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ANALYSIS OF NUTRIENT SUPPLIES FOR ALGAE IN ELEPHANT BUTTE RESERVOIR

Partial Technical Completion Report

Project No. A-040-NMEX

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New Mexico Water Resources Research Institute
in cooperation with
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ABSTRACT

Nutrient supplies for algae in Elephant Butte Reservoir were investigated by chemical analysis of water samples, laboratory bioassays with unialgal cultures, and field experiments in which the effect of nutrient additions on primary productivity of the natural phytoplankton population was determined. Laboratory experiments were conducted with unialgal cultures to quantify algal growth at various concentrations of nitrogen and phosphorus in a Rodhe's nutrient solution. Growth and nitrogen content of algae colonizing artificial substrates suspended in the Reservoir were determined throughout a one year period.

The average nitrate concentration was essentially the same in the lower (near the outlet) and upper Reservoir over a one year sampling period with a mean value of 0.59 mg/liter as nitrogen. The average total phosphate and orthophosphate concentrations were 0.395 and 0.090 mg/liter as phosphorus in the lower Reservoir while average values for total and orthophosphate in the upper Reservoir were 0.642 and 0.176 mg/liter as phosphorus. Condensed phosphates were not detected in water samples analyzed for this form of phosphorus at several times during the year.

Nitrogen was found to be a limiting nutrient for algal growth in all laboratory bioassays of Reservoir water conducted with green algae. Phosphorus was also found to be limiting in

most bioassays of Reservoir water conducted in the laboratory. In field experiments conducted in August, 1972 and July, 1973 nitrogen was found to severely limit primary productivity. In a July, 1973 field experiment phosphorus was also found to be severely limiting. Nutrients did not clearly limit productivity in a field experiment conducted in January, 1973, although this interpretation may have been complicated by possible toxic effects due to the addition of Na_2SiO_3 and iron and trace elements.

Growth of algae colonizing artificial substrates suspended in the Reservoir was correlated with the nitrate concentration of Reservoir water. The nitrogen content of the algal material varied from 0.95% to 4.89% on an ash free dry weight basis and was not correlated with the nitrate concentration of Reservoir water. These observations suggest that nitrogen was limiting accumulation of algal biomass on the artificial substrates.

Cultures of two predominate species of green algae occurring in the Reservoir, Chlamydomonas sp. and Platymonas sp., were found to produce acid and alkaline phosphatases when grown with growth limiting orthophosphate concentrations. These enzymes would enable algae to convert monoesters of phosphoric acid to orthophosphate which then could be utilized by the algae. This is significant because much of the soluble phosphorus in the Reservoir is not in the ortho form.

Growth of algae in nutrient solutions with varying nitrate

concentrations in the laboratory indicated that the nitrate levels in Reservoir water would severely limit growth if other nutrients were present at the levels supplied in the bioassays. Thus the nitrate concentrations measured in the Reservoir substantiated the observation that nitrogen was a limiting nutrient in field experiments and laboratory bioassays. The total phosphate concentration measured in Reservoir water was sufficient to support considerable algal growth based upon laboratory experiments; however, phosphorus was found to be limiting in most laboratory bioassays and in one field experiment. Apparently the total phosphate measured at some sampling times did not correspond to the phosphorus available for algal growth. Thus algal growth in Elephant Butte Reservoir seems to generally be limited by nitrogen while at times phosphorus is also limiting.

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INTRODUCTION

The problem of eutrophication of lakes and reservoirs is receiving increasing attention in relation to environmental pollution and maintenance of water quality suitable for human needs for domestic water supplies, recreation, and agricultural uses of water. This investigation was initiated to evaluate limiting nutrients for algal growth in Elephant Butte Reservoir which might explain the absence of algal blooms and also the infrequent occurrence of blue-green algae in this relatively nutrient rich, warm water Reservoir. Nutrient supplies for phytoplankton in the generally richer and more productive upper Reservoir were compared to the less productive lower Reservoir near the outlet in search of an explanation for the differences in primary productivity and phytoplankton abundance in the upper and lower Reservoir.

Nitrate, total phosphate and orthophosphate concentrations were determined in membrane filtered water from the upper and lower Reservoir to chemically characterize the supplies of these nutrients over a one year period.

Field and laboratory experiments were conducted to determine which nutrients limited phytoplankton growth and productivity. In field experiments the influence of nutrient additions on primary productivity of the natural phytoplankton population was determined. Unialgal cultures were used in laboratory bioassays of Reservoir water with various nutrient additions.

The growth of several species of algae, including two of

the predominate species isolated from Elephant Butte Reservoir, was measured in laboratory cultures with varying concentrations of nitrogen and phosphorus in the culture medium. The production of the enzymes acid and alkaline phosphatase, which are involved in the utilization of organic phosphate compounds was investigated in algal cultures grown with various phosphate concentrations.

The growth rate and nutrient contents of algae colonizing artificial substrates suspended in the Reservoir were determined to provide estimates of cellular levels of nutrients in algae growing in the Reservoir.

MATERIALS AND METHODS

Nitrate and phosphate content of Reservoir water

From August, 1972 through July, 1973, surface water samples were collected from the Reservoir on a monthly basis for analysis for the nutrients nitrogen and phosphorus. Samples were collected at the boomline near the dam, representing the lower Reservoir, and at a station representing the upper Reservoir. The upper Reservoir sample was obtained about 4 miles north of Castle Rock except during the summer of 1972 when storage in the Reservoir was very low, at which time the upper Reservoir sample was obtained at Castle Rock. These sites correspond to Stations I (boomline), IV (4 miles north of Castle Rock), and III (at Castle Rock) as sampled by Kidd and Johnson (1971).

Water samples were filtered through a 0.45 micron membrane filter soon after collection and refrigerated until the analyses were performed on the day after sample collection. Nitrate nitrogen was measured by an ultraviolet absorption procedure (APHA, 1971). Total and orthophosphate were determined by a molybdenum blue procedure (Hach).

Water temperature and transparency

The surface water temperature and Secchi disk transparency were determined when samples were collected for nutrient analysis.

Algal growth on artificial substrates

Plastic plates (14x10x0.3 cm) were suspended in the Reservoir just below the surface and at a depth of 1 meter at the boomline (Station I) to measure algal growth rates and nutrient contents. The plastic plates were ordinarily collected on a monthly basis, and returned to the laboratory until the algae was removed from the plates for analysis. Four replicate plates were suspended at each depth; however, as a result of equipment failure or in some cases vandalism some or all the plates were lost during several months.

The algae and associated sediment was scrapped off the plates with a razor blade and washed into a beaker with redistilled water. The water was driven off by heating the beaker at 55-60C on a sand bath and the dry weight of the algal residue was then determined. The ash-free dry weight of the residue was determined by heating a sub-sample in a muffle furnace at 550C overnight. The total nitrogen (including nitrate) content of the sample was determined by a Kjeldahl digestion procedure followed by distillation and titration of the ammonium (Humphries, 1956). The total phosphorus content of one group of samples was determined by a molybdenum blue procedure after the samples were digested with nitric and sulfuric acid (Chapman and Pratt, 1961).

Nutrient studies in the Reservoir

Nutrient studies were conducted by adding one or several nutrients to 2.0-2.3 liters of Reservoir water containing the natural population of planktonic organisms (Goldman, 1960). The

bottles of water were collected and incubated at the surface at the boomline for 2 to 10 days. Phytoplankton productivity in these bottles was measured by removing sub-samples from each large bottle initially and at intervals during the incubation period and measuring productivity over a 5 to 6 hour period by the ^{14}C method. Duplicate light bottles and a dark bottle containing ^{14}C were prepared from each large bottle. The ^{14}C procedure was identical to that used by Kidd and Johnson (1971) except that the radioactivity fixed on the filters was measured with a liquid scintillation counter in 10 ml of 0.4% PPO in toluene (Lind and Campbell, 1969).

Nutrients were added to Reservoir water in concentrations based on a modified Rodhe's No. 8 nutrient solution for the growth of algae (Table 1). Rodhe's No. 8 solution was selected because it has been reported to be a satisfactory solution for growth of a wide variety of algal species and it is a dilute nutrient solution thus more closely representing natural nutrient levels than the higher concentrations of nutrients commonly used in the laboratory for growth of algae. In addition to the inorganic salts added, a combination of the vitamins thiamine, biotin, and vitamin B_{12} was added to give final concentrations of 1000, 1.0, and 1.0 µg/liter respectively in Reservoir water.

In experiments conducted in August and October, 1971, treatments included a control without added nutrients, addition of the complete Rodhe's solution, and addition of each component

Table 1. Nutrient solutions for field and laboratory experiments

Modified Rodhe's No. 8 Nutrient Solution Salt	Concentration mg/liter
$Ca(NO_3)_2$	60.0
$MgSO_4$	5.0
Na_2SiO_3	20.0
K_2HPO_4	5.0
Iron and trace element solution	*

PG Nutrient Solution Salt	Concentration mg/liter
KNO_3	1,210.
$MgSO_4$	120.
NH_4Cl	53.
$Ca(NO_3)$	16.7
K_2HPO_4	973.
Iron and trace element solution	*

*1.0 ml/liter of the following stock solution of chelated iron and trace elements (in g/100 ml): H_3BO_3, 1.00; $CuSO_4 \cdot 5H_2O$, 0.15; Na_2EDTA, 5.00; $ZnSO_4 \cdot 7H_2O$, 2.20; $CaCl_2$, 0.50; $MnCl_2 \cdot 4H_2O$, 0.50; $FeSO_4 \cdot 7H_2O$, 0.50; $CoCl_2 \cdot 6H_2O$, 0.15; $(NH_4)_6 Mo_7O_{24}$, 0.01; (Stein, 1958).

(K_2HPO_4, $Ca(NO_3)_2$, $MgSO_4$, Na_2SiO_3, vitamins, and chelated iron and trace elements) individually to a bottle of Reservoir water. Subsequent experiments conducted in August, 1972 and January, 1973 included a control, addition of a complete Rodhe's solution, and additions of Rodhe's solution with one or two components deleted. In the final nutrient experiment conducted in July, 1973 the K_2HPO_4 and chelated iron and trace elements were used both at full and 10% of the Rodhe's concentration. In this experiment treatments included a control, addition of complete nutrient solutions, and additions of solutions with a single component omitted. Sodium silicate was not added to any of the bottles in this experiment.

In earlier experiments alkalinity was determined for the control sample on the initial day of the experiment and then on a sub-sample of water from each bottle on subsequent days. In the January and July, 1973 experiments alkalinity was determined on sub-samples from each bottle initially as well as on the following days.

Nutritional studies in the laboratory

Algae were cultured in the laboratory to establish satisfactory nutrient solutions for the growth of purchased algal cultures and unialgal cultures isolated from Elephant Butte Reservoir water. Algae were grown in 50 ml culture tubes with continuous fluorescent illumination at 500 footcandles and continuous aeration with a stream of 3% CO_2 in air. Forty ml of modified Rodhe's No. 8 and chelated iron and trace elements were used in most experiments (Table 1). Glassware and nutrient solutions were sterilized by

autoclaving and aseptic techniques were employed; however, cultures were not axenic.

Scenedesmus dimorphus, Chlamydomonas debaryana var. cristata, and Anabaena flos-aquae purchased from the Indiana University stock culture collection and Chlamydomonas sp. and Platymonas sp. isolated from Elephant Butte Reservoir were used in laboratory experiments. Growth of cultures was measured by cell counts using a hemocytometer, optical density measurements of suspended cells at 600 nm, or by determination of the dry weight of cells collected on a 0.45 micron membrane filter.

Initial experiments involved the measurement of the growth rate of various algal species in Rodhe's solution. Subsequent experiments involved measurement of the growth of purchased and isolated algal species in Rodhe's solution with varied concentrations of $Ca(NO_3)_2$ or K_2HPO_4. In these experiments $Ca(NO_3)_2$ was replaced by an equal molar concentration of $CaSO_4$ and K_2HPO_4 was replaced by an equal molar concentration of K_2SO_4.

In other experiments algal cultures were grown in membrane filtered (0.45 micron) and non-filtered Reservoir water to identify limiting nutrients. In these experiments Reservoir water was supplimented with the complete Rodhe's concentration of nutrients or Rodhe's solution with a single component deleted. Cultures were started by addition of a small volume, usually 0.1 ml, of complete Rodhe's solution containing actively growing algal cells.

Laboratory experiments were conducted to evaluate the capacity of algal cultures to produce the enzymes acid and alkaline phosphatase and thus to break down organic phosphate compounds (monoesters of phosphoric acid). The algae were cultured as in the nutritional studies, however; the PG medium with the level of K_2HPO_4 varied from 0.1 to 100% of full strength was used in place of Rodhe's medium (Table 1). Phosphatase activity was measured as the culture's ability to hydrolyze p-nitrophenyl phosphate over a 30 minute period following the procedure described by Kuenzler and Perras (1965). Phosphatase activity measured at pH 6.8 is ascribed to acid phosphatase and activity at pH 9.0 is considered to be alkaline phosphatase. Growth of these cultures was evaluated by optical density measurements at 600 nm.

RESULTS AND DISCUSSION

Nitrate and phosphate content of Reservoir water

The nitrate content of membrane filtered surface water is presented in Fig. 1 and 2 and Table 2 for the lower Reservoir (Station I) and the upper Reservoir (Station III or IV) from August, 1972 through July, 1973. Nitrate nitrogen ranged from 0.27-0.93 mg/liter and 0.24-0.95 mg/liter in the lower and upper Reservoir respectively. Mean values for the one year sampling period were 0.591 and 0.592 mg/liter for the lower and upper Reservoir respectively. Monthly sampling of both the lower and upper Reservoir indicate that highest nitrate nitrogen concentrations occurred in fall and winter with a decline to lower levels in March to May followed by an increase in early summer. That nitrate concentrations can change very rapidly is clearly indicated by the data obtained by sampling at two to three day intervals in August, 1972.

