

FACTORS AFFECTING THE VERTICAL DISTRIBUTION OF A BLUE-GREEN ALGA

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ANDREW ROBERT KLEMER

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ABSTRACT

Certain Oscillatoria species disappear from the surface layers and form metalimnetic or upper hypolimnetic population maxima during the summer stratification. In attempting to explain this phenomenon, some have focused their attention on the temperature and light conditions at which such population maxima develop, and have characterized these algae as cold-water shade plants. Proponents of this view would attribute anomalous epilimnetic appearances of these species to the existence of different physiological races in different lakes. Others have associated such epilimnetic populations with high nutrient levels in the lakes concerned, and have suggested that Oscillatoria's thermal and photic requirements may vary with nutrient concentrations. Although results from some laboratory experiments have supported the latter hypothesis, in situ experiments with metalimnetic Oscillatoria populations have provided either inconclusive or negative results.

Lake experiments, designed to test the above hypotheses, were conducted with metalimnetic populations of O. agardhii var. isothrix in Deming Lake, Minnesota. Destratification experiments were conducted to determine the response of this alga to epilimnetic conditions of light and temperature in nutrient-rich water. These experiments indicated that, given sufficient nutrient enrichment, metalimnetic populations of O. agardhii var. isothrix could increase in the epilimnion across gradients of light and temperature.

To identify the critical nutrients in the broad-spectrum enrichment of destratification, specific nutrient enrichment experiments were conducted with Oscillatoria samples transferred to the

epilimnion within plexiglass cylinders. In these experiments, sustained increases of Oscillatoria near the surface occurred only with added N. Enrichment with NH_4^+ -N produced more positive responses with Oscillatoria than NO_3^- -N, but even with ammonia enrichment, the nature as well as the magnitude of the response depended on the concentration employed. Further experiments with plexiglass cylinders were conducted to determine whether nitrate utilization in Deming Lake was limited by enzyme cofactors required for nitrate reduction. In these experiments supplemental Mo and Mn consistently enhanced responses to nitrate enrichment if only to a small degree.

P alone or with a N source stimulated the growth of green algae within plexiglass cylinders, but promoted an increase in O. agardhii var. isothrix within a large polyethylene tube previously enriched with NH_4^+ -N.

Clear and black polyethylene tubes were used to determine the response of Oscillatoria in a metalimnetic stratum to nutrient enrichment and light reduction within enclosed but otherwise undisturbed water columns. In black tubes, Oscillatoria appeared at the surface in increasing numbers with or without nutrient enrichment albeit greater increases occurred with enrichment. In the clear tube, O. agardhii var. isothrix increased at the surface after a doubling of the initial rate of enrichment. These experiments with undisturbed water columns provided evidence of vertical displacements of Oscillatoria as a result of increased buoyancy with either light reduction or nutrient enrichment. Subsequently, a buoyancy experiment suggested that light and nutrients interact in determin-

ing the buoyancy of O. agardhii var. isothrix. Thus the vertical distribution of this alga depends upon the combined effects of light and nutrients on buoyancy as well as growth. The apparent interaction between light and nutrients involved in buoyancy determination is best explained by Walsby's turgor-pressure hypothesis.

A consequence of the nutrient effect on the vertical distribution of O. agardhii var. isothrix is that nutrient enrichment increases the portion of a lake's photic zone that is inhabitable by this alga and thus renders the lake more susceptible to invasion. Oscillatoria may occur more readily in small protected lakes with less allochthonous enrichment because the stable water columns in such lakes permit reducing conditions and, hence, large reservoirs of available nutrients to develop at the base of the photic zone.

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INTRODUCTION

Numerous Oscillatoria species form metalimnetic or upper hypolimnetic population maxima during summer stratification. In small protected lakes with high relative depth these maxima occur as well-defined strata and frequently give rise to metalimnetic O₂ maxima (Eberly, 1959, 1964; Anderson, 1954; Duthie and Carter, 1970; Schindler et al., 1971). In larger lakes, such Oscillatoria population maxima are less well defined but usually occur within the metalimnion. Records of these occurrences include O. agardhii in Lough Neagh (Gibson, et al., 1971), O. agardhii var. isothrix in Windemere and Esthwaite Water (Lund, 1959), O. rubescens in Lake Zurich (Schroter, 1897) and Lake Maggiore (Ravera and Vollenweider, 1969) and O. agardhii and O. rubescens in Lake Washington (Edmondson, 1956). In these larger lakes, as in certain other Swiss (Hasler, 1947) and Norwegian (Skulberg, 1968) lakes, Oscillatoria populations appeared after increased nutrient enrichment and at times occupied the surface waters during the summer. Although more profound mixing may contribute to such surface appearances in larger lakes, Vollenweider (1950) has associated epilimnetic populations of O. rubescens with small productive lakes. In general, however, these Oscillatoria species occur throughout the water column during the spring and fall mixing, disappear from the surface during the summer stratification, and appear at the surface, often under ice, during winter. Recent studies in Minnesota have indicated that O. agardhii is perennial in certain lakes (Brook, et al., 1971).

The seasonal behavior of these Oscillatoria species has stimulated much work and even more speculation on their environmental

requirements. Ruttner (1963), noting the winter accumulation of O. rubescens at the surface and its summer increase at low temperatures in the metalimnion, described this alga as a cold stenotherm. Findenegg (1943) has emphasized the roles of both light and temperature in determining algal occurrence, and he has characterized O. rubescens as an oligothermal shade form. Both Ruttner (1937) and Findenegg approached this problem with emphasis on the temperatures at which population maxima occurred, but Findenegg also stressed the association of O. rubescens maxima with low light intensity. He grouped organisms into four categories according to their light and temperature requirements, and linked each category with one of the seasons. In the case of O. rubescens, its seasonal behavior marked it as a winter form really requiring low light and low temperature. Viewed in this manner, studies of seasonal variations in the vertical distribution of O. agardhii (Eberly, 1959) would indicate similar light and temperature requirements for this alga. More recently, Zimmerman (1969) concluded that O. rubescens is a eurythermal, oligophotic species and that light and temperature interact in determining its vertical distribution.

Another view is that light and temperature optima are not fixed, but may change with nutrient concentrations. As lakes stratify in summer, algae in the epilimnion are subject not only to higher light and temperature regimes but also to lower nutrient concentrations than algae in the metalimnion. Thus, Adam and Birrer (1943), studying O. rubescens in Lake Baldegg, attached more importance to the decline in nutrients than to the increase in temperature, and

Vollenweider (1950) suggested that O. rubescens may require more nitrate at higher light intensities. Vollenweider based this hypothesis in part on a study of the distribution of O. rubescens and also on the work of White (1937) with Lemna. Later, Eberly (1967) performed a series of laboratory experiments with several Oscillatoria species including O. rubescens and strains of O. agardhii. He grew these algae at 15 and 20°C in modifications of A.S.M. medium, and found that growth rates increased with temperature and with nutrient concentrations. In effect, he found, in his own work and that of Maddux and Jones (1964) with diatoms, support for the notion of variable optima inherent in Vollenweider's hypothesis.

In situ experiments with Oscillatoria populations have not supported Vollenweider's hypothesis. For example, Thomas (1964) used plexiglass cylinders to determine the response of O. rubescens to enrichment with nitrate and phosphate, and after analyzing an extensive series of experiments, he maintained that neither his lake experiments nor Staub's (1961) related laboratory work gave grounds for a conclusion about which substance stimulates the growth of O. rubescens. Other in situ experiments have involved epilimnetic exposures of O. agardhii collected from the metalimnion (English, 1962; Baker et al., 1969). Although these short-term exposures in B.O.D. bottles showed that O. agardhii is capable of higher photosynthetic rates than it exhibits in the metalimnion, they did not prove that this alga is capable of sustained growth in the epilimnion. On the contrary, the Baker et al. study produced evidence of light injury to the cold and dark-adapted alga.

Furthermore, extended surface exposures of O. agardhii in polyethylene bags and superficial destratifications of enclosed water columns have failed to demonstrate sustained growth near the surface (Brook et al., 1971).

The present study began as an attempt to test in situ the principal hypotheses advanced to explain the occurrence of certain Oscillatoria species, and in the process to find under what conditions, if any, metalimnetic Oscillatoria populations would flourish in the epilimnion. These in situ tests consisted of destratification and specific nutrient enrichment experiments conducted in Deming Lake, Minnesota during four consecutive summers starting in September of 1969.

METHODS AND MATERIALS

All experiments described in this study involved the manipulation of metalimnetic populations of Oscillatoria agardhii var. isothrix in Deming Lake, Minnesota. Several recent papers describe seasonal variations in the vertical distribution of Oscillatoria in this lake (Baker and Brook, 1971; Brook et al., 1971). Deming Lake is one of the small protected lakes (5.09 hectares) with high relative depth (6.48) referred to above. Hooper (1948, 1951) provides a detailed description of the lake and its geologic setting.

I. Destratification experiments

Destratifications were conducted within clear 6 mil polyethylene tubes, 1 meter in diameter and 6-8 meters in length (Goldman, 1962). Inner tubes, tied to a cable strung across the lake, held the polyethylene tubes at the surface. Aluminum hoops, taped to the tubing at meter intervals, provided lateral support, and aluminum collars, 10 in. deep, anchored the tubes at the base. Optical density profiles, obtained with a Schenk transmissometer, indicated that careful insertion of the tubes enclosed water columns with well-defined O.D. peaks similar to those in the lake itself.

An air compressor and a water pump, mounted on a pontoon boat, were used to mix the enclosed water columns. In September, 1969, destratifications were by compressed air. A hose joining the compressor to a perforated ring of hard rubber tubing introduced the compressed air at the base of the water column rendering it isothermal within 15 minutes. In July, 1970 and subsequently,

destratifications involved a combination of compressed air and pumped hypolimnetic water for more thorough enrichment. Turbidity profiles, chlorophyll determinations, and direct counts of O. agardhii var. isothrix were used to assess algal responses in each experiment.

A 0.6 neutral density filter served as a standard for the Schenk transmissometer. Chlorophyll determinations involved filtration of 0.2 to 0.5 l of sample plus $MgCO_3$ with glass fiber filters, desiccation of the filtered samples in a freezer, extraction in a tissue grinder with 90% acetone and spectrophotometry according to Lorenzen (1967). The method employed for direct algal counts was that of Utermohl (1958) using Lugol's preservative, sedimentation chambers, and a Unitron inverted microscope. Direct counts and measurements of Oscillatoria trichomes included more than 100 individuals per sample except for some surface samples with very few trichomes (Lund et al., 1958).

II. Specific nutrient enrichment experiments

Specific nutrient enrichment experiments were conducted with plexiglass cylinders approximately 3mm thick, 25cm in diameter and 5.7m long. Each of the five cylinders used in these experiments consisted of three 1.9m sections joined together with P.S.-30 acrylic adhesive. (Available from Cadillac Plastic Inc.) Polyurethane blocks cut to fit around the cylinders provided adequate flotation, and aluminum rods inserted through holes at the tops of the cylinders rested on the flotation blocks thus holding the cylinders in place. Nylon line, tied to the rods, fastened the floating cylinders to a steel cable strung across the lake. The experiments

began with the inspection of a recent turbidity profile and the selection of a depth within the metalimnetic Oscillatoria stratum. After measuring the temperature at this depth, each cylinder was positioned so that water from the stratum would replace water pumped from the top of the cylinder. In this manner water from the desired depth filled each cylinder until the temperature at the surface within the cylinder fell to that of the selected depth. Thomas (1964) described a similar pumping procedure in an account of his research with O. rubescens in Lake Baldegg.

The enrichment procedure involved stoppering a hose with a cork tied to a loop of nylon line, lowering the hose into the cylinder, and filling it with the appropriate nutrient solution. The loop ran through the cylinder; thus by holding the loop taut while withdrawing the hose, the nutrient solution was released. In addition to the experiments with plexiglass cylinders, two specific nutrient enrichment experiments involved clear and black polyethylene tubing, 1m in diameter. In these experiments the isolated water columns remained undisturbed except for the insertion of a hose during enrichment and sampling. A nonmetallic pump, a 5/8 in. hose, and a small diameter (6cm) discrete level sampler (Golterman, 1969) were used to sample the enclosed water columns.

Measures of algal responses to enrichment included in vivo chlorophyll fluorescence as well as direct counts and measurements of the volumes of Oscillatoria trichomes. A Turner 111 fluorometer, with modifications according to Lorenzen (1966) and with a high sensitivity sample holder, gave relative values of in vivo

chlorophyll fluorescence. Other instruments used for routine measurements included a thermistor thermometer, a silicon cell photometer devised by R. Maxwell and L. Schmid for use within cylinders, a Myron L Co. conductivity meter, and an Orion specific ion meter and NH_3 electrode.

