (%)	Significance* of differences between conditions
H	+14.8	.05
D	− 4.8	N.S.†
P	−10.1	N.S.†
N	−19.6	
H–D		.01
P–N		.02
H–P, H–N, D–P, D–N		.001

* Significance levels derived from an analysis of variance. † Not significant.

Comparable experimental situations were then used to explore the effects of exposing the same observers to three other test fields devised on the basis of the following speculation. In the absence of normal stimulation coming from patterned fields, there is reason to suspect that the visual nervous system exhibits spontaneous and patternless activity (*4*). Dominance of the visual system for long periods by such intrinsic noise may result in reduction of

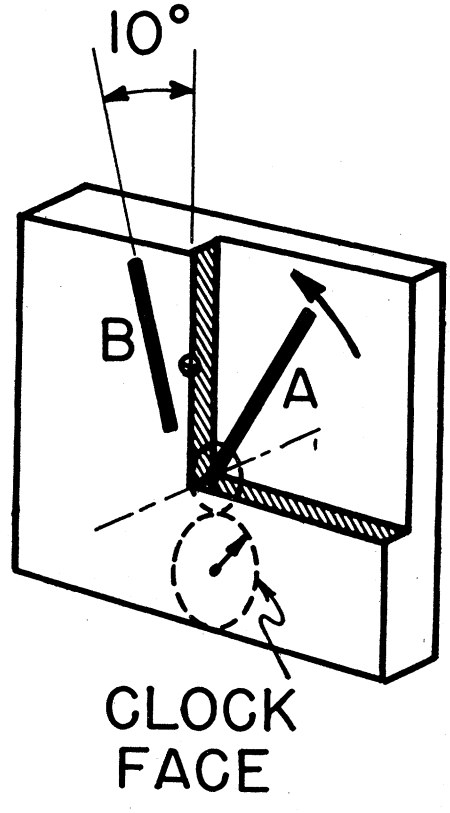

Fig. 1. Apparatus for measuring apparent speed. *A*, Rotating sweep; *B*, fixed marker.