Fig. 3 and 4 and Table 3 present data for total and orthophosphate concentration of membrane filtered surface Reservoir water for the lower and upper Reservoir from August, 1972 through July, 1973. The total phosphate concentration ranged from 0.058 to 1.45 mg/liter as phosphorus in the lower Reservoir and from 0.074 to 2.02 in the upper Reservoir. Mean values for the one year sampling period were 0.395 and 0.642 mg/liter for the lower and upper Reservoir stations respectively. Orthophosphate concentrations ranged from 0.00 to 0.255 mg/liter as phosphorus for the lower Reservoir and 0.005 to 1.04 mg/liter for the upper

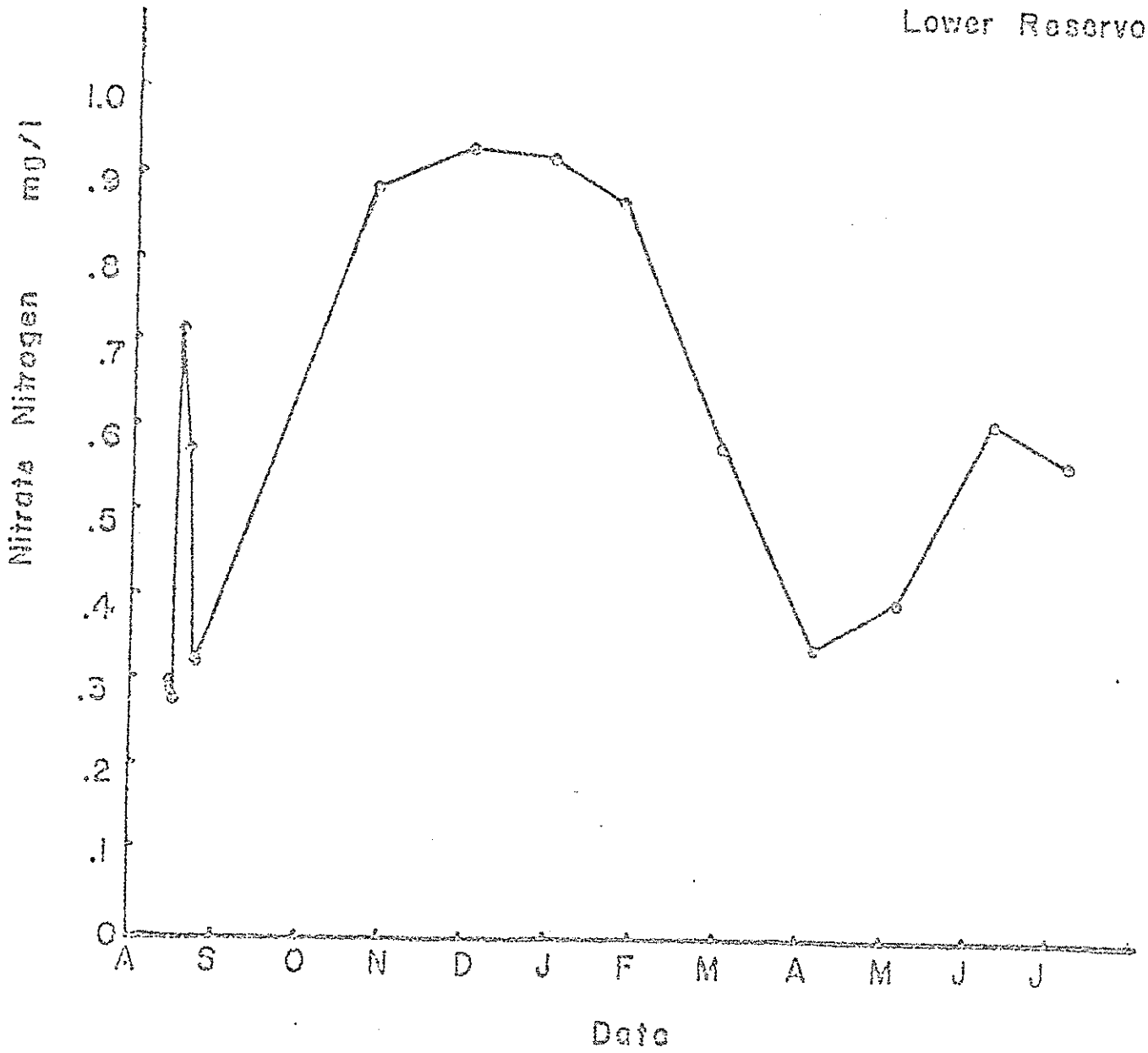


Fig. 1. Nitrate nitrogen content of water from the lower Reservoir (Station I) from August, 1972 through July, 1973. Points represent means for three replicate samples.

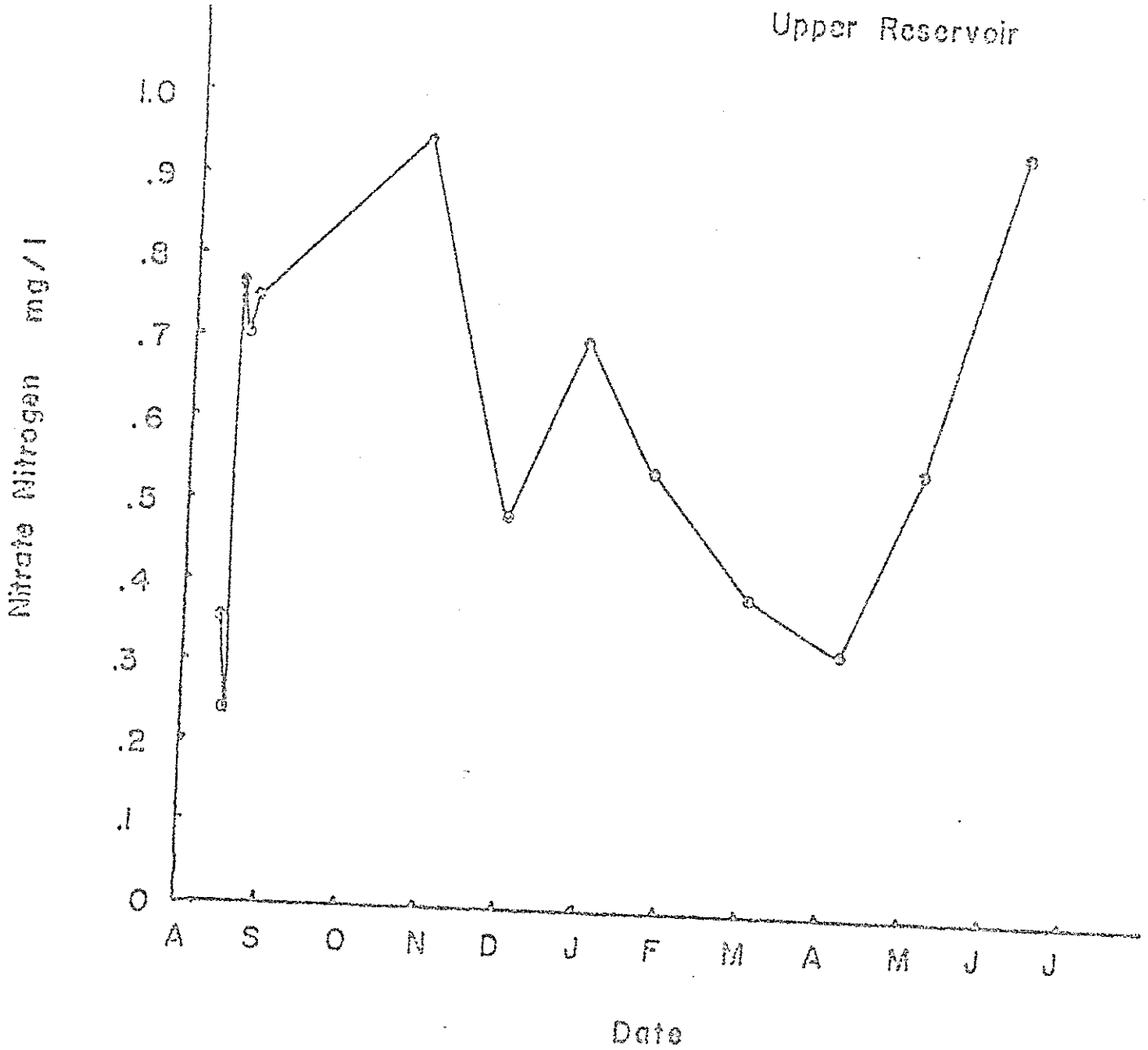


Fig. 2. Nitrate nitrogen content of water from the upper Reservoir from August, 1972 through June, 1973. Samples were obtained from Station IV except for August, 1972 when Station III was sampled. Points represent means of three replicate samples.

Table 2. Total dissolved nitrate nitrogen in the lower (Station I) and upper Reservoir (Station III or IV) from August, 1972 through July, 1973. Values are mean mg nitrogen/liter (N=3).

Date	Lower Reservoir	Upper Reservoir
8/14/72	0.296±0.008[1]	0.352±0.009
8/16	0.273±0.054	0.235±0.009
8/18	0.711±0.042	0.765±0.036
8/21	0.571±0.018	0.702±0.027
8/24	0.319±0.009	0.748±0.008
10/26	0.881±0.013	0.945±0.009
11/30	0.930±0.015	0.475±0.015
12/29	0.920±0.006	0.702±0.027
1/25/73	0.869±0.052	0.544±0.004[2]
3/1	0.582±0.060	0.392±0.008
4/5	0.345±0.016	0.326±0.010
5/3	0.400±0.030	0.553±0.008
6/8	0.615±0.056	0.947±0.033
7/6	0.567±0.020	- - -
$\bar{X}$	0.591	0.592

[1] Standard error.

[2] N=2.

Reservoir. The average orthophosphate concentrations were 0.090 and 0.176 mg/liter phosphorus respectively for the lower and upper Reservoir. The percentage of the total dissolved phosphorus in the ortho form ranged from 0 to 100% with a mean of 29% at the lower Reservoir station and from 1.5 to 84% with a mean of 23% at the upper end of the Reservoir.

At the lower Reservoir station the total phosphate concentration exhibited a very high value in late January and then declined to minimum values in April and May followed by somewhat higher values during the summer months. In the upper Reservoir the total phosphate concentration remained high (between 1 and 2 mg/liter as phosphorus) from the end of November through early March then dropped to a minimum level in April. This was followed by an increase to intermediate levels in May and the summer months.

In the lower Reservoir the orthophosphate level remained below 0.1 mg/liter except in August, 1972 when concentrations above 0.2 mg/liter were measured. In the upper Reservoir the orthophosphate concentration was above 0.8 mg/liter in late November and December and then declined to less than 0.2 mg/liter in January and March. Orthophosphate concentrations were quite low (below 0.03 mg/liter) from May through the summer months. With few exceptions the orthophosphate concentration did not reflect the large flucuations in the total phosphate concentration.

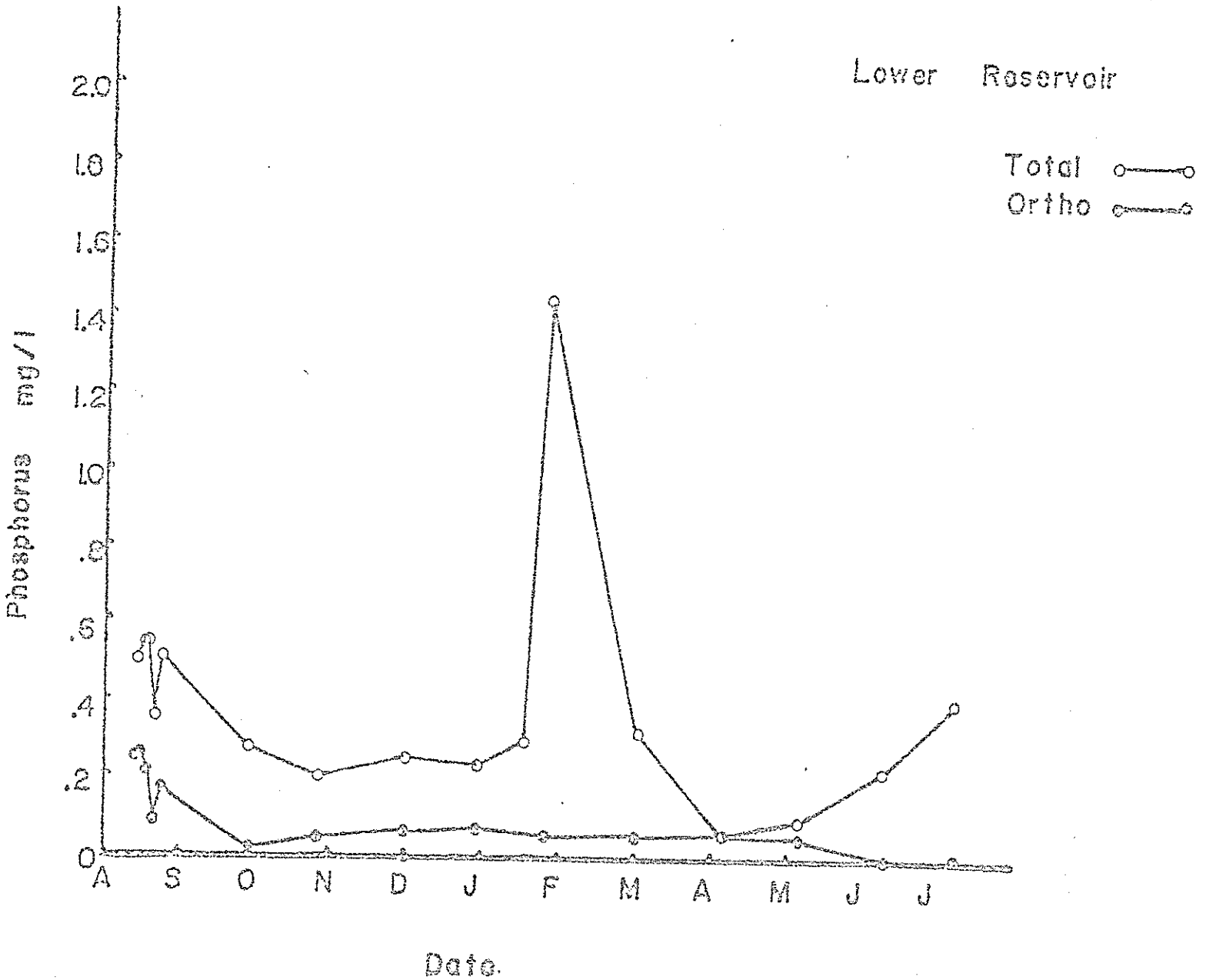


Fig. 3. Total phosphate and orthophosphate content of water as phosphorus from the lower Reservoir (Station I) from August, 1972 through July, 1973. Points represent means for three replicate samples.

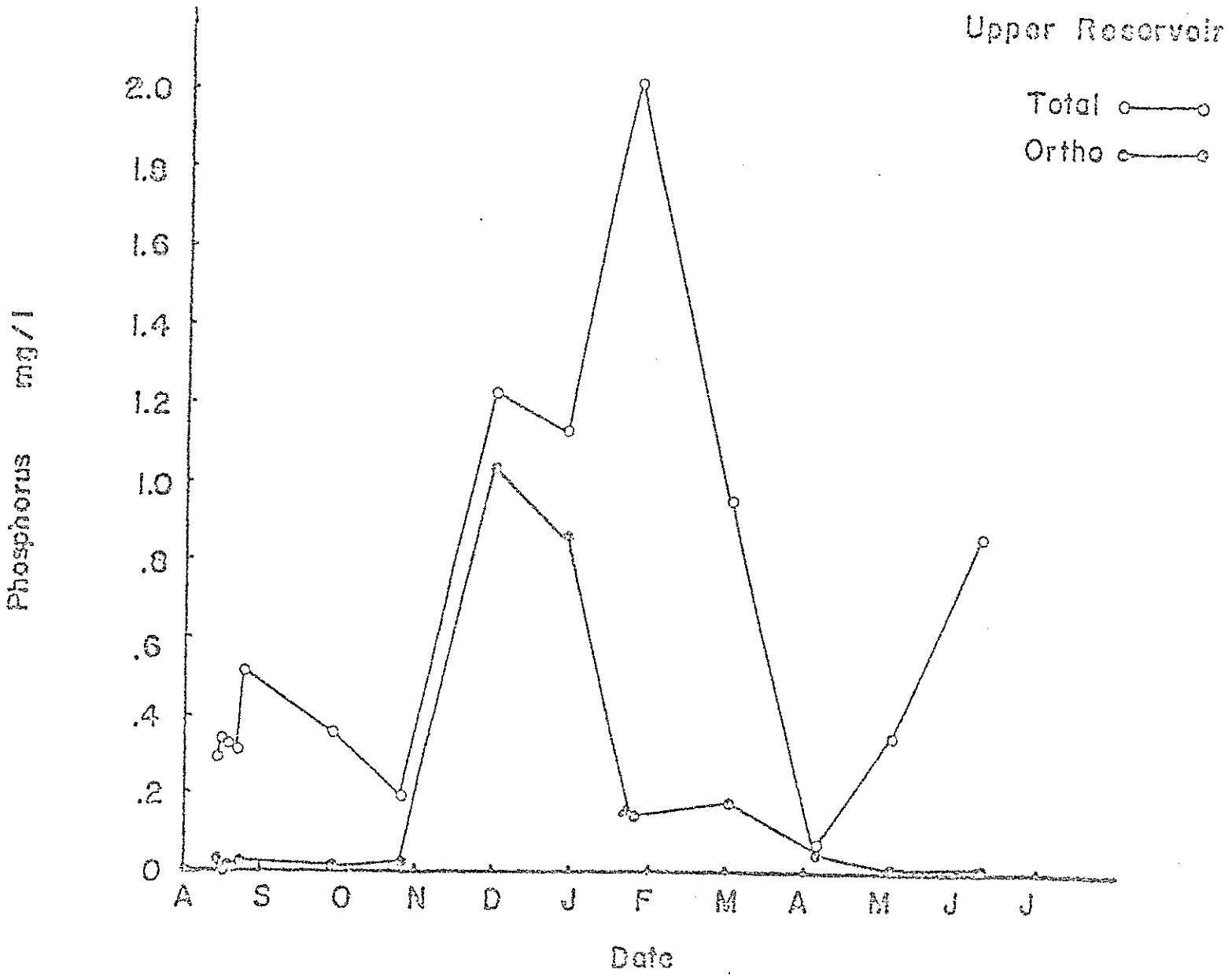


Fig. 4. Total phosphate and orthophosphate content of water as phosphorus from the upper Reservoir from August, 1972 through June, 1973. Samples were obtained from Station IV except for August, 1972 when Station III was sampled. Points represent means for three replicate samples.