III. Buoyancy experiment

A five gallon sample was taken from the O. agardhii var. isothrix maximum at 4.8m, mixed and decanted into liter bottles. Pairs of enriched and control bottles were suspended at selected depths in Deming Lake. After a period of exposure, the relative buoyancies of these Oscillatoria samples were determined by allowing subsamples to sediment in test tubes before separating the test tube contents into two or three levels with a pipette. A duplicate set of subsamples was subjected to nitrogen gas under pressure to collapse the algae's gas vacuoles. This was accomplished with a simplified version of an earlier apparatus used by Walsby (1971) to study the collapse of gas vacuoles under pressure. These subsamples were then allowed to sediment before being separated into upper and lower levels. Equal volumes from each level were treated with preservative and placed in sedimentation chambers for counts of their Oscillatoria contents. The number of Oscillatoria trichomes encountered in four transects at each level was expressed as a percentage of the total count for each test tube.

RESULTS

I. Destratification experiments

Destratification experiments were conducted to determine the response of Oscillatoria to epilimnetic conditions of light and temperature in nutrient-rich water. The limnological literature contains frequent references to the existence of nutrient reservoirs in the hypolimnia or monimolimnia of lakes and to their productivity-enhancing potential (Hooper, 1951; Hasler, 1964; Findenegg, 1965; Saraceni and Gerletti, 1968). Thus the destratifications described below had the combined effect of displacing metalimnetic Oscillatoria and nutrient-rich bottom water toward the surface of the enclosed water columns where epilimnetic conditions of light and temperature prevailed.

In September, 1969, a clear polyethylene tube was suspended in the lake so that its base rested in the sediment at a depth of 7m. Following destratification of the enclosed water column on September 12, chlorophyll 'a' increased for approximately a week, and Oscillatoria increased in abundance until September 27. Table 1 shows the changes in concentration of Oscillatoria and of chlorophyll 'a' expressed as hundreds of trichomes per ml and micrograms per liter respectively. Figure 1 shows that the O.D. also increased for about a week after the mixing and that the increase in Oscillatoria abundance occurred across gradients of temperature and light intensity. Within 48 hours of mixing, the temperature profile within the tube was almost identical to that of the lake, and although destratification and subsequent algal growth had reduced light transmission, Oscillatoria increased in abundance at light

Table 1. Concentrations of O. agardhii var. isothrix (10^2 trichomes per ml) and chlorophyll 'a' (ug per l) within a polyethylene tube after destratification of the enclosed water column. Chlorophyll concentrations are in parentheses.

Depth (m)	14IX	18IX	27IX
1	8.8(26.7ug)	17.2(38.5ug)	18.4(38.2ug)
3	10.4(30.7ug)	18.8(40.4ug)	20.2(41.7ug)
4.5	15.6(39.8ug)	15.8(41.0ug)	16.4(42.0ug)

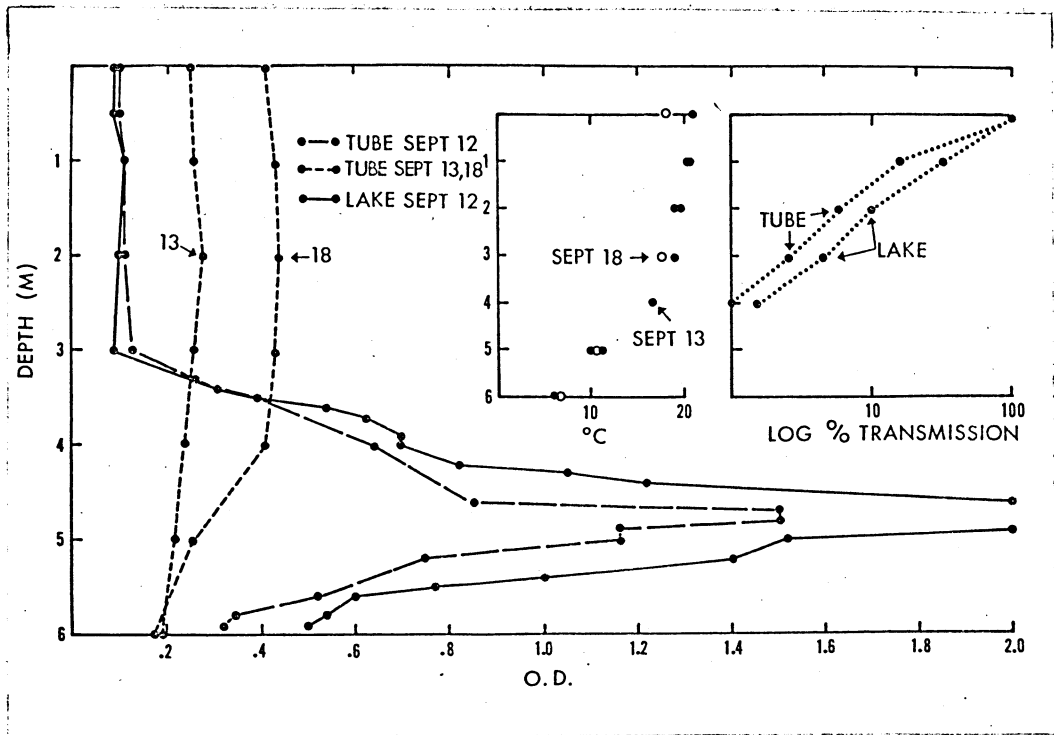


Fig. 1. O.D. profiles in Deming Lake and in a polyethylene tube before and at two dates after destratification of the enclosed water column on Sept. 12, 1969. Inset temperature profiles are from the lake and the tube 32 hours after destratification (closed circles) and from the lake 5 days later (open circles). Light transmission profiles are from the lake and the tube on Sept. 18.

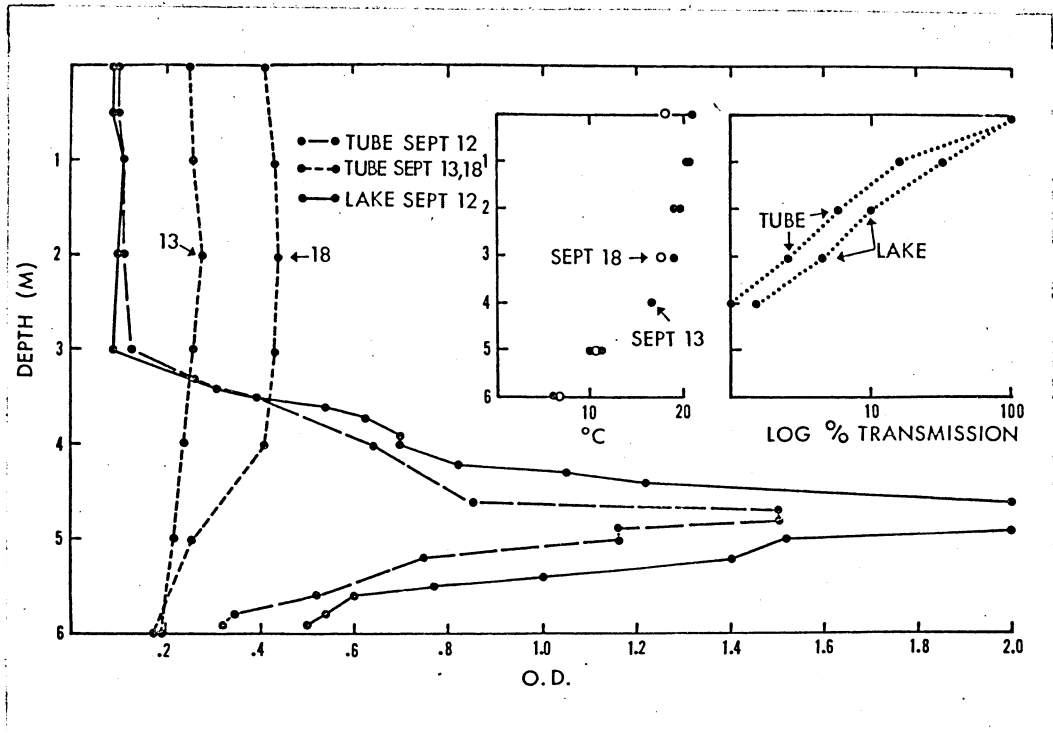


Fig. 1. O.D. profiles in Deming Lake and in a polyethylene tube before and at two dates after destratification of the enclosed water column on Sept. 12, 1969. Inset temperature profiles are from the lake and the tube 32 hours after destratification (closed circles) and from the lake 5 days later (open circles). Light transmission profiles are from the lake and the tube on Sept. 18.

intensities much higher than the metalimnetic population normally receives. Thus all three measures of algal response indicated a period of sustained growth throughout the photic zone after the brief period of mixing. However, these results were less than conclusive because Deming Lake, itself, began to mix in September. From September 16 to September 27, the Oscillatoria concentration in the open water increased from 40 to 1040 trichomes per ml; hence, the effects of seasonal mixing, though probably reduced within the tube, may have contributed to some of the increases in Oscillatoria numbers near the surface of the enclosed water column.

On July 11, 1970, a similar destratification was supplemented with water pumped from the mud-water interface into the polyethylene tube. Again, this time for approximately 9 days, O.D. increased throughout the enclosed water column (see Fig. 2). During this period, the one meter chlorophyll 'a' concentration increased from 21.9ug/l to 58.7ug/l while Oscillatoria abundance increased from 256 trichomes/ml or $1.57\text{mm}^3/\text{l}$ to 888 trichomes/ml or $7.30\text{mm}^3/\text{l}$. Although Oscillatoria trichome abundance peaked on July 20, Oscillatoria volume reached $9.3\text{mm}^3/\text{l}$ on July 23. Unlike the previous September, samples taken at intervals throughout this experiment indicated that O. agardhii var. isothrix was virtually absent from the surface of Deming Lake.

II. Specific nutrient enrichment experiments

Experiment 1

In the above destratification experiments, Oscillatoria increased in the enriched surface water of the polyethylene tubes.

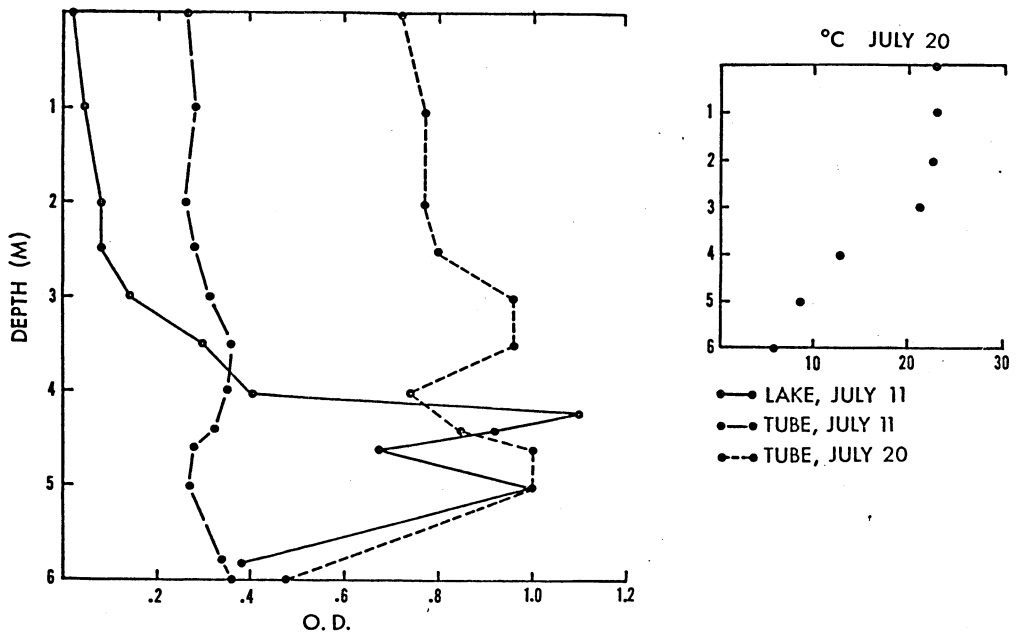


Fig. 2. O.D. profiles from Deming Lake and from a polyethylene tube just after destratification of the enclosed water column and 9 days later. The temperature profile, though from the lake, approximated that within the tube very closely.