Table 3. Total dissolved phosphate and orthophosphate as phosphorus. Values are mean mg phosphorus per liter (N=3).

Date	Lower Reservoir		Upper Reservoir	
	Total	Ortho	Total	Ortho
8/14/72	0.511±0.010[1]	0.243±0.010	0.296±0.025	0.029±0.011
8/16	0.548±0.064	0.255±0.046	0.345±0.029	0.005±0.003
8/18	0.553±0.048	0.216±0.003	0.327±0.030	0.020±0.008
8/21	0.347±0.017	0.084±0.003	0.315±0.022	0.018±0.011
8/24	0.531±0.016	0.162±0.000[2]	0.518±0.011	0.029±0.012
9/28	0.278±0.21	0.010±0.003	0.360±0.014	0.016±0.005
10/26	0.200±0.017	0.040±0.003	0.191±0.013	0.025±0.009
11/30	0.254±0.045	0.060±0.004	1.233±0.009	1.040±0.046
12/29	0.227±0.020	0.068±0.006	1.128±0.009	0.861±0.008
1/17/73	0.300±0.014	---	---	---
1/25	1.447±0.437	0.049±0.009	2.023±0.002[2]	0.152±0.013[2]
3/1	0.320±0.026	0.051±0.003	0.959±0.122	0.184±0.004
4/5	0.058±0.005	0.058±0.004	0.074±0.004	0.059±0.002
5/3	0.097±0.004	0.053±0.0	0.356±0.016	0.015±0.001
6/8	0.230±0.003	0	0.868±0.024	0.020±0.003
7/6	0.414±0.012	0	---	---
$\bar{X}$	0.395	0.090	0.642	0.176

[1] Standard Error

[2] N=2

In addition to determination of total and orthophosphate, condensed phosphate was determined in filtered water samples collected from the lower Reservoir in July, 1972 and from both lower and upper Reservoir stations in October and December, 1972 and January, 1973 (APHA, 1971). The measurement of condensed phosphate in the January 25, 1973 samples was of particular interest since both the lower and upper Reservoir stations exhibited the maximum total phosphate concentrations on this date while the orthophosphate concentration was quite low. Determinations on all of these samples indicated that the water did not contain detectable amounts of condensed phosphates (polyphosphates) and that the dissolved phosphate in the Reservoir was in the ortho and organic forms. Thus these samples did not contain detectable amounts of undegraded phosphate compounds derived from detergents.

Water temperature and transparency

Seasonal variation in surface water temperature in the lower Reservoir from August, 1972 through July, 1973 is depicted in Fig. 5. Temperature in the lower Reservoir ranged from a low of 6C in January to highs of 28C in July and August. Measurements in the upper Reservoir (Station IV) were made from late September through June with a minimum of 5C in January and a maximum of 25C in June (Fig. 5).

Secchi disk transparency for lower and upper Reservoir stations is depicted for August, 1972 through July, 1973 in Fig. 6. Secchi disk transparency was less than 100 cm in the lower Reservoir from August into January and then increased to

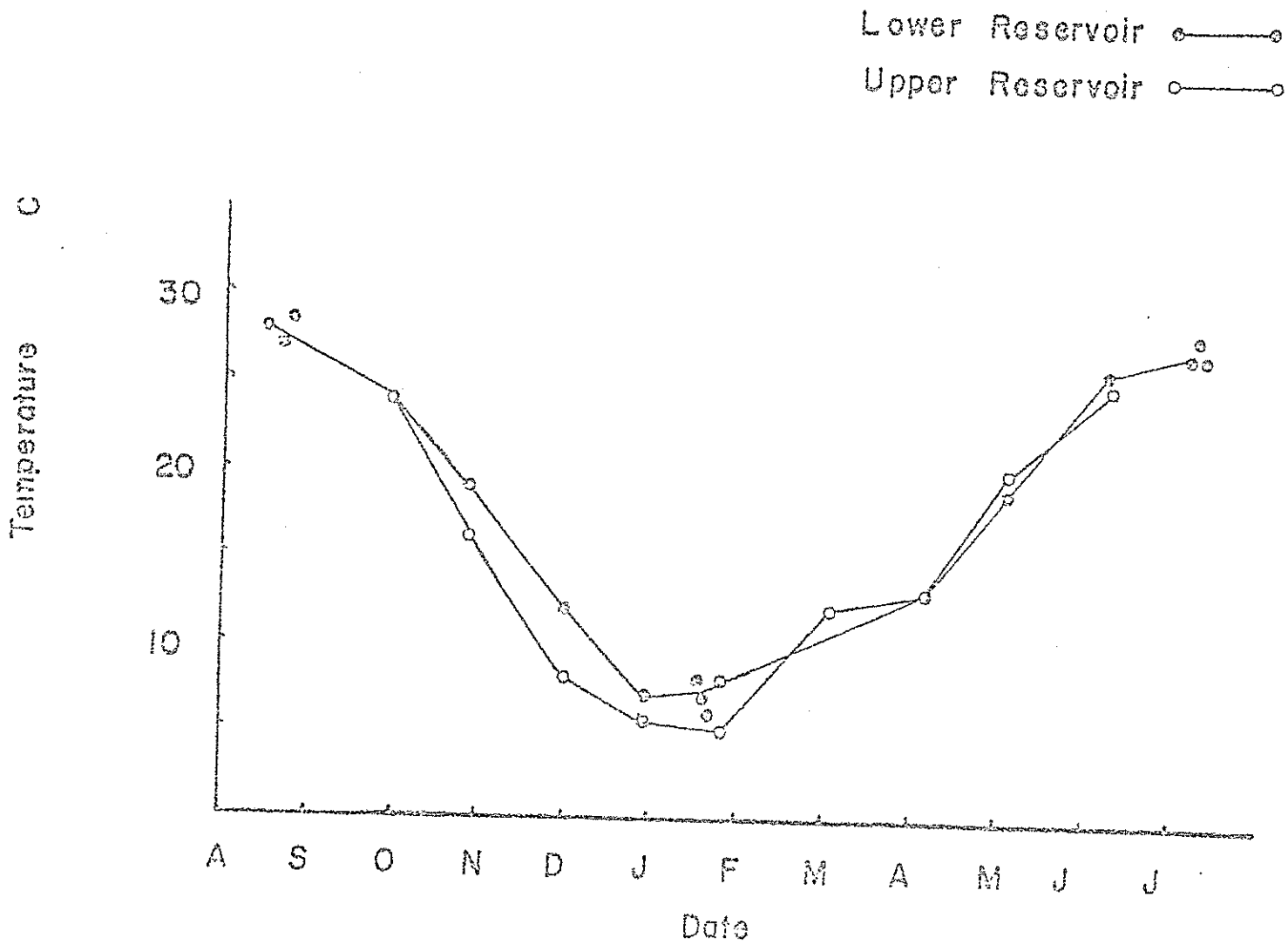


Fig. 5. Temperature of surface water of lower (Station I) and upper (Station IV) Reservoir from August, 1972 through July, 1973.

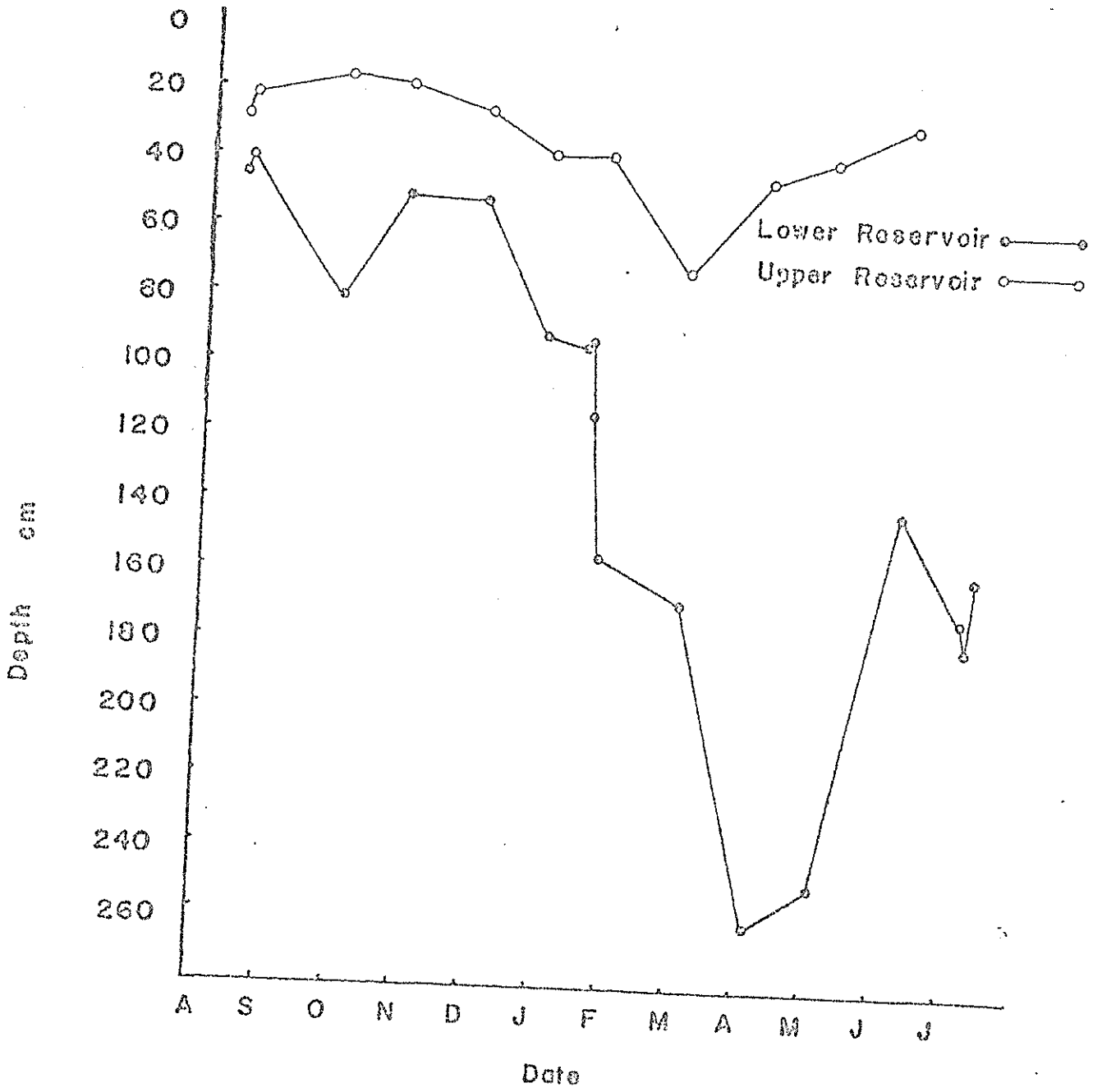


Fig. 6. Secchi disk transparency in lower and upper Reservoir from August, 1972 through July, 1973. The lower Reservoir was sampled at Station I and the upper Reservoir was sampled at Station IV except in August, 1972 when Station III was sampled.

maximum values in April and May (261 and 279 cm respectively) and then decreased in June and July. Transparency in the upper Reservoir was always very limited reaching a maximum of 71 cm in March. The large sediment load and reduced transparency is one of the dominant factors influencing productivity in the Reservoir (Kidd and Johnson, 1971).

Algal growth on artificial substrates

Fig. 7 and Table 4 summarize the results for algal growth measurements on artificial substrates suspended just below the surface and at a depth of 1 meter in the lower Reservoir (Station I). The sampling date indicates the date plates were removed from the Reservoir, hence growth on the plates reflects conditions for the time interval prior to removal of the plates. Plates were suspended in the Reservoir for one month except those sampled 24 August, and 28 September, 1972 which remained in the Reservoir 10 and 16 days respectively.

Two measures of total growth on the artificial substrates are reported in Table 4, total dry weight and ash free dry weight. Because of the large and variable amount of sediments associated with the algal mass, the ash free dry weight provides the best measure of algal growth and biomass. In Fig. 7 it is apparent that the ash free dry weight developed on the plates suspended at the surface was greatest with the September sampling and then decreased for the November and December samplings. This was followed by a slight rise in ash free dry weight in March, a

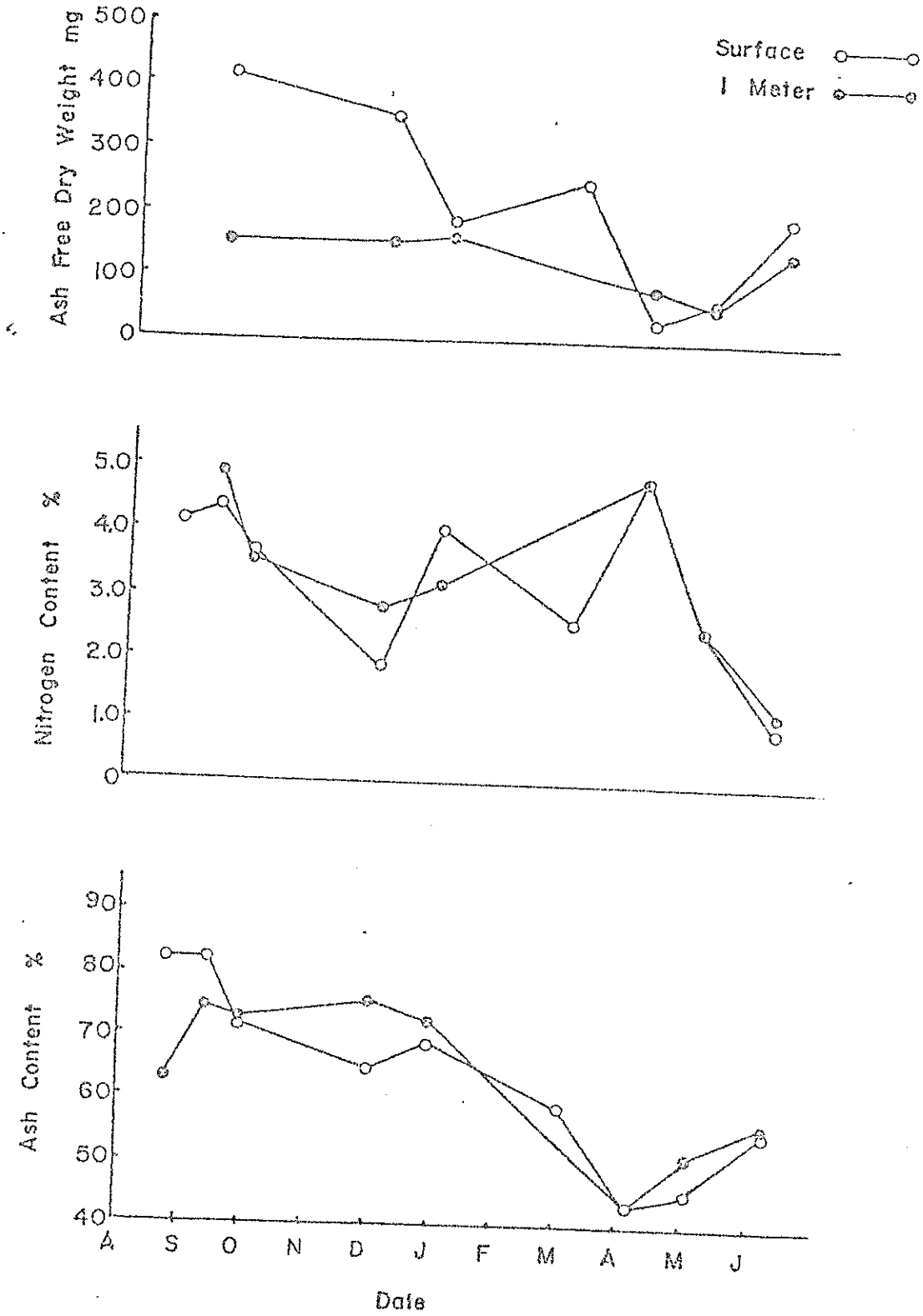


Fig. 7. Ash free dry weight, nitrogen content on an ash free dry weight basis, and percent ash content of algal growth on artificial substrates from August, 1972 through June, 1973. Ash free dry weight is given only for samples collected after 1 month of growth.