The pumping and mixing of hypolimnetic water resulted in broad-spectrum enrichment, but as such, these experiments gave no indication of the critical nutrient or nutrients involved. In order to assess the relative importance of essential nutrients, specific nutrient enrichment experiments were conducted. On June 19, 1971, four plexiglass cylinders were suspended in the lake and filled with water from approximately 4.8m. During the next eleven days, declining chlorophyll concentrations near the surface reflected the sinking out of algae within the cylinders (see Fig. 3). On June 30, the upper five meters of each cylinder were treated with one of the following nutrient additions:

1.0 ppm $\text{NH}_4\text{NO}_3\text{-N}$

0.05 ppm $\text{KH}_2\text{PO}_4\text{-P}$

1.0 ppm $\text{NH}_4\text{NO}_3\text{-N}$ + 0.05 ppm $\text{KH}_2\text{PO}_4\text{-P}$

Distilled water (control)

N and P were supplied in a ratio (20:1) approximating both the N/P ratios found in some natural blue-green populations (Birge And Juday, 1922) and the average N/P ratio found in natural fresh waters (Russel - Hunter, 1970). NH_4NO_3 was added to provide both a reduced and an oxidized N source. By design the actual concentrations employed were lower than those used in previous, inconclusive experiments, but, otherwise, their selection was arbitrary. Table 2 shows that from June 30 to July 3, chlorophyll fluorescence increased markedly in the upper four meters of both of the N-enriched cylinders, but declined slightly in the upper two meters of the P-enriched and the control cylinder. During this period, similar changes occurred at one meter in Oscillatoria abundance

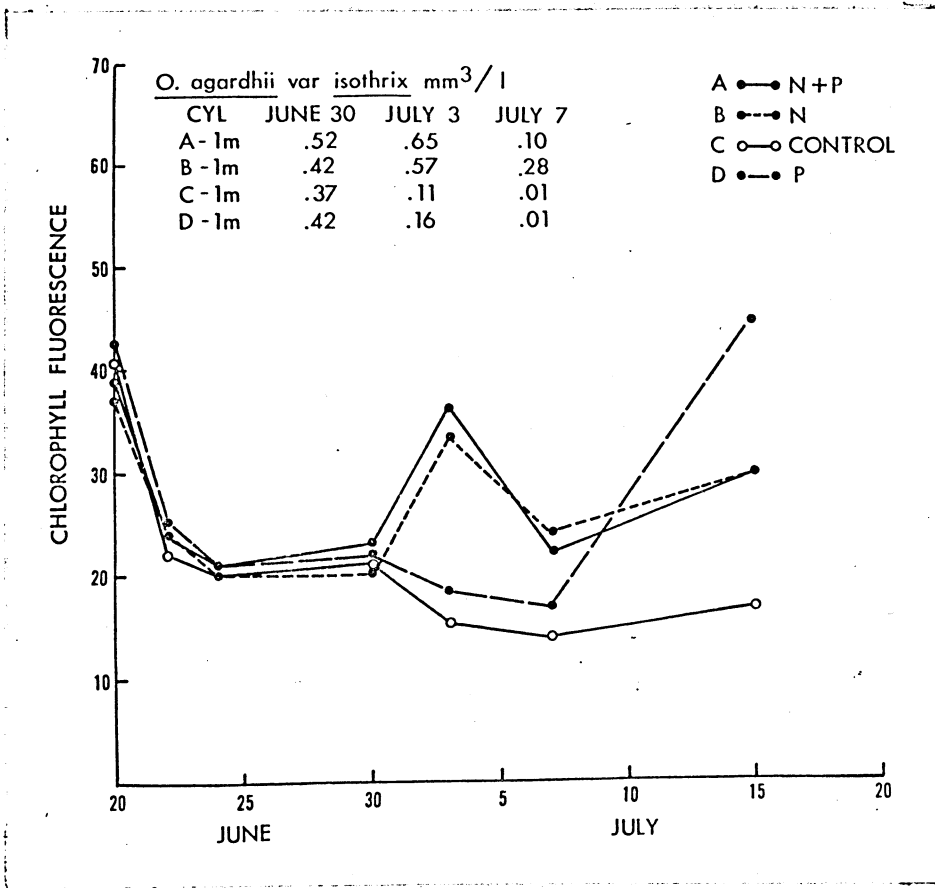


Fig. 3. Changes in chlorophyll fluorescence at 1 meter within plexiglass cylinders filled with metalimnetic water on June 19, 1971, and enriched on June 30 with 1.0 ppm $\text{NH}_4\text{NO}_3\text{-N}$ and 50 ppb P. The inset table shows corresponding changes in Oscillatoria abundance with different treatments.

Table 2. Relative values of chlorophyll fluorescence in plexiglass cylinders before and after enrichment.

Depth (m)	Control		P		N		N+P	
	30VI	3VII	30VI	3VII	30VI	3VII	30VI	3VII
1	21.0	15.5	22.0	18.5	20.5	33.5	23.0	36.5
2	22.0	19.0	23.0	22.0	22.0	38.0	24.5	39.0
3	32.0	32.0	34.0	37.0	32.0	61.0	35.0	65.0
4	49.5	53.5	51.5	56.0	46.5	66.5	47.0	69.0
Mean	31.0	30.0	32.5	33.5	30.0	50.0	32.5	52.5

expressed as volume and in chlorophyll fluorescence (see Fig. 3). Notably, in the non-N-enriched cylinders, the decline in Oscillatoria abundance was proportionately greater than the decline in chlorophyll fluorescence. This was due partly to residual or non-algal fluorescence and also to the presence of other algae that are apparently less sensitive to N limitation than Oscillatoria. By July 7, the N-enriched cylinders showed declines in both measures of algal response. The P-enriched and the control cylinder continued to show declines through July 7, but by the end of the experiment on July 15, chlorophyll fluorescence had increased strikingly in the P-enriched cylinder due to the growth of numerous green algae including species of Ankistrodesmus, Cosmarium, Kirchneriella, Oedogonium and Scenedesmus. By July 15, smaller increases in chlorophyll fluorescence had occurred in the control and in the N-enriched cylinders where O. redeckii and Anabaena spp. then dominated the phytoplankton. Some green algae also appeared in the N and P-enriched cylinder, but grew mostly on the cylinder wall and not as abundantly as in the P-enriched cylinder.

This experiment indicated that N was limiting for O. agardhii var. isothrix displaced from the metalimnion but not for various species of green algae that grew profusely in the P-enriched cylinder once the concentration of Oscillatoria had declined. The response of Oscillatoria to N and P was not significantly greater than its response to N alone, and in both cases, these responses to single enrichments were transient. In later experiments with large polyethylene tubes, repeated nutrient additions produced

sustained increases in Oscillatoria abundance, and P enrichment led to a significant increase in Oscillatoria numbers. In the plexiglass cylinders, the failure of N and P to elicit a greater response than N alone suggests that either Oscillatoria, limited primarily by N, retained stored P at the time of enrichment or that the P added was insufficient or not as available to Oscillatoria as to other algae. The adsorption of P by the walls of the plexiglass cylinders may have accounted for the growth there of green algae. This and other container effects are less significant in larger containers such as the polyethylene tubes referred to above. Finally, in this experiment the average trichome length was found to vary directly with response to enrichment. Therefore, volume determinations of Oscillatoria abundance permit more accurate comparisons among cylinders.

Experiment 2

Having observed positive responses by Oscillatoria to the addition of NH_4NO_3 , a subsequent experiment was conducted to compare the responses of this alga to additions of reduced and oxidized forms of N alone and with P. To this end, NH_4Cl , NaNO_3 , and KH_2PO_4 were used as sources of reduced N, oxidized N and P respectively. On July 15, five plexiglass cylinders were filled with metalimnetic water, and after four days, each of the cylinders was treated with one of the following additions:

1.0 ppm NO_3^- -N

1.0 ppm NO_3^- -N + 0.05 ppm PO_4^{3-} -P

1.0 ppm NH_4^+ -N

1.0 ppm NH_4^+ -N + 0.05 ppm PO_4^{3-} -P

Distilled water (control)

As shown in Table 3, the most marked increases in Oscillatoria abundance occurred in the cylinders receiving NH_4^+ -N either with or without P, and despite decreases above three meters, both NO_3^- -enriched cylinders exhibited smaller overall losses than the control cylinder. Figure 4 shows the patterns of change in chlorophyll fluorescence and Oscillatoria abundance expressed as volume at the one meter level in each of the cylinders. At the time of enrichment, the cylinders exhibited greater differences in algal abundance than in the previous experiment. An extremely high concentration of Oscillatoria was present in the surface meter of one of the cylinders. This may have happened as a result of pumping water from the cylinder before raising its base to the selected depth. Thus the water initially drawn into the cylinder would have come from a deeper, denser portion of the metalimnetic stratum. By chance the cylinder with the highest algal content received NO_3^- -N and P while the cylinder with the lowest received NH_4^+ -N and P. Despite large initial differences, both measures of algal abundance in these two cylinders approached similar values during the next eight days of the experiment. Chlorophyll fluorescence in the cylinder with NH_4^+ -N and P added rose steadily to match that in the cylinder enriched with NO_3^- -N and P which also increased after an initial decline. Although chlorophyll increased in both cylinders, Oscillatoria volume declined markedly in the cylinder enriched with NO_3^- and P and increased in the cylinder enriched with NH_4^+ and P to produce similar final volumes in these cylinders. As in the previous experiment, green algae,

Table 3. Concentrations of *O. agardhii* var. *isothrix* (10^2 trichomes per ml) in plexiglass cylinders before and after enrichment.

Depth (m)	NH_4^+		$\text{NH}_4^+\text{+P}$		Control		NO_3^-		$\text{NO}_3^-\text{+P}$	
	19VII	27VII	19VII	27VII	19VII	27VII	19VII	27VII	19VII	27VII
1	4.2	10.9	1.4	2.5	6.4	0.5	5.1	1.4	32.6	4.1
2	3.8	5.8	1.0	3.5	5.4	0.3	5.3	1.0	6.9	3.7
3	6.4	15.2	3.3	14.3	4.8	1.8	3.4	9.0	6.2	27.4
4	25.9	32.8	25.4	32.4	36.8	22.6	24.9	25.9	26.5	28.4
Mean	10.1	16.2	7.8	13.2	13.4	6.3	9.7	9.3	18.1	15.9

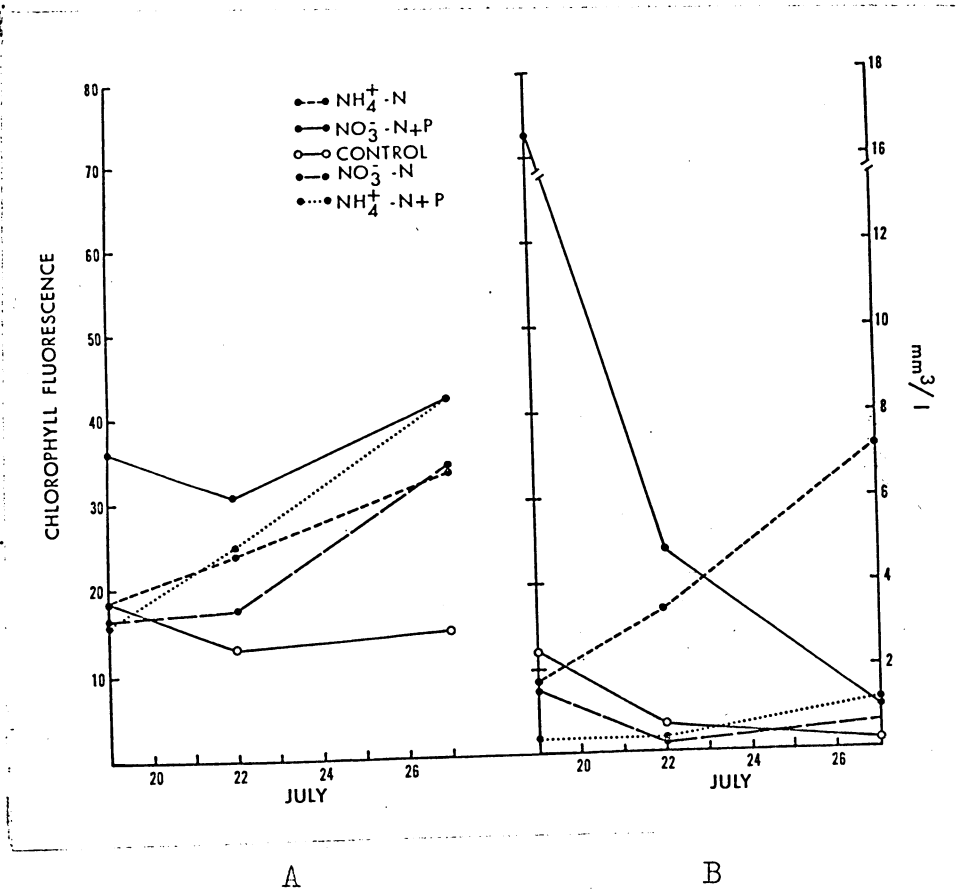


Fig. 4. A. Changes in chlorophyll fluorescence at 1 meter within plexiglass cylinders filled with metalimnetic water on July 15, 1971, and enriched on July 19 with 50 ppb P and 1.0 ppm N as NO_3^- or NH_4^+ . B. Corresponding changes in Oscillatoria abundance with N and P enrichment.

especially Oedogonium species, appeared in these phosphorus-enriched cylinders, and contributed to the observed increases in chlorophyll fluorescence.

The remaining cylinders showed smaller differences in their initial chlorophyll and Oscillatoria contents. Of these, the NO_3^- and the NH_4^+ -enriched cylinder showed increases in chlorophyll fluorescence. In the latter the response was immediate and sustained, whereas in the former chlorophyll fluorescence increased after a three day lag period. Oscillatoria abundance also increased steadily in the NH_4^+ -enriched cylinder, but in the NO_3^- -enriched cylinder, Oscillatoria showed a net decrease despite a slight increase during the last five days of the experiment. Both measures of algal response showed a decline in the control cylinder.

Although NH_4^+ enrichment produced more positive responses with Oscillatoria than NO_3^- , the final chlorophyll fluorescence values did not reflect this difference in response. Apparently, other algae present utilized the added NO_3^- more effectively than Oscillatoria. For example, green algae appeared in both P-enriched cylinders, but grew more abundantly in the cylinder that also received NO_3^- . However, chlorophyll fluorescence values did indicate time lags in the responses to NO_3^- addition. Thus rather than a lesser ability to utilize NO_3^- , Oscillatoria may have a greater need for an immediately available source of N at high light intensities than the algae that eventually utilized the NO_3^- -N. This interpretation would explain Oscillatoria's intermediate responses to NO_3^- enrichment as compared with its responses to NH_4^+ enrichment and to the absence of added N in the control (see Table 3).

Experiment 3

With clear-cut evidence that Oscillatoria responded better to NH_4^+ than to NO_3^- enrichment, an experiment was conducted to determine Oscillatoria's response to a range of NH_4^+ -N additions. In the two previous experiments, enrichment occurred eleven and four days after the transfer of the metalimnetic algae to the epilimnion. During the interim between transfer and enrichment, Oscillatoria and chlorophyll concentrations declined near the surface while, presumably, some degree of adaptation to epilimnetic conditions occurred. On August 11, 1971, I enriched the cylinders immediately after refilling them with metalimnetic water and before much sedimentation or surface adaptation could occur. The treatments consisted of the following additions:

0.5 ppm NH_4^+ -N

1.0 ppm NH_4^+ -N

1.5 ppm NH_4^+ -N

2.0 ppm NH_4^+ -N

Distilled water (control)

In the surface meter of the cylinders an immediate and general decline in chlorophyll fluorescence occurred over the first twenty-four hours (see Fig. 5-A). During the next twenty-four hours chlorophyll remained stable in the cylinder enriched by 1.0 ppm N, increased markedly in the cylinder with the 1.5 ppm N addition, and increased moderately in the cylinder with 2.0 ppm N added. As shown in Fig. 5-B, Oscillatoria abundance expressed as volume increased slightly with the addition of 1.0 ppm N and markedly with 1.5 ppm N, but in the cylinder with 2.0 ppm N added,

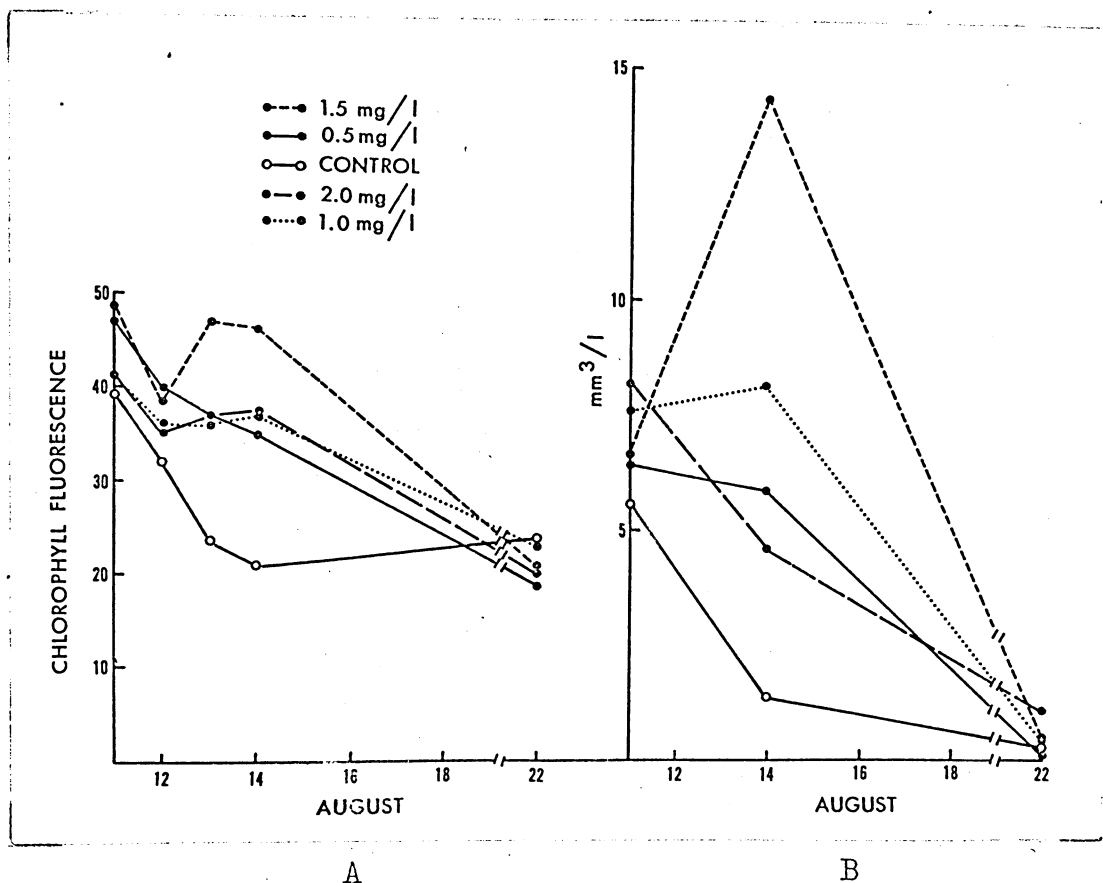


Fig. 5. A. Changes in chlorophyll fluorescence at 1 meter within plexiglass cylinders filled with metalimnetic water and enriched with a range of NH_4^+ -N concentrations on August 11, 1971. B. Corresponding changes in Oscillatoria abundance with the addition of different concentrations of NH_4^+ -N.

Oscillatoria declined as strikingly as in the control cylinder. Chlorophyll fluorescence as well as Oscillatoria abundance decreased steadily in the control cylinder and to a lesser extent in the cylinder with 0.5 ppm N added. Table 4 shows that between August 11 and 14, the greatest increases in Oscillatoria numbers occurred in the cylinders that received 1.0 and 1.5 ppm N. Although the mean abundance increased in every cylinder, only the cylinders with 1.0 and 1.5 ppm N added exhibited increases in the surface two meters of water. After August 14, all cylinders showed decreases in Oscillatoria abundance and chlorophyll fluorescence except the control which showed a slight increase in chlorophyll fluorescence.

NH_4^+ -N additions from 0.5 to 1.5 ppm N produced graded responses in Oscillatoria volume at a depth of one meter (see Fig. 5B). As shown in Table 4, graded responses in Oscillatoria abundance also occurred with depth in each cylinder. Oscillatoria did not show an increase above three meters in either the control or in the cylinder with 0.5 ppm N added. But in the cylinder with 1.0 ppm N added, Oscillatoria showed an increase at two meters, and in the cylinder with 1.5 ppm N, this alga increased at one meter. The negative response of Oscillatoria to the addition of 2.0 ppm NH_4^+ -N may have been the result of a container effect. In subsequent experiments, this effect was observed again in a plexiglass cylinder but never in the larger polyethylene tubes despite much higher NH_4^+ -N concentrations.

Table 4. Concentrations of O. agardhii var. isothrix (10^2 trichomes per ml) in plexiglass cylinders before and after enrichment.

Depth (m)	Control		0.5ppm N		1.0ppm N		1.5ppm N		2.0ppm N	
	11VIII	14VIII	11VIII	14VIII	11VIII	14VIII	11VIII	14VIII	11VIII	14VIII
1	13.4	4.0	14.9	10.8	16.0	14.1	15.2	19.3	16.6	9.9
2	11.2	6.2	20.1	8.2	13.4	17.2	18.0	17.2	14.6	7.5
3	11.6	12.3	12.2	18.3	12.2	37.4	30.8	32.0	19.2	21.4
4	10.5	32.3	20.3	33.4	19.6	35.2	29.2	52.4	19.2	31.9
Mean	11.7	13.7	16.9	17.7	15.3	26.0	23.3	30.2	17.4	17.7

Experiment 4

The above enrichment experiments indicated that N was limiting for Oscillatoria displaced from the metalimnion and that NH_4^+ -N elicited greater responses than NO_3^- -N. These findings prompted enrichment experiments with molybdenum and manganese in addition to nitrate to determine whether these cofactors in nitrate and nitrite reduction limited nitrate utilization in Deming Lake. On June 24, 1972, two levels of NO_3^- -N with and without Mo were added to the surface meter of plexiglass cylinders filled with metalimnetic water just prior to enrichment. The treatments were repeated every day until June 28, and samples were taken from just below the surface before enrichment. As shown in Table 5, Oscillatoria abundance as measured by volume declined throughout the experiment with only one notable exception. From June 29 to July 2, Oscillatoria volume increased slightly in the cylinder receiving a high level of NO_3^- -N plus Mo. An even slighter increase attributed to the cylinder receiving only the high level of NO_3^- -N may not be real.

In Table 6 are similar results obtained in a subsequent experiment involving enrichment with either a combination of NO_3^- , Mo, Mn, and P or with NH_4^+ every other day for four days (see also Fig. 6). In this experiment, the only indications of a trend reversal appeared in the cylinder receiving NO_3^- , Mo, and Mn. The cylinder with NO_3^- , both cofactors, and P retained a comparatively high Oscillatoria volume with a continuous but relatively slow rate of loss. After receiving a total of 3.0 ppm N by July 13, the ammonium-enriched cylinder showed an increase in

Table 5. Surface concentrations of O. agardhii var. isothrix (mm^3 per liter) within plexiglass cylinders.

Treatment	24VI	29VI	2VII
Control	30.0	0.7	0.7
0.5ppm N	28.6	3.6	3.2
1.0ppm N	27.5	1.9	2.2
0.5ppm N+ 0.01ppm Mo.	26.1	2.2	1.8
1.0ppm N+ 0.01ppm Mo.	23.3	2.1	3.8

Table 6. Surface concentrations of *O. agardhii* var. *isothrix*
(mm³ per liter) within plexiglass cylinders.

Treatment	11VII	13VII	14VII	20VII
10ppb Mo + 10ppb Mn + 50 ppb P (control)	19.0	5.4	4.9	0.55
1.5ppm NH ₄ ⁺ -N	26.2	13.5	5.5	1.61
1.5ppm NO ₃ ⁻ -N	19.6	11.9	11.5	0.94
1.5ppm NO ₃ ⁻ -N+ 10ppb Mo+10ppb Mn	27.2	11.8	16.4	1.45
1.5ppm NO ₃ ⁻ -N+ 10ppb Mo+10ppb Mn +50ppb P	25.0	16.6	12.3	1.64

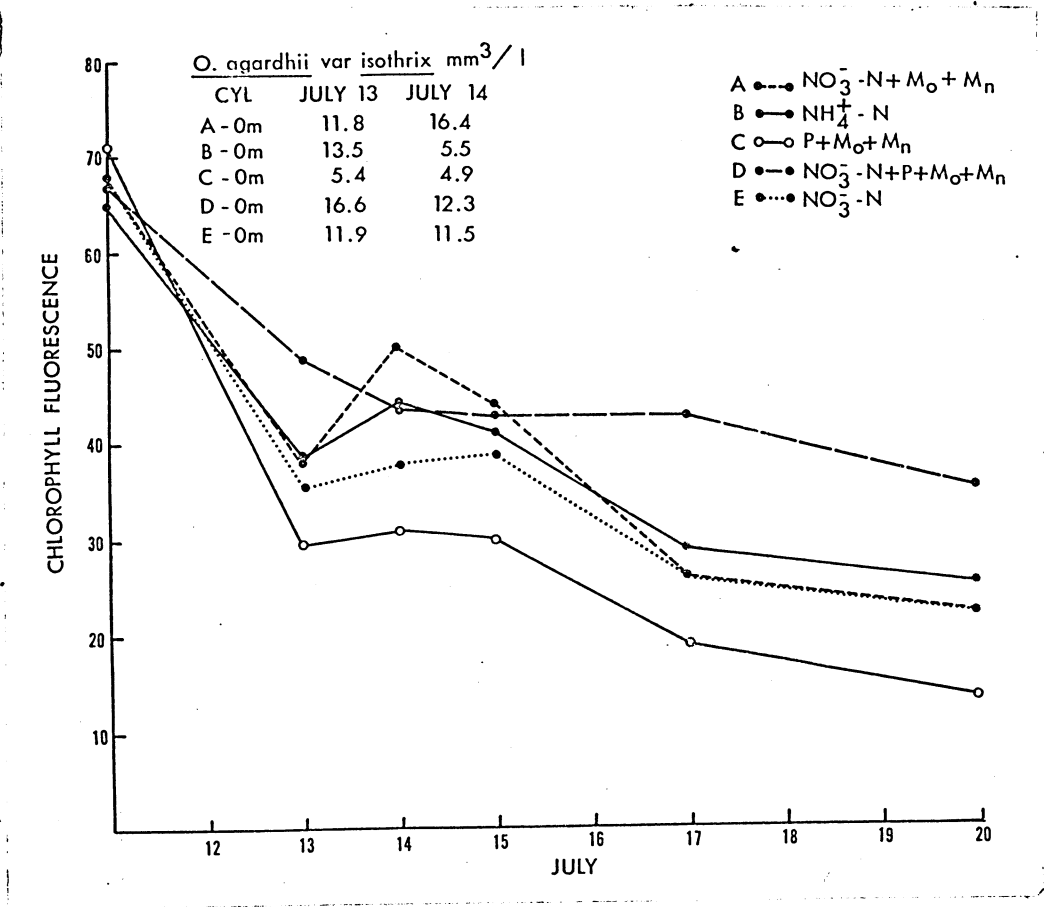


Fig. 6. Changes in chlorophyll fluorescence at the surface of plexiglass cylinders filled with metalimnetic water on July 11, 1972, and enriched every 2 days thereafter with a combination of 1.5 ppm NO₃⁻-N, 50 ppb P, 10 ppb Mo, and 10 ppb Mn or with 1.5 ppm NH₄⁺-N. The inset table shows changes in *Oscillatoria* abundance from July 13 to July 14.

chlorophyll fluorescence but a reduction in Oscillatoria volume by July 14. The final Oscillatoria volumes correlated rather well with the final values for chlorophyll fluorescence. Both measures of algal response were higher in cylinders receiving N, alone in either form or as NO_3^- in some combination with Mo, Mn, and P, than in the control cylinder receiving only Mo, Mn, and P. Enrichment with NH_4^+ and with the combination of NO_3^- , Mo, Mn, and P produced the highest final Oscillatoria volumes and fluorescence readings with the combination of NO_3^- with Mo and Mn, NO_3^- alone, and the control following in that order. Throughout the experiment, the N-enriched cylinders maintained higher Oscillatoria volumes through lower overall rates of loss than the control cylinder.