Table 4. Algal growth on artificial substrates suspended in the lower Reservoir. Plates were ordinarily collected after one month in the Reservoir. Values are means for the samples collected on each date. When samples were small replicate samples were combined for ash free dry weight and nitrogen determinations.

Date	Depth meters	Total Dry Wt. g	Ash Free Dry Wt. g	Ash Content %	N Content (total sample) %	N Content (ash free dry wt.) %	Plates Collected
8/24/72[1]	S[2]	0.452±0.042[3]	0.088	82.1	0.74	4.11	2
	1	0.053±0.027	0.018±0.006	63.2±7.4	--	--	1
9/12	S	2.340±0.014	0.415±0.006	82.3±0.1	0.78±0.00	4.39±0.01	2
	1	0.612±0.041	0.154±0.009	74.8±0.2	1.23±0.05	4.89±0.23	2
9/28[4]	S	0.590±0.022	0.167±0.008	71.7±0.4	1.04±0.01	3.67±0.03	2
	1	0.497±0.016	0.135±0.008	72.8±0.9	0.95±0.07	3.52±0.31	4
11/30	S	0.994±0.103	0.351±0.045	64.9±1.5	0.66±0.04	1.88±0.16	4
	1	0.638±0.047	0.156±0.017	75.6±1.4	0.67±0.04	2.77±0.22	4
12/29	S	0.665±0.138	0.190±0.038	69.0±2.5	1.21±0.13	4.01±0.60	4
	1	0.635±0.089	0.165±0.019	72.5±1.6	0.86±0.03	3.17±0.18	4
3/1	S	0.617	0.252	59.1	1.05	2.57	1
4/5	S	0.057±0.027	0.032	43.5	2.73	4.84	3
	1	0.151±0.018	0.086	43.4	2.74±0.17	4.83±0.29	4
5/3	S	0.150±0.014	0.068±0.007	54.3±2.5	1.13±0.14	2.50±0.44	4
	1	0.112±0.002	0.058±0.003	48.5±3.7	1.28±0.03	2.50±0.12	4
6/8	S	0.447±0.090	0.198±0.038	55.3±0.6	0.42±0.01	0.95±0.04	4
	1	0.342±0.039	0.148±0.018	56.6±0.3	0.52±0.03	1.19±0.08	3

[1]Plates were in Reservoir for 10 days.

[2]S = surface.

[3]Standard error.

[4]Plates were in Reservoir for 16 days.

decrease to very low levels in April and May, and then an increase in June.

The ash free dry weight produced on plates suspended at a depth of 1 meter varied only slightly in the September, November, December, and June samplings; however, as in the case of the surface samples very low values were obtained in April and May. The accumulation of algal biomass in September and November was two to three times greater on the surface plates than the plates suspended at 1 meter. In the other samplings the accumulation of algal biomass differed only slightly between surface and 1 meter plates except in April when more than twice the mass was accumulated at 1 meter compared to surface plates.

Total dry weights (Table 4) accumulating on the plates showed similar trends with time as did the ash free dry weights; however, considerable variation in the ash content was observed throughout the period of sampling (Table 4 and Fig. 7). The percent ash content of surface and 1 meter samples ranged from 63 to 82% in August through January and then decreased with a minimum percent ash content of 43.4% being observed in April. The percent ash content then gradually increased in May and June to 56.3%. The lower ash content of the March, April and May samples corresponded to a period of time in which Secchi disk transparency in the lower Reservoir (Fig. 6) increased reaching maximum values in April and May.

The nitrogen content of the algal mass on an ash free dry weight basis varied from 0.95 to 4.89% at different sampling dates (Table 4 and Fig. 7). On most sampling dates the nitrogen contents of surface and 1 meter samples were very similar. Samples with high nitrogen contents on an ash free basis (September and April) do not correspond to periods of higher nitrate concentration in the water nor do samples of lowest nitrogen contents (December and June) correspond to low nitrate concentrations. The low algal biomass in April cannot be attributed to limited nitrogen since this was at a maximum in the algal mass at this time although it decreased greatly in May, another period of low biomass. Possibly the minimal phosphate concentration in the lower Reservoir in April and May provides an explanation for the low yield of algal biomass in these months.

Correlations of the measurements of algal growth on artifical substrates and date, Secchi disk reading, surface water temperature, nitrate concentration, total phosphate concentration and ortho-phosphate concentration at Station I at the time the plates were sampled are presented in Table 5. Correlation coefficients greater than 0.482 are significant at the 95% level.

Of particular interest is the significant positive correlation of nitrate concentration and ash free dry weight of the algal samples ($r=0.506$). The nitrogen content of the algal material, either on a total weight basis or on an ash free dry weight basis, was not significantly correlated with the nitrate concentration of

Table 5. Correlation matrix between measurements on algal samples from artifical substrates suspended in the Reservoir and chemical and physical measurements made in the lower Reservoir. The variables are date of sample collection (A), depth of plate (B), total dry weight (C), ash free dry weight (D), percent ash content (E), nitrogen content on a total weight basis (F), nitrogen content on an ash free dry weight basis (G), Secchi disk transparency (H), surface water temperature (I), total dissolved nitrate (J), total dissolved phosphate (K), and dissolved orthophosphate (L). Values of 0.482 or greater are significant at the 95% level.

	A	B	C	D	E	F	G	H	I	J	K	L
A	--											
B	-.02	--										
C	-.396	-.311	--									
D	-.181	-.393	.893	--								
E	-.790	-.05	.621	.451	--							
F	.236	.031	-.346	-.407	-.599	--						
G	-.574	.067	.086	-.211	.194	.601	--					
H	.775	-.03	-.460	-.407	-.876	.690	-.04	--				
I	-.290	.070	.061	-.110	.242	-.358	-.001	-.264	--			
J	-.005	.000	.309	.506	.366	-.421	-.381	-.455	-.545	--		
K	-.798	-.03	.318	.195	.731	-.583	.177	-.816	.523	-.071	--	
L	-.633	.013	.011	-.209	.315	.000	.518	-.329	.122	-.349	.613	--

the water. These relationships would be expected if nitrogen were a limiting nutrient for algal growth, i.e., increases in the nitrate concentration would result in increased algal growth but there would not be an increase in the nitrogen content of the algal cells. Neither the total phosphate nor the orthophosphate concentration was significantly correlated to the total or ash free dry weight of algal material, suggesting that phosphorus did not in general limit algal growth.

As would be expected a significant negative correlation exists between the percent ash content of the algal samples and the depth of Secchi disk visibility ($r=-0.876$). There is also a negative correlation between Secchi disk transparency and the total phosphate concentration ($r=-0.816$). The total phosphate concentration is positively correlated with water temperature ($r=0.523$) while the nitrate concentration is negatively correlated with water temperature ($r=-0.545$). The orthophosphate concentration, as might be expected, is correlated with the total phosphate concentration ($r=0.613$).

The ash free dry weight of algal growth on the plates was regressed against the other variables listed in Table 5. The most significant variables influencing ash free dry weight were nitrate concentration, followed by depth, and temperature which when combined accounted for 46.4% of the variation in ash free dry weight ($F=3.75$, df 3 and 13, significant at the 95% level).

The results of total phosphorus analysis of algal samples from artifical substrates collected in September and November, 1972 are tabulated in Table 6. The phosphorus content ranged from 0.163% to 0.936% on an ash free dry weight basis. The decrease

Table 6. Total phosphorus content of algal samples from artificial substrates suspended in the lower Reservoir. Plates were ordinarily sampled after one month in the Reservoir

Date	Depth meters	P Content (total sample) %	P Content (ash free dry wt.) %	Plates Sampled
9/12/72	S[1]	0.091	0.517	2
	1	0.236	0.936	2
9/28[2]	S	0.087	0.305	2
	1	0.067	0.252	4
11/30	S	0.044	0.163	3
	1	0.051	0.212	4

[1]S=surface

[2]Plates were in Reservoir for 16 days

in phosphorus content of the samples on an ash free dry weight basis from September through November corresponds to decreases in total dissolved phosphate measured in lower Reservoir water from August through November (Fig. 3). Unfortunately, the analytical methods employed did not distinguish between the phosphorus in algal cells and that associated with sediments which made up the greatest portion of the dry weight of the material accumulating on the plates during these months.

The nitrogen and phosphorus content of algal cells growing on artificial substrates might provide a measure of the adequacy of the nutrients in Reservoir water during the growth of these cells. Gerloff (1969), has reported critical cell contents of nitrogen and phosphorus as 4.0% and 0.12% for the blue-green alga Microcystis aeruginosa and values of 1.3% and 0.12% for several species of aquatic higher plants. The predominate phytoplankton in Elephant Butte Reservoir are green algae (Kidd and Johnson, 1971) and presumably these species have colonized the artificial substrate. Critical values for nitrogen in green algae apparently have not been reported in the literature. If the critical values for nitrogen in green algae are at all similar to those reported for M. aeruginosa then some samples listed in Table 4 probably represent nitrogen limited growth. Algal samples collected in November, March, and May had relatively low nitrogen contents (ash free dry weight basis) of between 1.88% and 2.77% while June samples were 0.95% and 1.19%. Certainly the June samplesrepresented nitrogen deficient cells while this may have been the case for the November, March, and

May samples as well.

The phosphorus contents (ash free dry weight basis) of the algal samples listed in Table 6 are all above critical values reported for Microcystis aeruginosa or aquatic higher plants. Interpretation of the phosphorus content data in terms of critical levels is ambiguous because of the probable contribution of associated sediments to the phosphorus content of the algal samples.

Nutrient Studies in the Reservoir

In the first nutrient addition experiment, conducted on August 18-20, 1971, the effect on productivity of additions of the single components of Rodhe's solution, the iron and trace element solution, or vitamins individually or combined as a "complete" addition was compared to Reservoir water without added nutrients. By the final day of incubation with added nutrients it was evident that there was a two to three fold increase in productivity when all nutrients were added compared to the control without additions or any of the additions made singly. The additions made singly did not appear to increase productivity compared to the control.

A similar experiment was performed on October 1-3, 1971. On the initial day of the experiment the treatments did not alter the productivity compared to the control without additions. On the next day (day 1) of the experiment addition of the iron and trace element solution and also the vitamin solution resulted in increases in productivity compared to the control. On the final day (day 2) of the experiment the Na_2SiO_3 and vitamin treatment resulted in higher productivity than the control while the other single additions differed little from the control. In contrast to the August experiment, combination of all nutrients resulted in little increase in productivity compared to the control. Unfortunately the bottle treated with $Ca(NO_3)$ was lost after the initial day of the experiment. The productivity in all treated bottles and the control increased only slightly between day 0 and day 1 and then increased by a factor of two between day 1 and 2.

A third nutrient addition experiment was performed on August 14-24, 1972. In this experiment treatments consisting of the complete additions and the complete with single components deleted were compared to the control without added nutrients. The primary productivity measurements in this experiment are presented in Fig. 8. On day 0 the bottle with K_2HPO_4 deleted greatly exceeded the control, while the other nutrient addition bottles were somewhat below the control. On day 2 productivity in the control and the $Ca(NO_3)$ deletion treatment were very low (18.6 and 15.8 mg $C/m^3/hr$ respectively). The other treatments, including the K_2HPO_4 deletion, ranged from 138 to 240 mg $C/m^3/hr$. On day 4 the control and $Ca(NO_3)$ deletion remained at low levels, 19.8 and 38.9 mg $C/m^3/hr$. The K_2HPO_4 deletion showed another decrease in productivity to 89.5 mg $C/m^3/hr$ while the complete and other deletions were in a range of 325 to 475 mg $C/m^3/hr$. On day 7 the control and $Ca(NO_3)$ deletion remained at low levels while differences appeared among the iron and trace element deletion (487 mg $C/m^3/hr$), the $MgSO_4$ deletion (621 mg $C/m^3/hr$), the Na_2SiO_3 deletion (1,049 mg $C/m^3/hr$), and the complete (590 mg $C/m^3/hr$). The bottles with the K_2HPO_4 and vitamin deletions were lost from the float between day 4 and day 7.

At the time of the final sampling on day 10 marked decreases were observed in productivity of the iron and trace element deletion (200 mg $C/m^3/hr$), the $MgSO_4$ deletion (551 mg $C/m^3/hr$), the Na_2SiO_3 deletion (326 mg $C/m^3/hr$) and the complete (485 mg $C/m^3/hr$). The $Ca(NO_3)$ deletion remained at a low level while the control increased to its maximum value (86.1 mg $C/m^3/hr$). The data

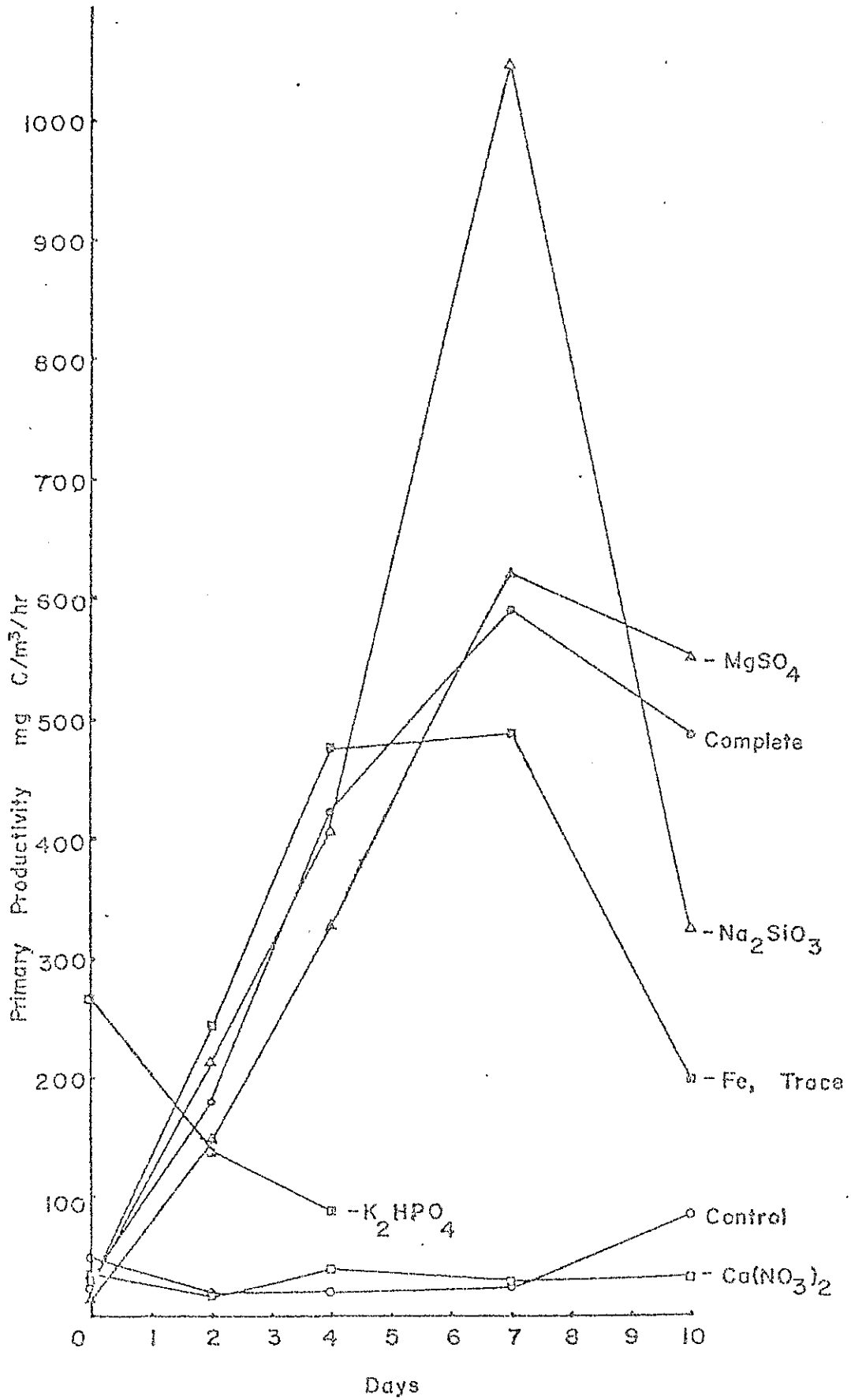


Fig. 8. Effects of nutrient additions on primary productivity in a field experiment conducted in the lower Reservoir, August 14-25, 1972. Values for the vitamin deletion were obtained for days 0 to 4 only and were similar to the complete.

on day 10 are probably not meaningful since even the complete nutrient addition treatment showed a decline in productivity compared to day 7.

The results in this type of experiment are most reliable at the earlier sampling dates as phytoplankton population changes would be expected to occur as a result of nutrient additions. As the experiment continues other factors may interfere with productivity, carbon dioxide may be depleted in the bottle or toxic metabolic products may be released. Determination of alkalinity on each day on which productivity was measured indicated that inorganic carbon was depleted to less than half the initial concentration by day 7 in the highly productive bottles (complete, and $MgSO_4$, Na_2SiO_3, and iron and trace element deletions). Except for the iron and trace element deletion further decreases in available carbon occurred by day 10 in these productive bottles. Changes in available inorganic carbon are corrected for in the calculation of carbon fixation; however, carbon may become a limiting nutrient and thus lower the rate of photosynthesis.

The data in Fig. 8 clearly demonstrate that nitrogen was a limiting nutrient; deletion of $Ca(NO_3)_2$ resulted in productivity levels similar to the control and far below the complete on days 2 through 10.

The apparent stimulation obtained when K_2HPO_4 was deleted is questionable because of the incorporation of extremely high levels of ^{14}C in the dark bottle on days 0, 2, and 4. The incorporation of

^{14}C in the light bottles was also extremely high on the first day of the experiment and the results indicate a promotion of primary productivity as a result of deletion of K_2HPO_4. This apparent promotion seems more likely to be an artifact than real. By day 4, althought the dark bottle activity was still high, productivity of the K_2HPO_4 deletion bottle was relatively low suggesting that phosphorus was by then limiting.

The results in Fig. 8 suggest that between days 4 and 7 some component of the iron and trace element solution became limiting.

Figure 9 presents the results of a similar deletion experiment conducted January 17-25, 1973. On day 0 the control bottle (without additions) exhibited the greatest productivity (97 mg $C/m^3/hr$). The bottles with Na_2SiO_3, both K_2HPO_4 and Na_2SiO_3, and iron and trace elements deletions were less productive, ranging from 42-53 mg $C/m^3/hr$. The other treatments, including the complete, were of even lower productivity ranging from 18-29 mg $C/m^3/hr$.

On day 2 productivity in the control bottle decreased dramatically to 8.9 mg $C/m^3/hr$. Productivity in all other bottles also decreased; however, all remained above the control bottle. On day 4 the productivity in the bottle with deletions of both K_2HPO_4 and Na_2SiO_3 and also the iron and trace element deletion decreased while productivity in other bottles remained nearly constant. Measurements on day 8 indicated that there were slight increases in productivity of the control bottle and the bottle

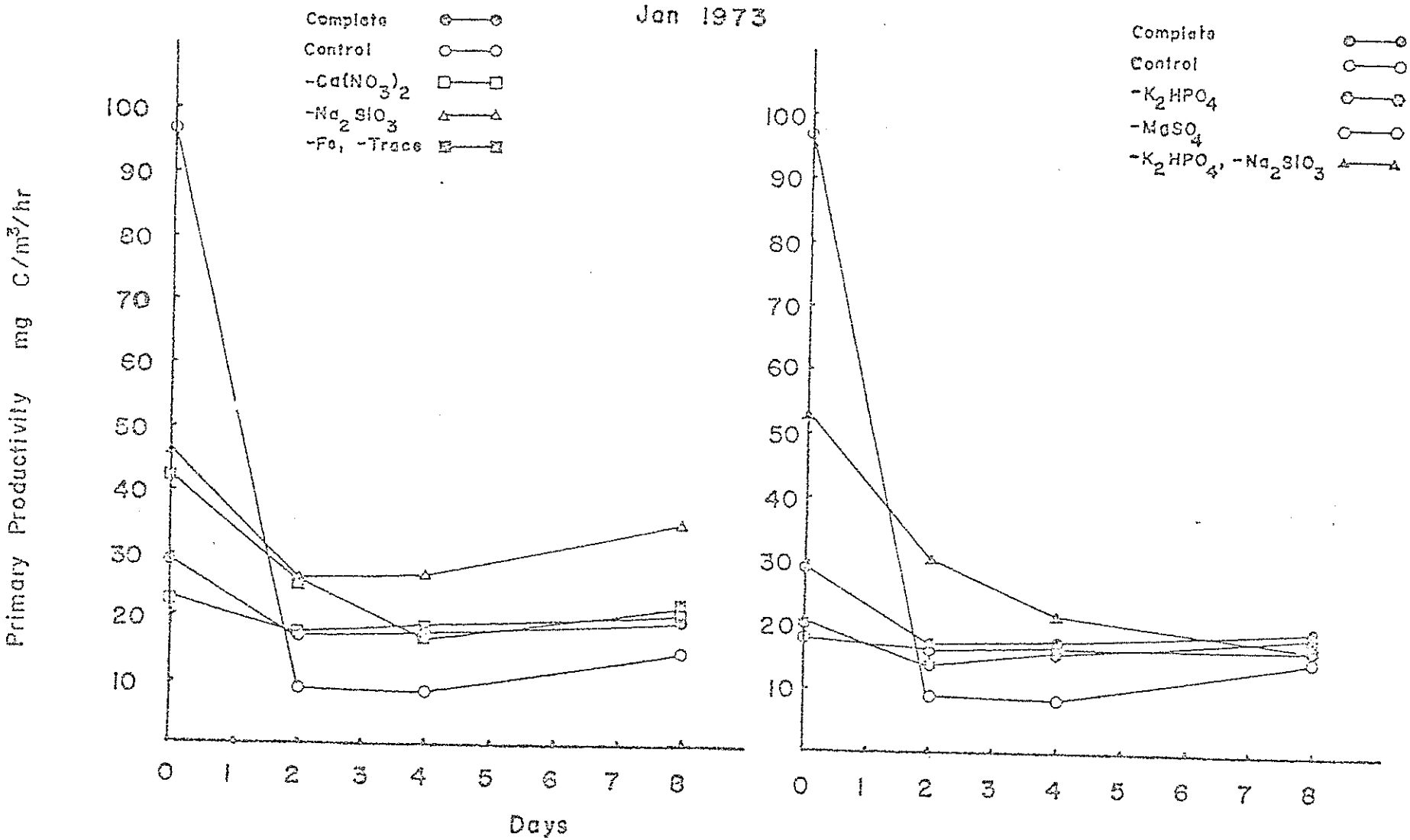


Fig. 9. Effects of nutrient additions on primary productivity in a field experiment conducted in the lower Reservoir, January 17-25, 1973. Data for the complete and control are plotted on both graphs.

with Na_2SiO_3 deleted to 14.5 and 34.8 mg $C/m^3/hr$ respectively. Productivity in other bottles changed but little between days 4 and 8.

These results suggest that initially all the nutrient additions depressed productivity compared to the control and the presence of Na_2SiO_3 and the iron and trace element components are partly or totally responsible for this depression.

Some nutrient(s) may have been depleted in the control bottle resulting in its low productivity when measured on day 2. It must be noted, however, that the original control bottle was lost after the sub-samples for productivity measurements were removed on day 0 and another sample of Reservoir water was collected for use as the control late in the afternoon of day 0. It is possible that the phytoplankton population in this second control sample differed appreciably from the population collected in the morning and this may account for the much lower productivity value obtained on day 2 compared to the initial day.

On day 4 only the control without added nutrients was appreciably below the complete additions value. The bottle with Na_2SiO_3 deleted as well as the bottle with both Na_2SiO_3 and K_2HPO_4 deleted were more productive than the complete and other nutrient treatments. On day 8 a small increase in productivity of both the control and the bottle with Na_2SiO_3 deleted was observed while the values for other nutrient treatments changed little and remained similar to the value for the complete nutrient additions.

Compared to the experiment conducted in August, 1972 productivity remained quite low and inorganic carbon measured as alkalinity was not appreciably depleted during the experiment.

The results of the January experiment suggested that productivity may have been stimulated by nutrient additions; however, the identity of the limiting nutrients was masked by inhibitory effects due to the addition of Na_2SiO_3 and iron and trace elements.

The final nutrient experiment was conducted in the Reservoir on July 6-11, 1973 and the results are presented in Table 7. The initial day's data were highly variable probably due to loss of ^{14}C from the bottles during several days of storage of the radioisotope in the bottles prior to use in the experiment. These data are not included in Table 7. Between day 2 and day 5 all but three of the large bottles were lost as a result of vandalism hence the experiment was terminated after sampling on day 5.

The effects of certain nutrient treatments are quite clear on day 2. Primary productivity of the control without added nutrients was 42.4 mg $C/m^3/hr$ while the complete nutrient addition resulted in a value of 124.4 mg $C/m^3/hr$. Low levels of productivity were measured when $Ca(NO_3)_2$ and K_2HPO_4 were omitted from the complete (18.1 and 17.5 mg $C/m^3/hr$ respectively) indicating that both nitrogen and phosphorus were limiting nutrients at the time of this experiment. Deletion of $MgSO_4$ or vitamins did not limit productivity compared to the complete.

On day 2 similar levels of productivity were measured for all complete addition bottles, whether the K_2HPO_4 and iron and

Table 7. Effects of nutrient additions on primary productivity in an experiment conducted July 6-11, 1973. Values are mean primary productivity in mg $C/m^3/hr$.

Treatment	Day 2 (N=2)	Day 5 (N=3)
No Additions	42.4±15.6[2]	--
Complete[1]	124.4±2.2	--
Complete - $Ca(NO_3)_2$	18.1±1.1	--
Complete - $MgSO_4$	134.4±7.4	259.1±4.3
Complete - K_2HPO_4	17.5±3.4	26.3±0.3
Complete - vitamins	153.0±27.9	--
Complete (100% K_2HPO_4)	123.6±5.1	--
Complete (100% iron, trace)	108.0±1.0	--
Complete (100% K_2HPO_4, 100% iron and trace elements)	130.0±1.5	--

[1]This bottle and the deletion bottles contained 10% of the K_2HPO_4 concentration and 10% of the iron and trace element concentrations of Rodhe No. 8 (Table 1). Na_2SiO_3 was not added in this experiment.

[2]Standard error.

trace elements were added at 10% of Rodhe's concentration or at the full Rodhe's concentration. This observation indicates that these additions were not toxic as was suggested in some earlier experiments.

On day 5 the $MgSO_4$ deletion bottle showed increased productivity to 259. mg $C/m^3/hr$ while the K_2HPO_4 deletion remained low at 26.3 mg $C/m^3/hr$. The alkalinity measurements on this date indicated that the available inorganic carbon had decreased in the $MgSO_4$ deletion bottle to 68% of its initial value.

In this experiment the results obtained from the complete with iron and trace elements deleted were questionable and have been omitted from Table 7 . On both day 2 and day 5 filters from the dark bottles from this nutrient treatment exhibited unusually high activities while filters from the light bottles were even higher. These effects suggest that chemical or physical factors other than phytoplankton photosynthesis were involved in the fixation of radiocarbon on these filters.

Nutritional studies in the laboratory

Figure 10A depicts the growth as dry weight for unialgal cultures of *Scenedesmus dimorphus*, *Chlamydomonas debaryana* var. *cristata* and *Anabaena flos-aquae* in Rodhe's medium with concentrations of phosphorus varied from 0 to 0.888 mg/liter. The latter corresponds to the concentration of phosphorus provided in full strength Rodhe's medium. Growth of *C. debaryana* and *A. flos-aquae* increased linearly through the range of concentrations

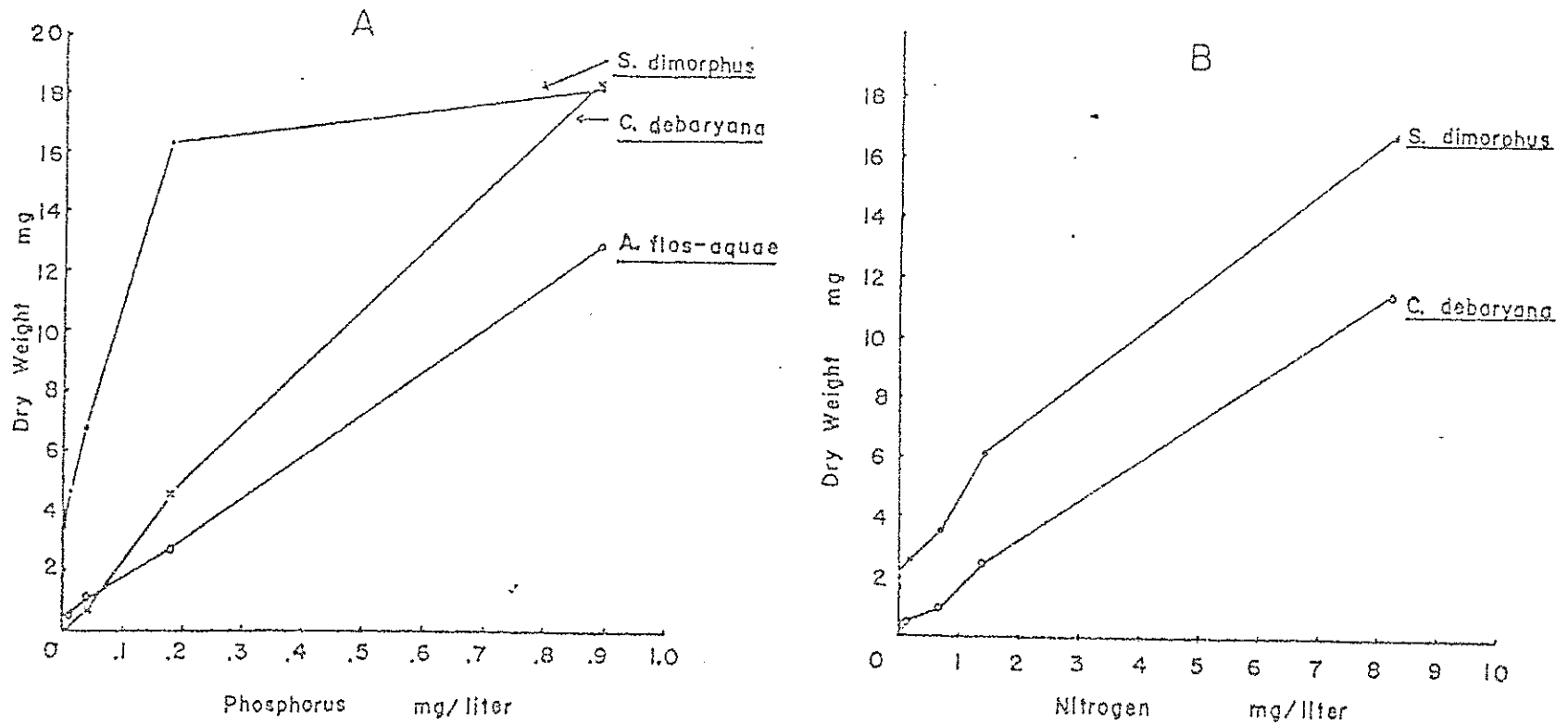


Fig. 10. A. Growth of Scenedesmus dimorphus, Chlamydomonas debaryana var. cristata, and and Anabaena flos-aquae with increasing concentrations of phosphorus added to Rodhe's medium (N=2).