Although only to a small degree, supplemental Mo and Mn consistently enhanced the responses of Oscillatoria to NO_3^- enrichment. Notwithstanding the small size and short-lived nature of this effect, parallel changes in chlorophyll fluorescence and Oscillatoria volume suggest that it was real. From July 13 to July 14, although virtually no increase (<5%) occurred in the concentration of Oscillatoria trichomes at the surface of the cylinder receiving NO_3^- and both co-factors, the average trichome length of the Oscillatoria in this cylinder increased by 39%. This increase in trichome length and, consequently, Oscillatoria volume was significant at the 0.001 level, and accompanied a parallel increase in chlorophyll fluorescence.

Experiment 5

The enrichment experiments described to this point have involved the disruption of Oscillatoria strata and the transfer of

metalimnetic algae to the epilimnion. On June 24, 1972, two polyethylene tubes were suspended in the lake. These tubes were similar to those used in the destratification experiments except that one of the tubes was black. Although the light field within the black polyethylene tube was not uniform near the surface, light attenuation even through the center of the enclosed water column was such that the photic zone at noon was approximately half that of the lake. Table 7 presents light and temperature data from Deming Lake and both polyethylene tubes. Temperature profiles within the cylinders whether clear or black were always nearly identical to those of the lake. From the time of insertion, the enclosed water columns received no disruptive treatment other than that incurred during enrichment and sampling. Starting on June 28, the upper four meters of the tubes were enriched with 1.0 ppm NH_4^+ -N every two days with exceptions noted below. Prior to each enrichment, samples were taken from 0, 2, and 4 meters. By July 2, the concentration of O. agardhii var. isothrix expressed as numbers of trichomes per ml was increasing rapidly at the surface of the black tube. This increase continued at a high rate until July 25 when the tube submerged slightly exchanging water with the lake (see Fig. 7). After eight days and three enrichments with no increase of Oscillatoria at the surface of the clear tube, the enrichment rate was doubled. During the next six days, Oscillatoria increased markedly at the surface of this tube. On July 14, enrichment was halted temporarily, and the increase of Oscillatoria at the surface was slowed. When enrichment resumed

Table 7. Light and temperature measurements in Deming Lake
and in polyethylene tubes on July 25, 1972

Depth (m)	Relative radiation (%)			Temperature (°C)		
	lake	clear tube	black tube	lake	clear tube	black tube
0	100.0	100.0	100.0	23.4	23.4	23.4
1	47.3	35.6	14.3	22.2	22.1	22.0
2	22.4	14.0	3.5	21.7	21.8	21.7
3	11.9	8.8	0.6	18.5	18.6	18.4
4	4.6	3.6	0.1	10.6	10.8	10.7
5	0.8	1.0	-	7.0	7.5	7.3

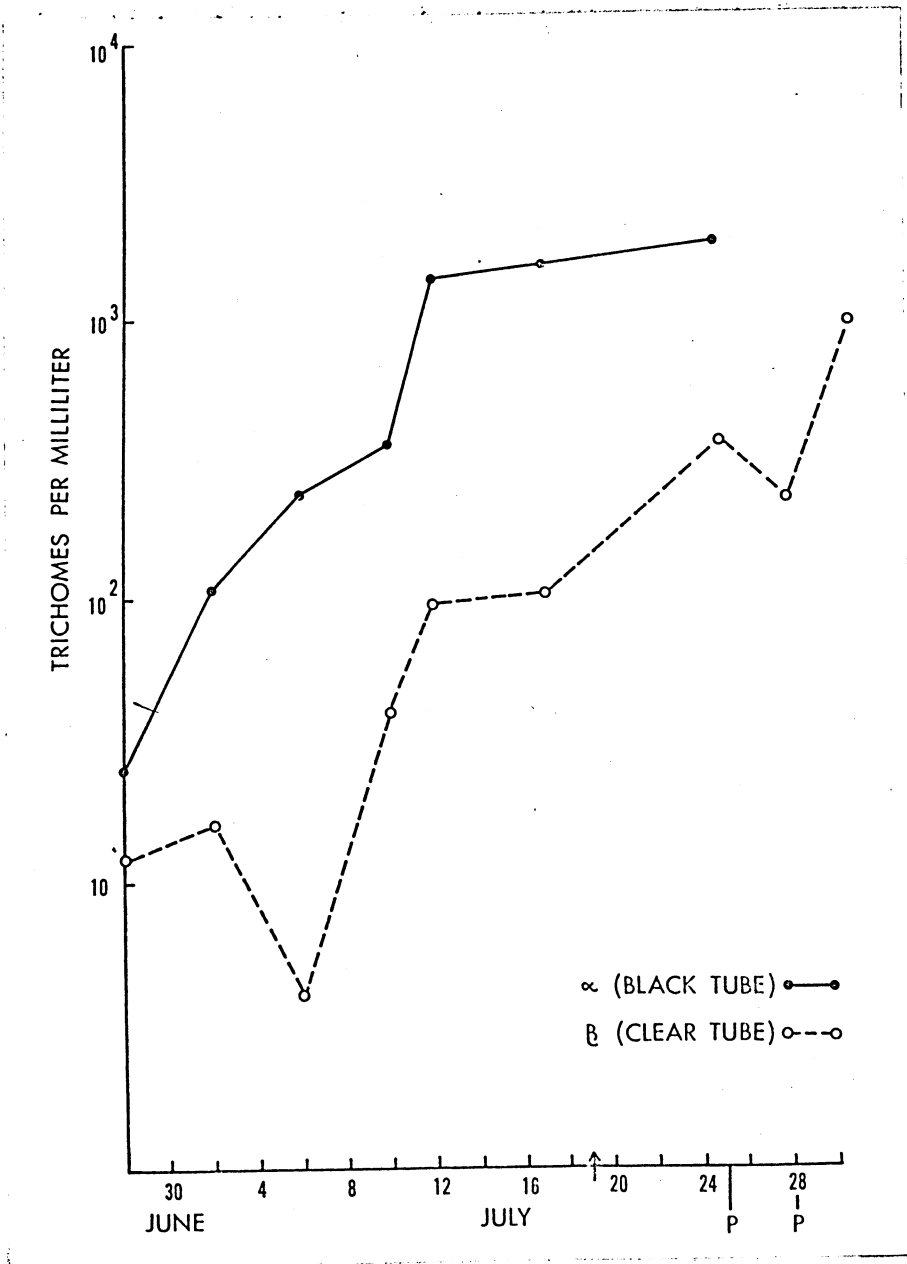


Fig. 7. Changes in the surface concentration of *Oscillatoria* trichomes in clear and black polyethylene tubes enriched as follows: alpha, 1.0 ppm NH_4^+ -N every 2 days from June 28 to July 12; beta, same as alpha until July 6, 1.0 ppm NH_4^+ -N daily from July 6 to July 14, and 1.5 ppm NH_4^+ -N on July 17 and 19. Arrow indicates last N addition. P's indicate 50 ppb P additions to beta.

Table 8. Concentrations of O. agardhii var. isothrix (10^2 trichomes per ml) in Deming Lake and in two enriched polyethylene tubes.

Black polyethylene tube (alpha)						
Depth (m)	Date					
	28VI	6VII	10VII	12VII	17VII	25VII
0	0.3	2.4	3.6	14.2	16.2	19.2
2	0.2	1.6	4.0	4.5	11.1	11.4
4	11.4	11.7	7.0	5.8	5.5	4.3
Mean	4.0	5.2	4.9	8.2	10.9	11.6

Clear polyethylene tube (beta)								
Depth (m)	Date							
	28VI	6VII	10VII	12VII	17VII	25VII	28VII	31VII
0	0.1	0.0	0.4	1.0	1.1	3.7	2.3	9.9
2	0.2	0.1	0.5	1.0	1.6	1.5	2.2	2.4
4	7.1	6.2	7.2	7.4	7.3	7.8	7.8	7.8
Mean	2.5	2.1	2.7	3.1	3.3	4.3	4.1	6.7

Table 8. Continued.

Deming Lake					
Depth (m)	Date				
	28VI	6VII	10VII	25VII	31VII
0	0.3	0.1	0.	0.1	0.0
2	-	-	0.1	0.0	0.0
4	-	-	5.5	4.9	5.2
Mean			1.9	1.7	1.7

on July 17, the Oscillatoria concentration increased again but at a lower rate than initially. By July 19, the clear tube had received a total of 15.0 ppm NH_4^+ -N, and on July 25, it retained 8.9 ppm NH_4^+ -N at the surface. Thus with NH_4^+ -N apparently present in excess, 50 ppb PO_4^{3-} -P were added to upper four meters of the clear tube on July 25 and 28. Within three days of the second P addition, a large increase of Oscillatoria was noted at the surface (see Fig. 7).

As shown in Table 8, the concentration of O. agardhii var. isothrix in the black tube increased steadily at two meters as well as at the surface through most of July. Although the mean concentration in the upper four meters of the black tube almost trebled over this period, the increase in the upper two meters appeared to occur partly at the expense of the four meter level. Table 7 shows that this depth within the black cylinder was receiving at most 0.1% of the surface radiation at the close of the experiment. By contrast a slight increase in Oscillatoria abundance at four meters accompanied the less marked surface increases in the clear tube, but even in the clear tube, the rate of increase in numbers of Oscillatoria was greater towards the surface. Table 7 shows that these surface populations of Oscillatoria developed in both tubes despite thermal gradients as pronounced as in the lake, itself, where, in contrast, Oscillatoria maintained a rather stable vertical distribution during this experiment (see Table 8).

To compare the responses of Oscillatoria to light reduction with and without nutrient enrichment, a pair of black polyethylene tubes was inserted on August 5, 1972. One was used as a control and the other was enriched to a depth of four meters with 1.5 ppm NH_4^+ -N every three days until August 11. As shown in Figure 8, the enriched tube showed a slightly greater rate of increase in the concentration of Oscillatoria trichomes at the surface. On August 13, the enriched tube had 60% more trichomes at the surface than the control, and by August 21, the difference had increased to 75% of the control. On August 21 and 24, a colleague inadvertently enriched the control tube with 2.0 ppm NH_4^+ -N, and by August 26, the newly enriched tube had 140% more trichomes at the surface than the tube enriched initially.

Whereas both light intensity and nutrients seem to play a role in determining the vertical distribution of O. agardhii var. isothrix, these roles appear to involve more than growth limitation. In the initially unenriched black tube, light reduction alone resulted in surface increases in Oscillatoria abundance that, if attributed entirely to in situ growth, would have required doubling times of less than 1.5 days. Because the unenriched surface water of Deming could scarcely have supported growth of this magnitude, recruitment from the metalimnetic stratum must have occurred. The even higher rates of increase observed at the surface of the enriched polyethylene tubes, both clear and black, exceeded the growth rate of O. rubescens at optimal culture conditions (Meffert, 1971). Hence the results of the preceding experiments suggest that both light reduction and nutrient

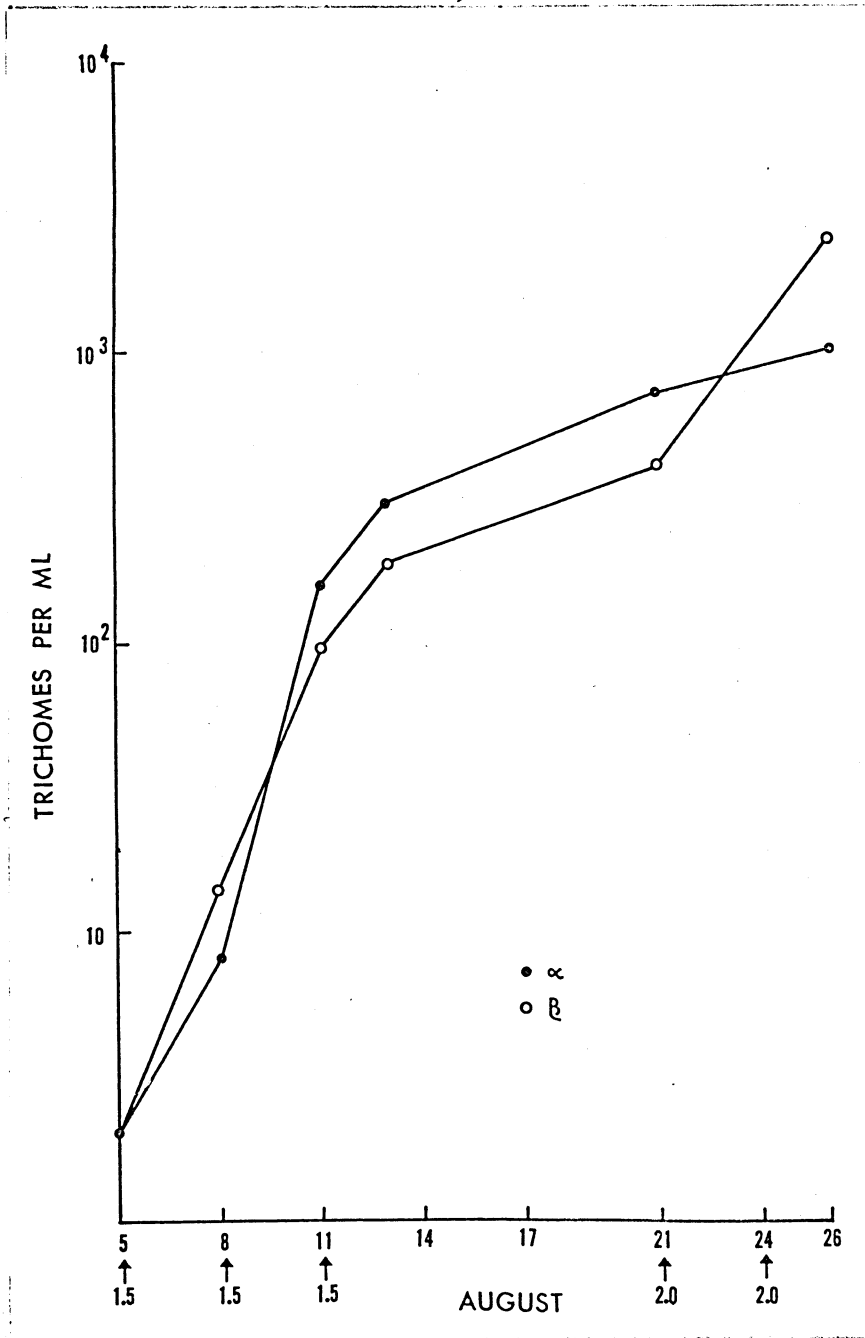


Fig. 8. Changes in the surface concentration of *Oscillatoria* trichomes in two black polyethylene tubes enriched as follows: alpha, 1.5 ppm $\text{NH}_4^+\text{-N}$ on August 5, 8, and 11; beta, 2.0 ppm $\text{NH}_4^+\text{-N}$ on August 21 and 24.

enrichment can enhance buoyancy in this alga to the extent that considerable vertical displacements result effecting drastic changes in vertical distribution not strictly dependent on mixing or in situ growth. Figure 9 shows the Oscillatoria population maximum at mid-summer poised in counter-gradients of light and nutrients (NH_4^+ -N as an example) that, so the above experiments seem to indicate, determine its position. Depth profiles of other essential nutrients, including P and Fe, in Deming Lake have approximately the same form as the NH_4^+ -N depth profiles, at least, in the upper ten meters (Baker, 1971). In the following experiment, the nutrient effect on buoyancy in Oscillatoria was examined more closely.

III. A buoyancy experiment

This experiment was part of a series conducted with A. E. Walby to study the effects of light and nutrients on the buoyancy of O. agardhii var. isothrix as well as the relationship of buoyancy to gas vacuolation in this alga. On September 6, 1972, homogeneous samples taken from the metalimnion at 4.8 m, were suspended in pairs of enriched and control bottles at 3m, 4m, 5m, and 6m. in Deming Lake. The enriched bottles had received 1.0 ppm NH_4^+ -N just prior to suspension. Table 9 presents some light and temperature data from Deming Lake obtained during the course of this experiment. After two days at the selected depths, the bottles were collected, examined and subsampled for relative buoyancy determinations. Upon collection we noted that the algae had clumped and sedimented somewhat in the bottles suspended above

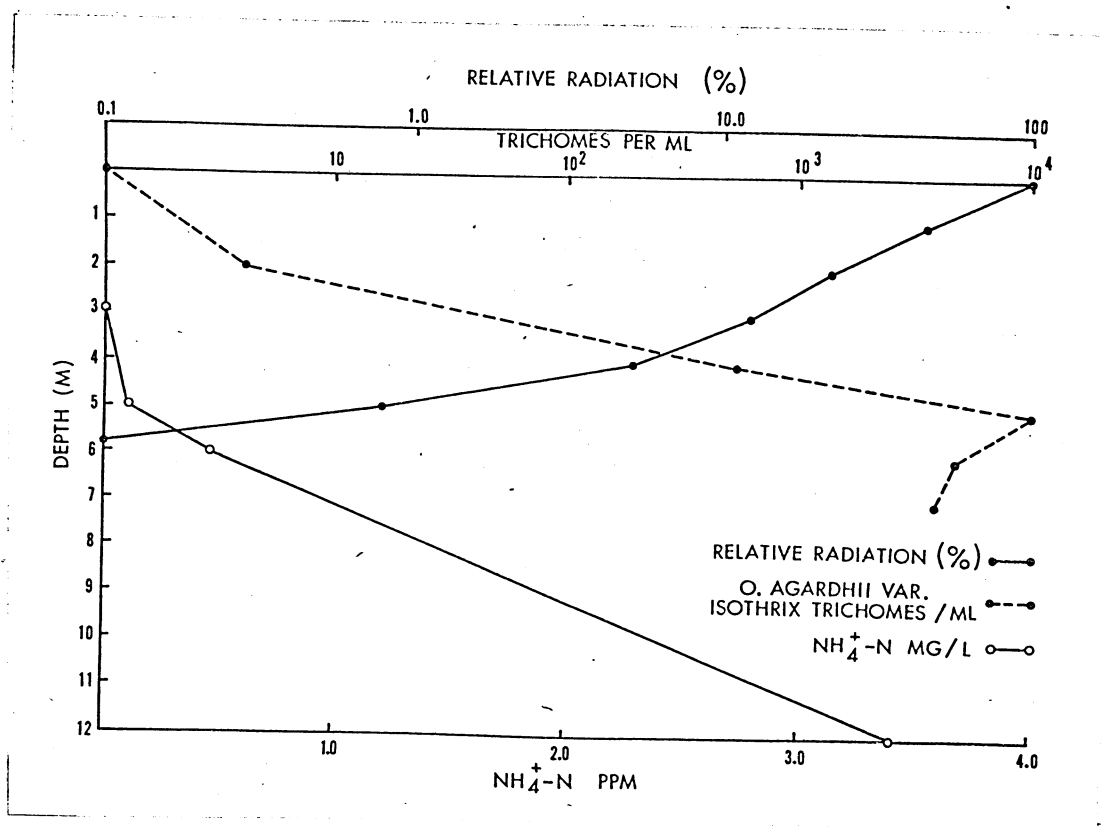


Fig. 9. Depth profiles of light transmission, Oscillatoria concentration and NH_4^+ -N concentration in Deming Lake on July 31, 1972. Both light transmission and Oscillatoria abundance are plotted on log scales.

Table 9. Light and temperature data from Deming Lake,
September 7, 1972

Depth (m)	Relative radiation (%)	Temperature (C)
0	100.0	19.0
1	46.4	17.9
2	25.0	17.0
3	10.7	16.8
4	5.3	16.0
5	0.6	10.6
6	0.1	6.3

5m, and had collected at the surface to a slight degree in the 5m and 6m bottles. The NH_4^+ -enriched samples suspended above five meters had fewer, smaller clumps and less sedimented algae than the controls. No obvious differences were noted between the enriched and control bottles at 5 and 6m.

The subsamples taken for buoyancy determinations were transferred to test tubes. Four subsamples were taken from each control bottle and two from each enriched bottle. Half of the control and enriched subsamples were stored in the dark at 12°C and allowed to sediment overnight. The remaining subsamples were subjected to sufficient pressure to collapse algal gas vacuoles, and then stored overnight under the same conditions as the others. After twelve hours storage, the unpressurized set of subsamples was carefully separated into top, middle and bottom portions, whereas the pressurized set was shaken and allowed to sediment for six hours before separation into top and bottom portions. Table 10 presents the distribution of Oscillatoria trichomes in each test tube expressed as percentages of the total count per test tube. In those subsamples to which pressure was not applied, buoyancy increased with depth of suspension to 5m. At 6m, the controls showed a decrease in buoyancy compared to the controls at 5m, whereas the NH_4^+ -enriched subsamples showed an even greater buoyancy at 6m than at 5m. In fact at all depths of suspension the enriched subsamples exhibited greater buoyancy than the controls in the absence of pressure, whereas the pressurized subsamples showed no notable variations in buoyancy with either treatment.

Table 10. Distribution of O. agardhii trichomes with and without collapsed gas vacuoles within test tubes after two days exposure at different depths in Deming Lake with and without ammonia enrichment

		Unpressurized			Pressurized		
Depth (m)	Level	Controls		NH ₄ ⁺ -N	Controls		NH ₄ ⁺ -N
3	Top	18.6	19.5	34.0	15.0	11.0	13.4
	Middle	20.4	21.0	16.5			
	Bottom	61.0	59.5	49.5	85.0	89.0	86.6
	TOTAL	2900	2936	2976	1496	1976	2288
4	Top	27.4	22.9	40.1	12.8	14.0	20.0
	Middle	15.7	29.5	18.3			
	Bottom	56.9	47.6	41.6	87.2	86.0	80.0
	TOTAL	3436	3376	3260	2344	2256	2512
5	Top	42.8	46.4	52.1	18.6	15.8	19.6
	Middle	25.1	16.3	21.3			
	Bottom	32.1	37.3	26.6	81.4	84.2	80.4
	TOTAL	2900	2644	2596	1696	1792	1800
6	Top	27.9	29.4	59.8	13.1	11.8	12.4
	Middle	38.1	32.4	18.1			
	Bottom	34.0	38.2	22.1	86.9	88.2	87.6
	TOTAL	2876	2888	2748	1584	1992	1744

DISCUSSION

Of the principal factors thought to determine the vertical distribution of "metalimnetic" Oscillatoria species, light and nutrients seem to play the most direct roles in the case of O. agardhii var. isothrix in Deming Lake. Both light reduction and nutrient enrichment under experimental conditions permitted this alga to extend its distribution across thermal gradients. Light reduction in the black polyethylene tubes clearly resulted in enhanced buoyancy with much of the Oscillatoria increase near the surface attributable to recruitment from the metalimnetic stratum. In related studies of stratified Oscillatoria populations, Thomas (1950) found that, during a seiche in Lake Zurich, an O. rubescens stratum overcame the thermocline tilt and took up a horizontal position apparently in response to the light gradient. But he also observed that "light conditions" drove this alga from layers rich in nitrate as a result of upwelling during the seiche. In subsequent experiments with O. rubescens in Lake Baldegg, Thomas (1964) found that this alga tended to rise to the surface in his small-diameter (5.4-5.6cm) cylinders. He observed this phenomenon through the spring and early summer of 1958, but only scarcely or not at all during the latter half of that summer. Although these surface accumulations were most evident when ambient concentrations of N and P were high, the effect was greatest in unenriched dark and clear cylinders, less evident in cylinders enriched with only phosphate or nitrate, and least in a cylinder with added phosphate and nitrate. Such surface accumulations of Oscillatoria were not observed in unenriched

clear plexiglass cylinders or polyethylene tubes in Deming Lake. Species differences aside, a number of differences in the experimental conditions make direct comparisons difficult. For example, Thomas' cylinders were much smaller and provided more stability than the cylinders used in Deming Lake. But greater stability, alone, could not have accounted for the surface accumulations, or they would have continued through the summer. Secondly Thomas did not fill his cylinders with water from a selected stratum until late in the summer. Thirdly Lake Baldegg is a eutrophic lake with a considerable supply of sewage, and according to Thomas (1964), O. rubescens never disappeared from the surface during the summer of 1958. Furthermore, the ambient nutrient concentrations were higher in spring and early summer when surface accumulations occurred than in late summer when they did not. On the other hand, O. rubescens exhibited less buoyancy in the enriched than in the unenriched cylinders; thus, the case for a positive nutrient effect is not clear-cut. In fact, Thomas reported that neither additions of nitrate or phosphate nor a combination of the two increased the growth rate of O. rubescens in his cylinders. Perhaps nutrient additions above certain concentrations inhibit these Oscillatoria species in such small cylinders. At first Thomas enriched the upper 3m of his cylinders with 2.3 to 4.0 ppm NO_3^+ -N and 140 to 330 ppb PO_4^{\equiv} -P, but by the end of August he was using about 8.1 ppm NO_3^+ -N and 580 ppb PO_4^{\equiv} -P. On two occasions in Deming Lake plexiglass cylinders were enriched with more than 2 ppm NH_4^+ -N. In both cases, O. agardhii var. isothrix sedimented as rapidly as in the control cylinders, whereas this effect was not evident in the

larger polyethylene cylinders at even higher ammonia concentrations. Similarly, large additions of nitrate (5-10 ppm N) to plexiglass cylinders in Deming Lake gave negative results during the summer of 1970. Finally, although Oscillatoria grows in laboratory cultures, these algae do seem sensitive to container effects in situ. For example, Thomas (1964) noted that O. rubescens survived much better in his cylinders than in bottles where they tended to decompose, and in Deming Lake, O. agardhii var. isothrix increased for longer periods in the larger polyethylene tubes than in plexiglass cylinders where, at times, other algae became dominant. Also, container size places limits on the duration of experiments and on the length of sampling intervals. Thomas sampled his cylinders every two weeks and may have missed transient responses to enrichment such as those which occurred in plexiglass cylinders in Deming Lake (see Fig. 5).