B. Growth of Scenedesmus dimorphus and Chlamydomonas debaryana var. cristata with increasing concentrations of nitrogen added to Rodhe's medium (N=2).

of phosphorus while growth of S. dimorphus increased linearly up to a concentration of 0.178 mg/liter and then increased only slightly at a phosphorus concentration of 0.888 mg/liter. The failure of S. dimorphus' growth to increase linearly over this concentration range is surprising and this experiment should be repeated; however, other types of experiments demonstrated that this species was striking efficient in the utilization of low concentrations of phosphorus.

Figure 10B presents the results of experiments on the growth S. dimorphus and C. debaryana with the nitrogen concentration in Rodhe's medium varied from 0 to 8.22 mg/liter. The latter concentration corresponds to that of the full strength medium. This experiment was not done with the blue-green alga A. flos-aquae since it utilizes atmospheric nitrogen and in various experiments we observed that it grew equally as well with or without a source of nitrate. Growth of both S. dimorphus and C. debaryana increased with the concentration of nitrogen supplied.

Two of the predominate species of green algae found in Elephant Butte Reservoir (Kidd and Johnson, 1971) were isolated and grown in the laboratory under conditions of varying phosphorus or nitrogen supply in Rodhe's medium. Growth of Platymonas sp. and Chlamydomonas sp. with varying phosphorus supply is depicted in Fig. 11A and varying nitrogen supply in Fig. 11B. Growth increased with the concentration of phosphorus or nitrogen supplied. Considerably more dry weight was produced by Platymonas sp. than by Chlamydomonas sp. at the various concentrations of phosphorus and nitrogen supplied.

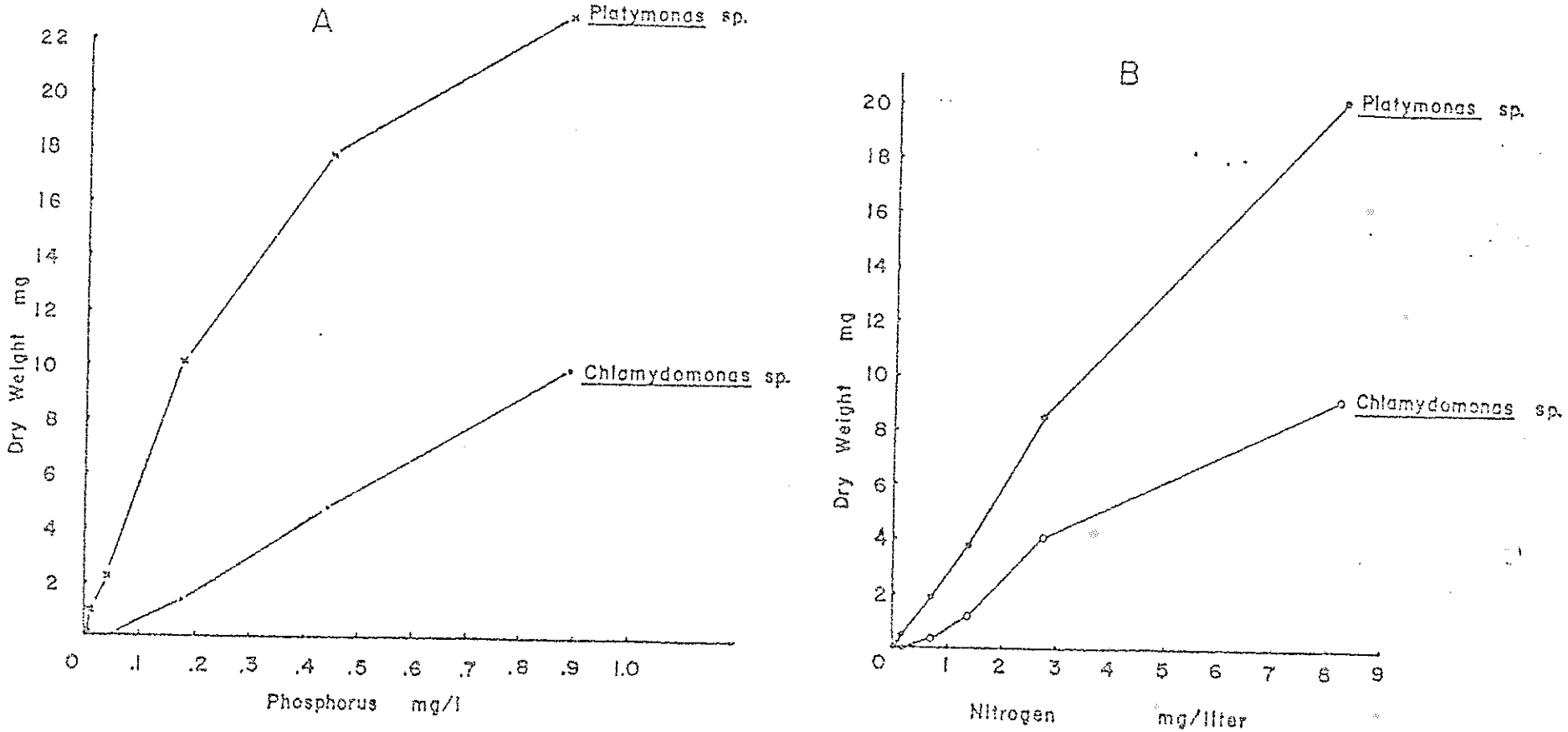


Fig. 11. A. Growth of Platymonas sp. and Chlamydomonas sp. with increasing concentrations of phosphorus added to Rodhe's medium (N=2).
B. Growth of Platymonas sp. and Chlamydomonas sp. with increasing concentrations of nitrogen added to Rodhe's medium (N=2).

The average total dissolved phosphate concentrations measured in the lower and upper Reservoir were 0.395 and 0.642 mg/liter as phosphorus respectively (Table 3). The results in Figures 10A and 11A indicate that these concentrations of phosphorus would be limiting for laboratory growth of C. debaryana, A. flos-aquae, Platymonas sp., and Chlamydomonas sp. if other nutrients were present in Reservoir water at Rodhe's medium concentrations. The average nitrate nitrogen concentration of water from both the lower and upper Reservoir was 0.59 mg/liter (Table 2). This concentration would very severely limit growth of all four species of green algae in laboratory culture experiments if the other nutrients were present in Reservoir water at Rodhe's medium concentrations. Of the two nutrients measured in Reservoir water, one would expect nitrogen to impose the greatest limitation on growth of green algae under laboratory culture conditions. The total dissolved phosphate in the Reservoir water was more variable both with time and between the lower and upper Reservoir. The total phosphorus concentration in most samples was below the 0.888 mg/liter supplied by Rodhe's medium; however, it was not as severely limiting as was nitrogen based on Rodhe's concentrations of these two nutrients. It should be noted that Rodhe's medium is a very dilute medium and that large increases in growth of many species of algae would be obtained in a more concentrated culture medium.

The first laboratory bioassay of filtered Reservoir water was conducted using a control of Reservoir water without added nutrients, a complete with additions of Rodhe's concentrations of all nutrients, and additions of each of Rodhe's components individually. Very little growth of C. debaryana or S. dimorphus was observed in Reservoir water alone (controls) while cultures grew well when supplimented with the complete Rodhe's medium. Single nutrient additions failed to increase growth above that of the control except for S. dimorphus which grew moderately well when only $Ca(NO_3)_2$ was added to Reservoir water. These results suggested that nitrogen and one or more additional nutrients were limiting for the laboratory growth of algal cultures in Reservoir water.

Figure 12 illustrates the results of laboratory growth experiments conducted using membrane filtered water obtained from the lower Reservoir in October, 1971. Deletion of either $Ca(NO_3)_2$ or K_2HPO_4 from the complete Rodhe's suppliment severely limited growth of S. dimorphus and C. debaryana to levels similar to the control without added nutrients. Deletion of $MgSO_4$, iron and trace elements, or Na_2SiO_3 (not plotted) did not limit growth compared to the complete. Results with A. flos-aquae indicated that of the nutrient deletions, only omission of K_2HPO_4 severely depressed growth. Chemical analysis of the filtered water indicated that there was a total phosphate concentration of 0.061 mg/liter, as phosphorus and a nitrate concentration of 0.475 mg/liter, as nitrogen (Table 8) both of which would severely limit algal growth.

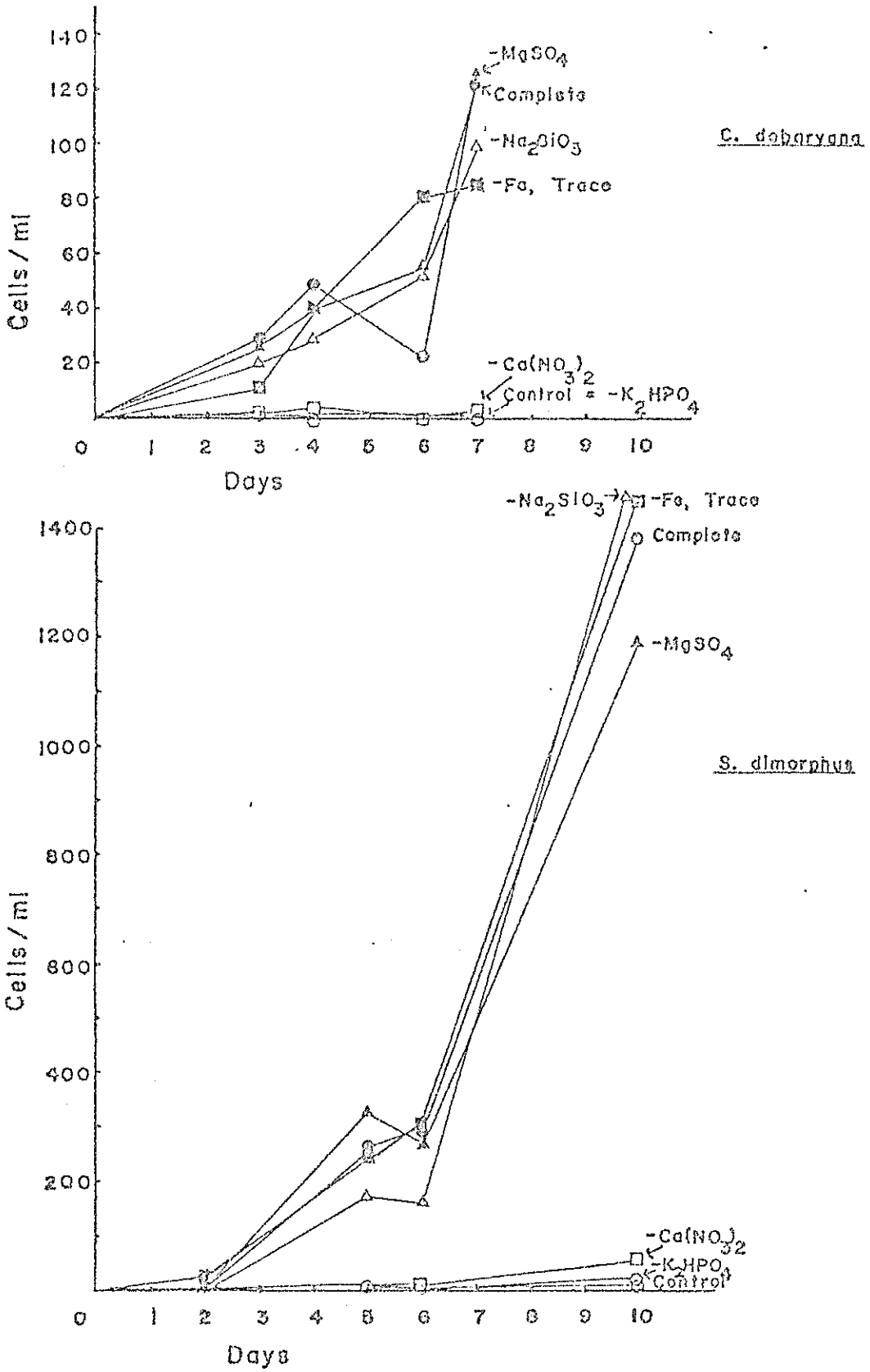


Fig. 12. Nutrient additions and growth of Chlamydomonas debaryana var. cistata and Scenedesmus dimorphus in lower Reservor water collected in October, 1971 (N=2).

Table 8. Total dissolved nitrate nitrogen and total dissolved phosphate as phosphorus in water used in laboratory bioassays and field experiments.

DATE	STATION	NITROGEN mg/liter	TOTAL PHOSPHATE mg/liter
10/71	I	0.475	0.061
3/08/72	I	0.240	0.094
3/08/72	IV	0.536	0.213
8/14/72	I	0.296	0.511
8/14/72	III	0.352	0.296
1/17/73	I	0.869[1]	0.300
7/06/73	I	0.567	0.414

[1]Sample obtained 1/25/73.

The possible contribution of the particulate matter in Reservoir water to algal nutrition was evaluated by identical growth experiments with S. dimorphus and C. debaryana using a portion of the same water sample collected in October, 1971, which had not been subjected to membrane filtration. Essentially identical results were obtained using filtered and unfiltered water thus indicating that the particulate material did not significantly contribute to the nutrient supply of the water under the conditions of these experiments.

That the greatly depressed algal growth occurring when $Ca(NO_3)_2$ and K_2HPO_4 were deleted from the additions to Reservoir water was due to limiting nitrogen and phosphorus rather than calcium and potassium is illustrated in Fig. 13 for S. dimorphus. Filtered lower Reservoir water from October, 1971, was supplimented with the complete Rodhe's medium, complete with $Ca(NO_3)_2$ replaced by an equivalent amount of $CaSO_4$ or $NaNO_3$, and complete with K_2HPO_4 replaced by an equivalent amount of K_2SO_4 or Na_2HPO_4. Growth was comparable to the complete when $NaNO_3$ replaced $Ca(NO_3)_2$, however; $CaSO_4$ was ineffective in replacing $Ca(NO_3)_2$. When Na_2HPO_4 replaced K_2HPO_4, growth was similar to the complete, while very little growth occurred when K_2SO_4 replaced K_2HPO_4. Similar results were obtained in an identical experiment conducted with C. debaryana. The same experiment was conducted with A. flos-aquae and the results indicated that phosphorus was the limiting element for growth of this species. The growth of the blue-green species was not affected by the presence or absence of nitrate.

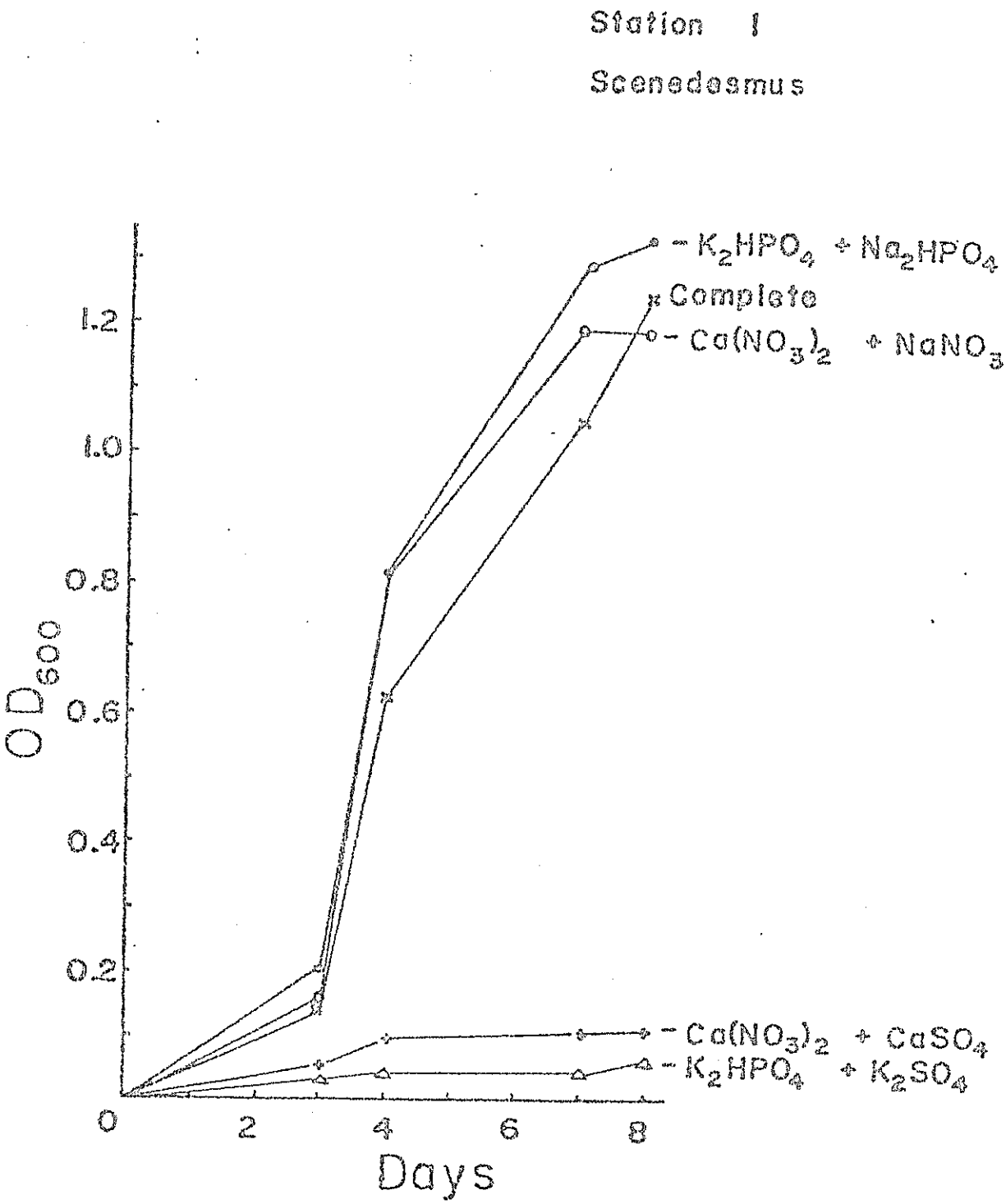


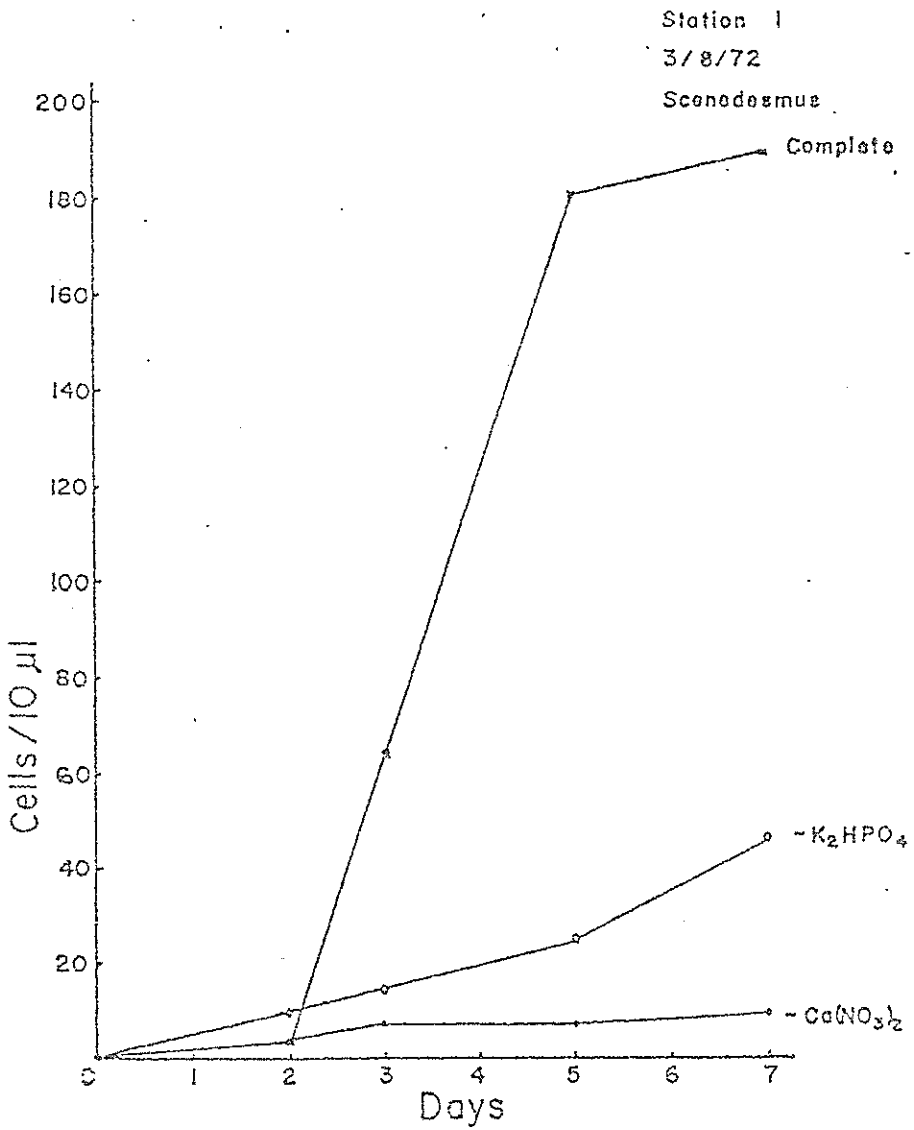
Fig. 13. Growth of Scenedesmus dimorphus in lower Reservoir water collected in October, 1971 with nutrient additions and substitutions (N=2).

Laboratory bioassays were conducted on filtered water collected from the lower and upper Reservoir on March 8, 1972. The total phosphate concentration of the water was 0.094 and 0.213 mg/liter as phosphorus and the nitrate concentration was 0.240 and 0.536 mg/liter as nitrogen for the lower and upper Reservoir samples respectively (Table 8).

Figure 14 presents the significant results obtained with S. dimorphus as the test organism. With water from the lower Reservoir deletion of $Ca(NO_3)_2$ resulted in very limited growth, while growth with K_2HPO_4 deleted was less severely limited compared to the complete additions. Considering growth in water from the upper Reservoir, only deletion of $Ca(NO_3)_2$ limited growth of S. dimorphus, the cell counts being virtually the same as for the complete when K_2HPO_4 was deleted.

Significant results for C. debaryana are depicted in Fig. 15. In water from both the lower and upper Reservoir deletion of either $Ca(NO_3)_2$ or K_2HPO_4 greatly limited growth of this species; however, deletion of K_2HPO_4 resulted in somewhat less growth depression in the upper Reservoir water compared to the lower. Growth of A. flos-aquae was limited only by deletion of K_2HPO_4 and this was much more striking in lower Reservoir water than in upper Reservoir water (Fig. 16).

The growth of the three algal species was not limited when $MgSO_4$ or iron and trace elements were deleted from the medium. Deletion of $Ca(NO_3)_2$ did not limit growth of the nitrogen fixing A. flos-aquae. These data have not been included in Fig. 14-16.



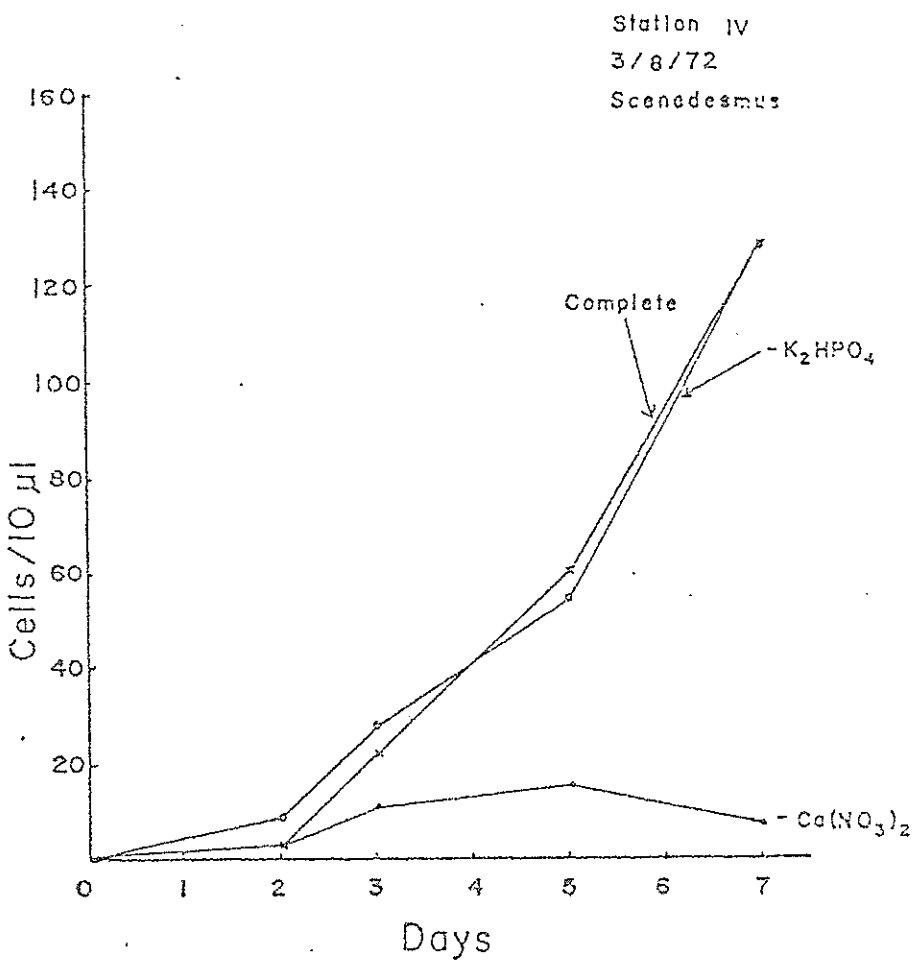
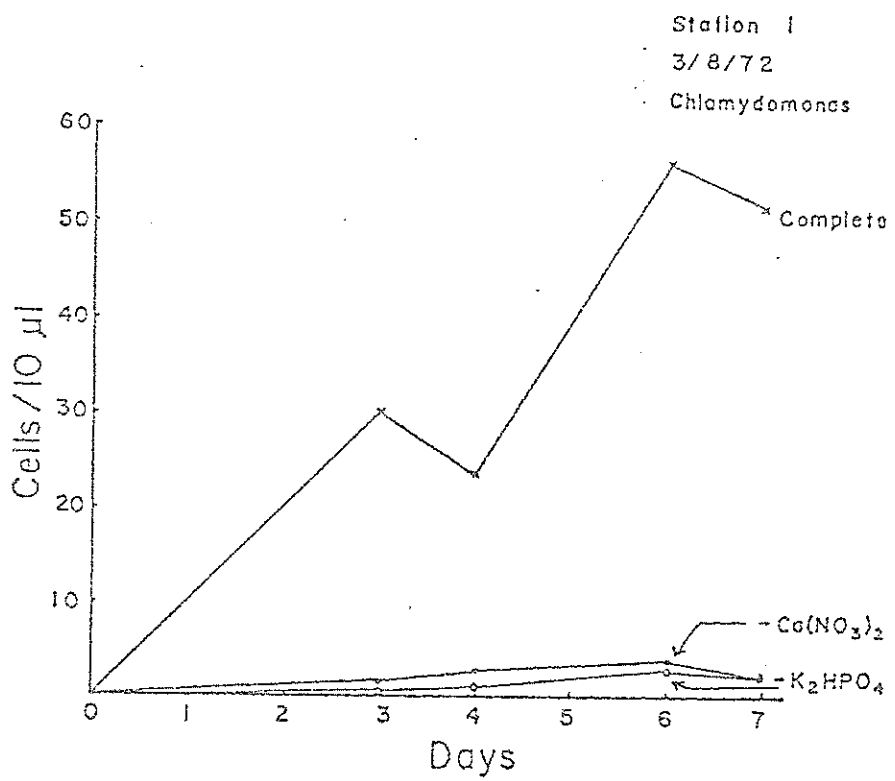


Fig. 14. Nutrient additions and growth of Scenedesmus dimorphus in lower and upper Reservoir water collected March 8, 1972 (N=2).



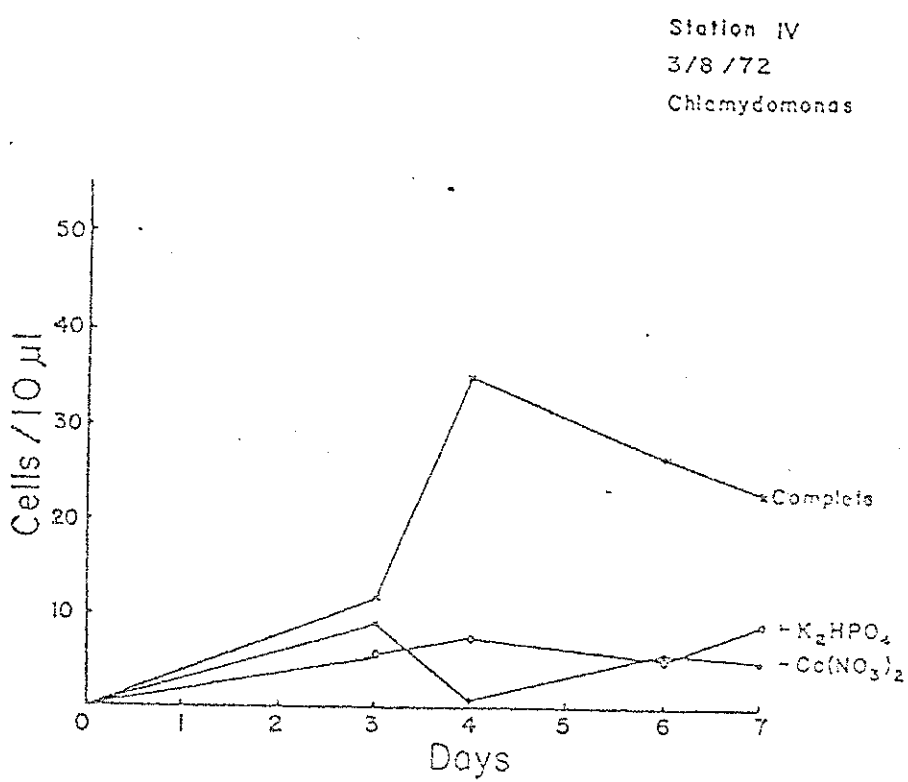


Fig. 15. Nutrient additions and growth of Chlamydomonas debaryana var. cristata in lower and upper Reservoir water collected March 8, 1972 (N=2).