In any case experiments with black polyethylene tubes in Deming Lake have provided evidence of a relationship between light intensity and the buoyancy of O. agardhii var. isothrix. In so doing, these experiments have corroborated the laboratory findings of Walsby (1969, 70, 71) with Anabaena flos-aquae. Walsby has investigated buoyancy regulation in this alga and has found that gas-vacuole formation and, consequently, buoyancy vary inversely with light intensity. Whereas gas-vacuole formation proceeds to the greatest extent at low light intensity, high light intensity seems to inhibit this process and, at least in certain cases, causes the collapse of previously formed gas vacuoles. According to Walsby (1972), high light intensity acts through the photosynthetic mechanism to

collapse some of the weaker gas-vacuole components, known as gas vesicles, by generating osmotically-active photosynthates and, thereby, increasing cell turgor pressure. The rise in turgor pressure and resultant loss of buoyancy following exposure to high light intensity failed to occur in the absence of CO_2 or in the presence of the photosynthetic inhibitor N-(3,4 dichlorophenyl)-N', N'-dimethyl urea (DCMU) thus implicating photosynthesis in this process. In addition to the turgor-pressure mechanism, light intensity may effect changes in gas-vacuole concentration by simply stimulating growth while inhibiting or merely failing to stimulate gas-vacuole formation. In a non-limiting situation, greater growth at higher light intensities would dilute the concentration of gas vacuoles even if gas-vacuole formation proceeded at a constant rate, that is, independently of light intensity.

In recent papers, Fogg and Walsby have described how such a buoyancy-regulating mechanism would operate to give gas-vacuolate blue-green algae access to adequate nutrients and light (Fogg, 1969; Walsby, 1970; Fogg and Walsby, 1971). In the dim but nutrient-rich environment at the base of the photic zone, gas-vacuole formation would cause the alga to rise in the photic zone allowing it to photosynthesize at higher light intensities until growth and/or increased turgor pressure reduced the alga's gas-vacuole concentration. As the dilution or collapse of gas vacuoles proceeded, the alga would return to the nutrient-rich depths to restock and begin the cycle again. Ganf reported evidence of such a cycle in the diurnal migration of Microcystis in Lake George,

Uganda (Fogg and Walsby, 1971). Fogg and Walsby (1971) have also suggested that some such buoyancy-regulating mechanism may act to poise metalimnetic populations of Oscillatoria at a point in the water column where growth conditions are optimal. They conjectured that relatively small-scale displacements occur about this optimal point due to the high form resistance of Oscillatoria trichomes, whereas, in accordance with Stoke's law, larger aggregates such as those of Anabaena, Microcystis, and Aphanizomenon range further and accumulate more frequently at the surface. But once at the surface, these larger aggregates run the risk of photosynthetic inhibition and loss of buoyancy regulation; thus, though limited to relatively small-scale adjustments, Oscillatoria spp. may possess more refined control of their buoyancy.

The results of the buoyancy experiment showed that buoyancy in the Oscillatoria samples increased with depth of suspension in the photic zone. At the base of the photic zone at 5m, both controls and the enriched sample gave evidence of positive buoyancy, whereas only the enriched sample showed increased buoyancy after two days exposure in virtual darkness at 6m. Notably, the NH_4^+ -enriched samples exhibited greater buoyancy than the controls at all depths of suspension in this experiment. Also in the earlier specific nutrient enrichment experiments, N enrichment, especially with NH_4^+ -N, increased not only growth but also buoyancy as evidenced by reduced sedimentation rates and greater population increases near the surface. The latter were most evident in the NH_4^+ and P enrichment of the "undisturbed" water column enclosed in clear polyethylene tubing. Thus both nutrient enrichment and light-intensity reduction increase the buoyancy of Oscillatoria, but such responses to light and nutrients

are not peculiar to gas-vacuolate blue-green algae. Munk and Riley (1952) found that increased sinking rates accompanied nutrient depletion in diatom cultures. Steele and Yentsch (1960) found increased buoyancy in Skeletonema costatum with placement in the dark and/or with nutrient enrichment, but noted a reversal of this effect with extended darkness. Furthermore, they observed that actively growing laboratory populations exhibited slower sinking rates than slowly dividing or senescent populations, and concluded that algal buoyancy depends on the general physiological condition of the cells. On this basis, they proposed an explanation for seasonal variations in the vertical distribution of chlorophyll, but made no further attempt to explain the mechanism underlying the effects of light and nutrients on buoyancy. Eppley, Holmes and Strickland (1967) observed that non-growing cultures of several marine diatoms and flagellates sank faster than growing cultures. They also found that the nutrient composition of the culture media influenced the sinking rate of Ditylum brightwelli and Stephanopyxis turris. In sedimentation experiments, more than a third of the cells of these two species exhibited neutral buoyancy in von Stosch's medium. Later, Smayda (1970) presented evidence that nutrients reduce the sinking rate of Rhizosolenia setigera and that the effect is age dependent. Recently Kiefer et al. (1972) invoked the findings of Steele and Yentsch to explain deep phytoplankton maxima in Lake Tahoe. In this situation, the phytoplankton maxima consisted mainly of diatoms that apparently sank out of the upper photic zone where nitrogen was limiting into nutrient-rich water near the base of the photic zone where, presumably, their buoyancy increased.

Despite the frequency of published references to nutrient effects on algal buoyancy, attempts to uncover the physiological mechanisms involved have not progressed much beyond Steele and Yentsch's (1960) analysis. For marine diatoms Gross and Zeuthen (1948) and others have suggested selective ion accumulation or discrimination as a means of buoyancy regulation. Among the ions considered advantageous to accumulate is the relatively light ammonium ion (Krogh, 1939; Cooper et al., 1929; Gross and Zeuthen, 1948). This scheme involves the exchange of K^+ for NH_4^+ and the storage of NH_4^+ at a very low pH in large vacuoles, but the mechanism is of dubious significance in freshwater which has a density only 0.003% greater than that of pure water (Lund, 1959; Fogg, 1965). According to Smayda (1970), the ionic discrimination theory may have limited significance even among marine algae. Recently, Conway and Trainor (1972) reported that Scenedesmus cultures grown in NH_4NO_3 -Bristol's medium exhibited a positive buoyancy compared to cultures grown in ordinary Bristol's medium with $NaNO_3$ as the only N source. They observed that cultures in the latter medium had more prominent vacuoles and suggested the possibility of a relationship between larger vacuoles and greater cell density in these cultures.

As for the role of nutrients in the buoyancy regulation of gas-vacuolate blue-green algae, Meffert (1971) found that nutrient deficiency increases the tendency of gas vacuoles in O. rubescens to collapse during centrifugation. She observed further that cultivation at a higher light intensity increases this effect whereas nutrient addition decreases it. In describing his turgor-

pressure hypothesis, Walsby maintained that "... the turgor-pressure rise could result from the amount of carbon fixed in photosynthesis exceeding that which can be assimilated by cells whose net growth-rate is limited by other factors such as temperature or the availability of particular nutrients" (Dinsdale and Walsby, 1972). Thus in a nutrient-limited situation, unassimilated photosynthate would accumulate, increase turgor pressure and, perhaps, collapse gas vesicles. Presumably, as light intensity and photosynthesis increase, the threshold of limitation or critical concentration for some nutrients would increase as well; hence, an alga would require higher nitrogen concentrations, for example, to keep pace with carbon fixation at higher light intensities. Of course, carbon limitation would itself limit the buildup of photosynthates, and carbon addition would aggravate rather than alleviate such a problem. Whatever the mechanism, the data in Table 10 strongly suggest that light and nutrients interact in determining the buoyancy of O. agardhii var. isothrix. Although temperature also varied with depth of suspension in this experiment, the prevailing effect of light is evident in a comparison of Tables 9 and 10 and in the results obtained with black tubes (Figs. 7 & 8).

In the enrichment experiments in Deming Lake, N was the primary limiting nutrient with P limitation apparently dependent upon the exhaustion of stored P. Like other blue-green algae, Oscillatoria has a high cellular N content. Staub (1961) determined that, under optimal experimental conditions, N constituted 5.5% of the dry weight of O. rubescens. When supplied with NO_3^- -N

over an "optimal range" of concentrations (10-28 ppm N), the cellular N content of O. rubescens remained stable at 5.5% whereas higher concentrations resulted in increased cellular N but decreased yield. In comparing his laboratory findings to analyses of the N content of a natural population and its medium, Staub found that although the N content of O. rubescens was usually over-optimal, the N concentrations in the lake water (Lake Zurich) were even lower than the laboratory concentrations required for an optimal cellular N content. Among other things, this may mean that, as Vollenweider (1968) suggested, more N is available than is analytically detectable, or that these algae had access to higher concentrations at greater depths where they had taken up "excess" N. In the latter case, O. rubescens could have incorporated some of the "excess" N into gas vacuoles which have membranes composed entirely of protein (Jones and Jost, 1970) and which may have returned this alga to shallower, less nutrient-rich layers.

As noted in the results, ammonia elicited greater responses than nitrate in enrichment experiments. Perhaps, for O. agardhii var. isothrix in Deming Lake, ammonia is simply a more readily available source of N than nitrate. Nitrate utilization requires a reduction process involving inducible enzymes that require a threshold concentration of NO_3^- for induction and cofactors such as Mo and Mn before they can carry out their catalytic functions, whereas ammonia requires no reduction and, hence, no cofactors or time lag before assimilation. If Mo, for example, were limiting, it would determine the magnitude of response to nitrate enrichment even in a situation of extreme nitrogen limitation. However, even

without Mo limitation, a low ambient nitrate concentration with a correspondingly low concentration of enzymes involved in nitrate reduction would result in a sluggish response to nitrate enrichment while enzyme induction was taking place. Although the experimental evidence that Mo limits nitrate utilization in Deming Lake is not compelling, the hypothesis is at least plausible. Deming Lake is a seepage lake encircled by alder shrubs, and the N-fixing bacteria in the root nodules of these plants compete for Mo entering the lake basin.

In Thomas' experiments (1964) as in mine, P-enrichment within plexiglass cylinders appeared to favor the growth of green algae. Thomas also reported that green algae grew well on the inner walls of his cylinders and that very small green algae utilized nitrates more effectively than other algae when the phosphate concentration was low. In contrast, the addition of P with excess N already present promoted an increase in O. agardhii var. isothrix within a clear polyethylene tube (see Fig. 7). Evidently, after more than a week in the "epilimnion" of the ammonia-enriched tube, these Oscillatoria had depleted their store of excess P.