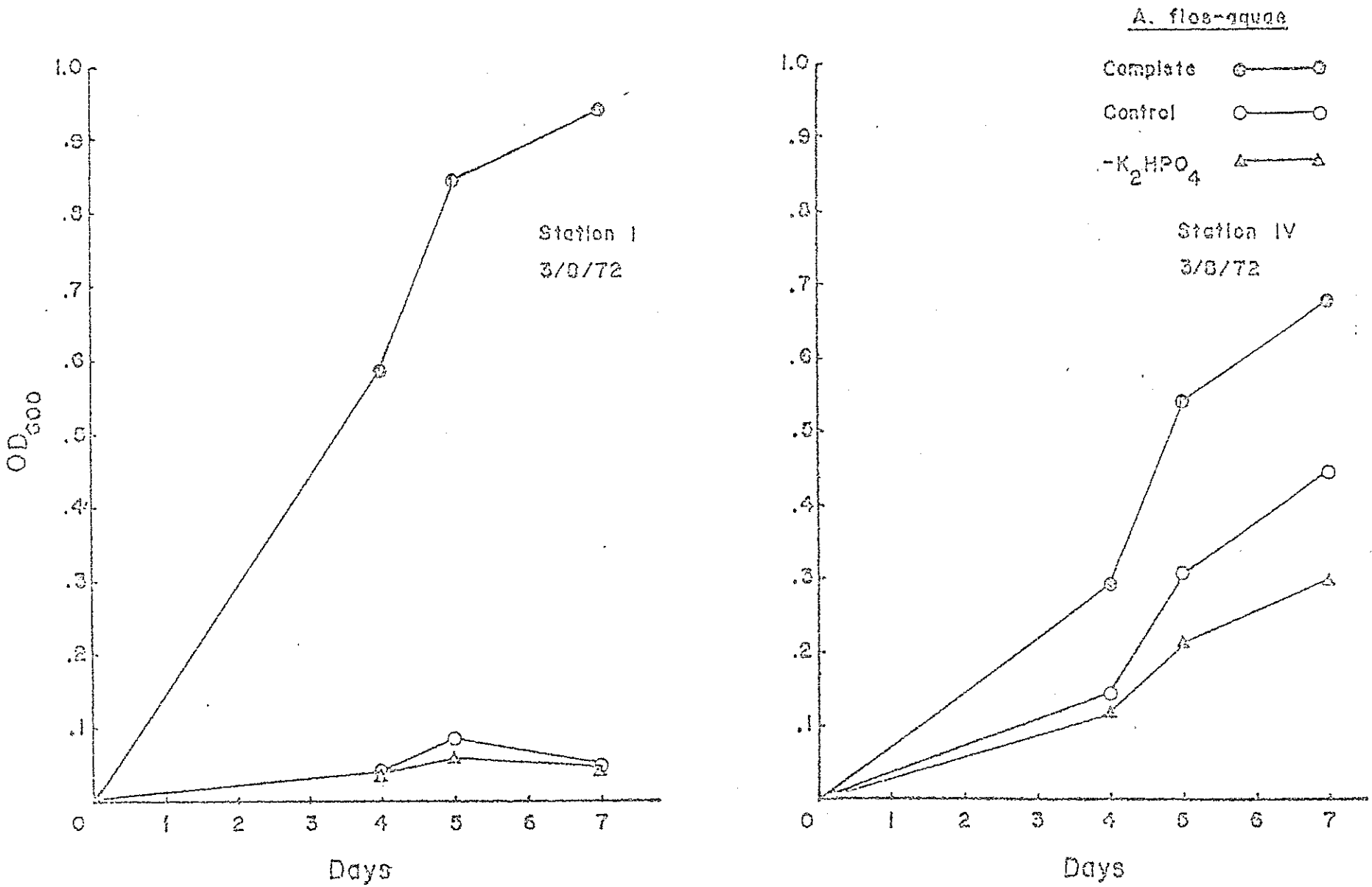


Fig. 16. Nutrient additions and growth of Anabaena flos-aquae in lower and upper Reservoir water collected March 8, 1972 (N=2 except for the $-K_2HPO_4$ culture for Station I where N=1).

Controls without added nutrients supported very little growth except when A. flos-aquae was grown on water from the upper Reservoir. Cell counts of S. dimorphus cultures in controls from both the lower and upper Reservoir were very similar to counts for the $Ca(NO_3)_2$ deletions suggesting that nitrogen was the most significant limiting nutrient for this species in both water samples. Control water from the lower Reservoir inoculated with C. debaryana supported almost no growth while the cell counts for control water from the upper Reservoir were quite similar to the $Ca(NO_3)_2$ deletion. Results for controls have been plotted only for A. flos-aquae (Fig. 16). The control growth curves for this species are similar to growth curves obtained when K_2HPO_4 was deleted.

The bioassay results are in agreement with trends suggested by the chemical analysis of water samples (Table 8) and the growth response of these species to increasing concentrations of nitrogen and phosphorus (Fig. 10A,B). Nitrate nitrogen was very low in samples from both lower and upper Reservoir water and was severely limiting in the bioassays. Phosphorus was limiting for all three species in the lower Reservoir sample. Phosphorus was limiting for C. debaryana and A. flos-aquae cultured in the upper Reservoir sample; however, this nutrient did not limit the growth of S. dimorphus which is apparently more efficient in the utilization of phosphorus.

Table 9 presents final dry weights of algal cultures grown on membrane filtered water collected from the lower and upper Reservoir on August 14, 1972. Growth of C. debaryana and S. dimorphus without added nutrients or with either K_2HPO_4 or $Ca(NO_3)_2$ deleted from Rodhe's medium was very limited in water from both the lower and upper Reservoir. Growth of A. flos-aquae was very restricted in the controls and in the K_2HPO_4 deletion cultures in both water samples. The nitrate concentrations were 0.296 and 0.352 mg/liter as nitrogen and the total phosphate concentration was 0.511 and 0.296 mg/liter as phosphorus for the lower and upper Reservoir samples respectively (Table 8). The nitrate concentrations would certainly be very limiting for algal growth while the phosphate concentrations should not limit growth as severely. Based on the response of these three species to various concentrations of phosphorus (Fig. 10A), there should have been only moderately limited growth of C. debaryana and A. flos-aquae and very little limitation on the growth of S. dimorphus in water containing 0.511 mg/liter phosphorus. In this experiment the phosphorus analysis of the water was not accurately reflected by the laboratory bioassay. Gerloff and Skoog (1954) noted that as much as several mg/liter of phosphorus could be lost by precipitation when an artificial medium was autoclaved. It is possible that the phosphate in the Reservoir water was precipitated as a result of addition of Rodhe's medium and autoclaving and thus became unavailable to the algae. The data in Table 9 also suggest that deletion of iron and trace elements may have restricted growth of C. debaryana in water from the upper Reservoir and

Table 9 . Effects of nutrient additions on growth of algal cultures in filtered Reservoir water in the laboratory. Water samples were collected from the lower (Station I) and upper (Station III) Reservoir on August 14, 1972. Values are mean dry weights in mg (N=2).

Species	Additions	Station I	Station III
Chlamydomonas debaryana var. cristata	Complete	17.88±0.38[1]	17.59±0.34
	-$Ca(NO_3)_2$	1.57±0.28	0.99±0.36
	-K_2HPO_4	1.42±0.66	0.42±0.12
	-iron, trace	18.72±1.33	10.26±1.16
	-$MgSO_4$	16.69±0.12	15.13±0.36
	No additions	0.74±0.17	0.18±0.04
Scenedesmus dimorphus	Complete	24.76±0.79	22.40±1.06
	-$Ca(NO_3)_2$	2.10±0.52	1.77±0.35
	-K_2HPO_4	2.36±0.10	2.76±0.82
	-iron, trace	20.56±0.60	21.44±1.92
	-$MgSO_4$	25.98±1.58	25.26±0.44
	No additions	1.44±0.005	4.30±3.04
Anabaena flos-aquae	Complete	12.16±2.94	17.00±6.19
	-K_2HPO_4	0.18±0.08	1.48±1.02
	-iron, trace	5.81±1.74	6.78±0.47
	-$MgSO_4$	6.95±3.46	7.96±0.24
	No additions	0.51±0.19	0.37±0.04

[1]Standard error.

growth of A. flos-aquae in both water samples. Growth depressions when iron and trace elements were deleted were not nearly as severe as when nitrogen or phosphorus was deleted from the medium.

The results of these bioassays of water from the lower Reservoir can be compared to the field experiment with the natural phytoplankton population conducted with water samples collected on the same date (Fig. 8). In the field study nitrogen was clearly indicated to be limiting primary productivity early in the experiment while the results were uncertain for phosphorus. Some component of the iron and trace element addition appears to have become limiting between day 4 and 7.

Phosphatase activity was detected in cultures of the two algal species isolated from Elephant Butte Reservoir as well as in cultures of S. dimorphus, C. debaryana, and A. flos-aquae. The results of phosphatase assays on cultures of Platymonas sp., Chlamydomonas sp., and S. dimorphus are presented in Table 10. That significant phosphatase activity was measured only when algae were grown at lower phosphorus concentrations which limited algal growth is illustrated by the data for Platymonas sp. and Chlamydomonas sp. Of these two species, Chlamydomonas sp. produced the greatest phosphatase activity at phosphorus concentrations of 0.173 and 1.73 mg/liter. Acid phosphatase activity greatly exceeded alkaline phosphatase activity for Chlamydomonas sp. while the activities of the two phosphatases of Platymonas sp. were at approximately the same level. The ability of these two species to produce phosphatases under conditions of limited

Table 10. Effect of phosphorus concentration on growth and acid and alkaline phosphatase activity of algal cultures. Algae were cultured in PG solutions with other conditions as described in Materials and Methods.

Species	Phosphorus Conc. mg/liter	Growth OD_{600}	Assay pH	Phosphatase Activity Units/OD_{600}
P[1]	0.173	0.225	6.8	2.38
	0.173	0.225	9.0	3.56
	1.73	0.67	6.8	0.498
	1.73	0.67	9.0	0.748
	17.3	6.50	6.8	0.037
	17.3	6.50	9.0	0.073
	173.	4.72	6.8	0
	173.	4.72	9.0	0.027
	Phosphorus Conc. mg/liter	Growth OD_{600}	Assay pH	Phosphatase Activity Units/OD_{600}
C	0.173	0.225	6.8	54.9
	0.173	0.225	9.0	5.04
	1.73	0.90	6.8	8.53
	1.73	0.90	9.0	2.19
	17.3	5.65	6.8	0
	17.3	5.65	9.0	0
	Phosphorus Conc. mg/liter	Growth OD_{600}	Assay pH	Phosphatase Activity Units/OD_{600}
S	1.73	6.70	6.8	1.67
	1.73	6.70	9.0	0.648

[1] P - *Platymonas* sp.
C - *Chlamydomonas* sp.
S - *Scendesmus dimorphus*

phosphorus supply would be important for algae growing in Elephant Butte Reservoir since on many sampling dates only a small portion of the total dissolved phosphate was in the ortho form while a much larger quanity was in the organic form. The orthophosphate concentrations characteristic of Elephant Butte Reservoir are low enough to promote synthesis of these enzymes by Chlamydomonas sp. and Platymonas sp.

CONCLUSIONS

Laboratory bioassays conducted at various times during the year and field experiments conducted during the summers of 1972 and 1973 have indicated that nitrogen is severely limiting for phytoplankton growth in Elephant Butte Reservoir. This conclusion is supported by the low concentration of nitrate nitrogen measured in Reservoir water throughout the year. The nitrate form was reported to be the most abundant form of inorganic nitrogen in Elephant Butte Reservoir and the only form consistently present in measureable concentrations (Kidd and Johnson, 1971).

Phosphorus was found to be limiting in most laboratory bioassays; however, this may have been a result of precipitation of phosphorus as the water was autoclaved with added nutrients. In a field experiment conducted in July, 1973, phosphorus was found to be severely limiting even though chemical analysis of Reservoir water indicated that sufficient total phosphate was present to support considerable algal growth. This enigma suggests that the total phosphate measured on this date

was not all available to the phytoplankton. Orthophosphate was not detectable in this water sample so the available phosphorus may have been much less than that measured by the total phosphate analysis.

The 0.089 mg/liter of phosphorus added to complete bottles with 10% of the usual Rodhe's concentration of K_2HPO_4 was sufficient to support productivity at a maximum level on day 2 of this experiment. This implies that the available phosphorus was indeed very low and not indicated by the total phosphate concentration measured (0.414 mg/liter as phosphorus).

The total phosphate of water samples collected on several dates was found to be almost entirely in the organic and ortho forms and condensed phosphates were not detected. The identification of phosphorus as a limiting nutrient in the field experiment conducted in July, 1973 indicated that on this date the compounds measured as total phosphate were largely not available to the phytoplankton even though two predominate algal species from Elephant Butte Reservoir were found to produce phosphatase enzymes capable of hydrolyzing monoesters of phosphoric acid. This points to a need to characterize the precise chemical nature of the total dissolved phosphate in addition to its orthophosphate component. Possibly the orthophosphate measurement provides the best indication of the phosphorus available for phytoplankton in this Reservoir.

A field experiment conducted in January, 1973, a time

when nitrate and total phosphate concentrations in Reservoir water were relatively high, indicated that nutrient levels did not clearly limit primary productivity under the existing conditions, although toxic effects may have complicated this interpretation.

Both laboratory and field experiments demonstrated that $MgSO_4$, iron, and trace elements concentrations were adequate in Reservoir water to support much higher levels of phytoplankton populations than the nitrogen and/or phosphorus supply. Furthermore, primary productivity was not increased in field experiments by the addition of Na_2SiO_3 or vitamins.

Differences in phytoplankton productivity and abundance reported by Kidd and Johnson (1971) for the lower and upper Reservoir are probably explainable by relatively small differences in nutrient concentrations in the generally nutrient poorer lower Reservoir and the richer upper Reservoir near the inlet of the Rio Grande. Under conditions of a severe nutrient limitation an increase in the supply of the limiting nutrient could result in a linear increase in phytoplankton numbers or productivity. An interaction between two limiting nutrients, nitrogen and phosphorus, would further enhance this effect.

In this investigation it was observed that the blue-green alga Anabaena flos-aquae grew well in filtered Reservoir water supplimented with K_2HPO_4 and did not require a source of combined nitrogen. It is thus surprising that blue-green algae are relatively rare in Elephant Butte Reser-

voir (Kidd and Johnson, 1971). Recently Shapiro (1973) has suggested that blue-green algae tend to replace green algae under conditions of limited CO_2 availability often associated with eutrophic conditions and high pH values. If this is the case, the abundant bicarbonate supply in Elephant Butte Reservoir may favor the green algae rather than the less desireable blue-green species and thus explain the predominance of green algae in this Reservoir.

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