Although light and nutrients play the most direct roles in determining the vertical distribution of O. agardhii var. isothrix, temperature plays several indirect roles. Physically, thermal stratification separates the epilimnion from the nutrient-rich bottom water, and physiologically, rising temperature hastens the depletion of nutrients in the epilimnion. Another possible physiological effect of increased temperature on algae is lowered nutrient uptake efficiency. Kinetic studies of both nutrient uptake and

growth at different nutrient levels yield K_S values, analogues of the Michaelis constant, that provide measures of uptake or growth efficiencies at low nutrient levels. J. Shapiro (1972) has found that K_S values for uptake of P increase with temperature in a variety of algae. Unless proportional increases occur in the corresponding half maximal uptake velocities of these algae, increased temperature may result in reduced uptake efficiencies at low P levels. Carter and Lathwell (1967) have found two reaction sites for P uptake in excised corn roots, and have shown that the " K_M " of the site dominant at low P concentrations increases with temperature while the corresponding half-maximal velocity does not. Thus higher temperatures nearer the surface reduce nutrient levels and may reduce an alga's efficiency in utilizing nutrients at low levels. Algae with high K_S values for critical nutrients are at a disadvantage in nutrient-depleted situations such as the epilimnia of well-stratified lakes and, therefore, may have to occur near the chemocline in the metalimnion or upper hypolimnion. Perhaps differences in species distributions along chemical gradients reflect differences in K_S values for limiting nutrients. Applied to stratified Oscillatoria populations, this might mean that species occurring deep in the metalimnion do so because their effective K_S 's (their K_S 's as affected by temperature and perhaps also light) for critical nutrients are too high at epilimnetic conditions to utilize the low concentrations there. In fact, the epilimnetic O. limnetica exhibited much lower K_S values for P (1.5 ppb) than two metalimnetic species, O. rubescens (24-37 ppb) and O. agardhii var. isothrix

(66-67 ppb), grown under similar conditions in Dr. J. Shapiro's lab. Because only a small quantity of O. rubescens was available at the time of these determinations, the K_S values for this alga are suspect; the actual values may be closer to those for O. agardhii var. isothrix. These values suggest that low P concentrations could limit O. agardhii var. isothrix and O. rubescens with respect to both growth and buoyancy without limiting other Oscillatoria species, such as O. limnetica and O. redeckii, whose distributions extend into the epilimnion. Stratified algae may well exhibit similar differences in K_S values for N sources. Some preliminary experiments with samples from the metalimnetic Oscillatoria stratum in Deming Lake have indicated relatively high values of K_S for growth with NH_4^+-N (1.4-5.4 μM). Also, results from these experiments have suggested that increases in light intensity have a proportionately greater effect on K_S than on u_{max} in these samples, but other factors may have intervened to limit growth at higher light intensities.

As implied in the introduction, appearances of Oscillatoria species, O. rubescens in particular, have come to be associated with lake enrichment or eutrophication. The results presented here indicate that nutrients affect the vertical distribution of O. agardhii var. isothrix in Deming Lake by limiting growth and buoyancy. A consequence of this nutrient effect on the vertical distribution of Oscillatoria is that nutrient enrichment increases the portion of a lake's photic zone that is inhabitable by this alga and renders the lake more susceptible to invasion. In this manner nutrient enrichment enlarges the potential habitat of this

alga and, if Walsby's mechanism applies, of other metalimnetic Oscillatoria species as well. Oscillatoria may occur more readily in small protected lakes with less allochthonous enrichment because the stable water columns in such lakes permit reducing conditions and, hence, large reservoirs of available nutrients to develop at the base of the photic zone.

LITERATURE CITED

- Adam, F. and A. Birrer. 1943. Biologisch-chemische Studien am Baldeggersee. Mitt. naturf. Ges. Luzern 14.
- Anderson, G.C. 1954. A limnological study of the seasonal variations of phytoplankton populations. Ph.D. Thesis. Univ. Wash. 268pp.
- Baker, A.L., A.J. Brook and A.R. Klemer. 1969. Some photosynthetic characteristics of a naturally occurring population of Oscillatoria agardhii Gomont. Limnol. Oceanogr. 14:327-333.
- Baker, A.L. 1971. Personal communication.
- Baker, A.L. and A.J. Brook. 1971. Optical density profiles as an aid to the study of microstratified phytoplankton populations in lakes. Arch. Hydrobiol. 69:214-233.
- Birge, E.A. and C. Juday. 1922. The inland lakes of Wisconsin. The plankton. I. Its quantity and chemical composition. Wisconsin Geological and Natural History Survey, Bull. No. 64, Scientific Series, No. 13:1-222.
- Brook, A.J., A.L. Baker and A.R. Klemer. 1971. The use of turbidimetry in studies of the population dynamics of phytoplankton populations with special reference to Oscillatoria agardhii var. isothrix. Mitt. Internat. Verein. Limnol. 19:244-252.
- Carter, O.G. and D.V. Lathwell. 1967. Effects of temperature on orthophosphate absorption by excised corn roots. Plant Physiol. 42:1407-1412.
- Conway, K. and F.R. Trainor. 1972. Scenedesmus morphology and flotation. J. Phycol. 8:138-143.
- Cooper, W.C., M.J. Dorcas, and W.J.V. Osterhout. 1929. The penetration of strong electrolytes. J. Gen. Physiol. 12:427-433.
- Dinsdale, M.T. and A.E. Walsby. 1972. The interrelations of cell turgor pressure, gas-vacuolation, and buoyancy in a blue-green alga. Journal of Experimental Botany 23:561-570.
- Duthie, H.C. and J.C.H. Carter. 1970. The meromixis of Sunfish Lake. Jour. Fish Res. Bd. Can. 27:(2):847-856.
- Eberly, W.R. 1959. The metalimnetic oxygen maximum in Myer's Lake. Invest. Indiana Lakes Streams 5:1-46.
- _____ 1964. Further studies on the metalimnetic oxygen maximum with special reference to its occurrence throughout the world. Invest. Indiana Lakes Streams 6(3):103-139.
- _____ 1967. Problems in the laboratory culture of planktonic blue-green algae. In: Environmental Requirments of Blue-green Algae. F.W.P.C.A., Northwest Region, Corvallis, Oregon. 7-34.

- Edmondson, W.T., et al. 1956. Artificial eutrophication of Lake Washington. *Limnol. Oceanogr.* 1:47-53.
- English, S.M. 1962. A limnological study of Sandy Lake, Pa., with special reference to algal stratification and productivity. Ph.D. Thesis, Univ. of Pittsburg, Pittsburg, Pennsylvania.
- Eppley, R.W., R.W. Holmes and J.D.H. Strickland. 1967. Sinking rates of marine phytoplankton measured with a fluorometer. *J. exp. mar. Biol. Ecol.* 1:191-208.
- Findenegg, I. 1943. Untersuchungen uber die okologie und die Produktionsverhaltnisse des Planktons im Karntner Seengebiets. *Int. Rev. Hydrobiol.* 43:368-429.
- _____ 1965. Factors controlling primary productivity, especially with regard to water replenishment, stratification, and mixing. *Mem. Ist. Ital. Idrobiol.*, 18 Suppl.:105-119.
- Fogg, G.E. 1965. *Algal Cultures and Phytoplankton Ecology*. Univ. Wisconsin Press, Madison, Wisconsin.
- _____ 1969. The physiology of an algal nuisance. *Proc. Roy. Soc. B.* 173:175-189.
- _____ and A.E. Walsby. 1971. Buoyancy regulation and the growth of planktonic blue-green algae. *Mitt. Internat. Verein. Limnol.* 19:182-188.
- Gibson, C.E., R.B. Wood, E.L. Dickson, and D.H. Jewson. 1971. The succession of phytoplankton in L. Neagh 1968-70. *Mitt. Internat. Verein. Limnol.* 19:146-160.
- Goldman, C.R. 1962. A method of studying nutrient limiting factors in situ in water columns isolated by polyethylene film. *Limnol. Oceanogr.* 7:99-101.
- Golterman, H.L., Edit. 1969. *Methods for Chemical Analysis of Fresh Waters*. IBP Handbook No. 8. Blackwell Scientific Publications, Oxford and Edinburgh.
- Gross, F. and E. Zeuthen. 1948. Buoyancy of plankton diatoms; a problem of cell physiology. *Proc. Roy. Soc. Edinburgh B.* 135:382-389.
- Hammer, U.T. 1964. The succession of 'bloom' species of blue-green algae and some causal factors. *Verh. int. Ver. Limnol.* 15:829-836.
- Hasler, A.D. 1947. Eutrophication of lakes by domestic drainage. *Ecology* 28:383-395.
- _____ 1964. Experimental limnology. *BioSci.* 14:36-38.
- Hooper, F.F. 1948. The summer standing crop of a small Minnesota lake. Ph.D. Thesis. Univ. of Minn. 220pp.

- _____ 1951. Limnological features of a Minnesota seepage
Lake. Amer. Mid. Nat. 46:462-481.
- Jones, D.D. and M. Jost. 1970. Isolation and chemical characterization of
gas vacuole membranes from Microcystis aeruginosa Kuetz emend.
Elenkin. Arch. Mikrobiol. 70:43-64.
- Kiefer, D.A., O. Holm-Hansen, C.R. Goldman, R. Richards and T. Berman.
1972. Phytoplankton in Lake Tahoe: Deep-Living Populations.
Limnol. Oceanogr. 17:418-422.
- Krogh, A. 1939. Osmotic Regulation in Aquatic Animals. Cambridge
Univer. Press, London, 242pp.
- Lorenzen, C.J. 1966. A method for the continuous measurement of
in vivo chlorophyll concentration. Deep-Sea Research, 13:223-227.
- _____ 1967. Determination of chlorophyll and phaeo-pigments:
spectrophotometric equations. Limnol. Oceanogr. 12:343-346.
- Lund, J.W.G. 1959. Buoyancy in relation to the ecology of the
freshwater phytoplankton. Br. phyc. Bull. 1:1-17.
- _____, C. Kipling and E.D. Le Cren. 1958. The inverted micro-
scope method of estimating algal numbers and the statistical
basis of estimates by counting. Hydrobiologia 11:143-170.
- Maddux, W.S. and R.F. Jones. 1964. Some interactions of temperature,
light intensity, and nutrient concentration during the continuous
culture of Nitzschia closterium and Tetraselmis sp. Limnol.
Oceanogr. 9:79-86.
- Meffert, M.E. 1971. Cultivation and growth of two planktonic Oscillatoria
species. Mitt. Internat. Verein. Limnol. 19:189-205.
- Munk, H.W. and G.A. Riley. 1952. Absorption of nutrients by aquatic
plants. J. Mar. Res. 11:215-240.
- Ravera, O. and R.A. Vollenweider, 1968. Oscillatoria rubescens D.C.
as an indicator of Lago Maggiore eutrophication. Schweiz.
Zs. Hydrologie 374-380.
- Russel-Hunter, W.D. 1970. Aquatic Productivity. Macmillan, New York.
- Ruttner, F. 1937. Limnologische Studien an einigen Seen der Ostalpen.
Archiv. Hydrobiol. 32:167-319.
- _____ 1963. Fundamentals of Limnology. Toronto.
- Saraceni, C. and M. Gerletti. 1968. How deep waters in a large
lake (Lake Maggiore, North Italy) may affect primary productivity.
Mem. Ist. Ital. Idrobiol. 23:141-159.
- Schindler, D.W., F.A.J. Armstrong, S.K. Holmgren, and G.J. Brunskill.
1971. Eutrophication of Lake 227, Experimental Lakes Area,

- N.W. Ontario, by addition of Phosphate and Nitrate. Journal Fish. Res. Bd. Can. 28(11):1763-1782.
- Schroter, C. 1897. Die Schwebeflora unserer Seen. Neujahrsblatt Zurich Naturf. Ges. 57pp.
- Skulberg, O.M. 1968. Studies on eutrophication of some Norwegian inland lakes. Mitt. Internat. Verein. Limnol. 14:187-200.
- Smayda, O.J. 1970. The suspension and sinking of phytoplankton in the sea. Oceanogr. Mar. Biol. Ann. Rev. 8:353-414.
- Staub, R. 1961. Ernährungsphysiologisch-autökologische Untersuchungen an der planktischen Blaualge Oscillatoria rubescens D.C. Schweiz. Z. Hydrol. 23:83-198.
- Steele, J.H. and C.S. Yentsch. 1960. The vertical distribution of chlorophyll. J. Mar. Biol. Ass. U.K. 39:217-226.
- Thomas, E.A. 1950. Auffällige biologische Folgen von Sprungschichtneigungen im Zurichsee. Schweiz. Z. Hydrol. 12:1-24.
- _____ 1964. Nährstoffexperimente in Plankton-Test-Loten. Verh. int. Ver. Limnol. 15:342-351.
- Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton Methodik. Int. Ver. Theor. Angew. Limnol. Verh. 17:47-71.
- Vollenweider, R.A. 1950. Oekologische Untersuchungen von planktischen Algen auf experimentelles Grundlage. Schweiz. Z. Hydrol. 12:194-262.
- _____ 1968. The scientific basis of lake and stream eutrophication, with particular reference to phosphorous and nitrogen as eutrophication factors. Tech. Rep. to O.E.C.D., Paris, DAS/CSI/68 No. 27 (mimeograph). 182pp.
- Walsby, A.E. 1969. The permeability of blue-green algal gas-vacuole membranes to gas. Proc. R. Soc. B. 173:235-255.
- _____ 1970. The nuisance algae: curiosities in the biology of planktonic blue-green algae. Proc. Soc. Wat. Treat. Exam. 19:359-373.
- _____ 1971. The pressure relationships of gas vacuoles. Proc. R. Soc. Lond. B. 178:301-326.
- White, H.L. 1937. The interaction of factors in the growth of Lemna. XI. The interaction of nitrogen and light intensity in relation to growth and assimilation. Ann. Bot. (n.s.) 1:623-647.
- Zimmermann, U. 1969. Okologische und physiologische Untersuchungen an der planktonischen Blaualge Oscillatoria rubescens D.C. unter besonderer Berücksichtigung von Licht und Temperatur. Schweiz. Z. Hydrol. 31:1-58.