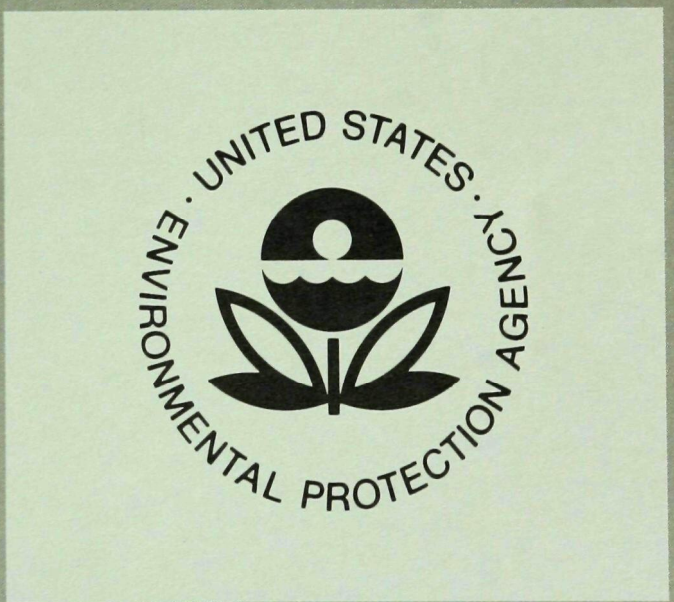
Ecological Research Series

ALGAL NUTRIENT AVAILABILITY AND LIMITATION IN LAKE ONTARIO DURING IFYGL Part III



Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

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ALGAL NUTRIENT AVAILABILITY AND LIMITATION IN LAKE ONTARIO DURING IFYGL

Part III. Algal Nutrient Limitation in Lake Ontario During IFYGL

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FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry---physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota develops methods, conducts laboratory and field studies, and extrapolates research findings

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report describes the potential significance of algal nutrients in limiting algal growth in Lake Ontario and its major tributaries.

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PREFACE

This project was conducted as part of the International Field Year for Great Lakes Research and consisted of three separate parts, all directed toward providing information needed to assess the factors limiting algal growth in Lake Ontario and the amounts of nitrogen and phosphorus in tributary drainage which would likely become available in the lake. Part I is concerned with a comprehensive study of the amounts of phosphorus entering Lake Ontario from U.S. tributaries which will likely become available in the lake. Particular attention is given to the particulate and organic forms of phosphorus in the major U.S. tributaries to the lake. Part II is concerned with a study of the amounts of available nitrogen entering Lake Ontario from the U.S. tributaries. Part III is concerned with the factors limiting algal growth in Lake Ontario and in the major U.S. tributaries. This report presents Part III of this study. Parts I and II are published as separate reports by the U.S. Environmental Protection Agency under the title, Algal Nutrient Availability and Limitation in Lake Ontario During IFYGL, with the following subtitles:

Part I: Available Phosphorus in Urban Runoff and Lake Ontario Tributary Waters

Part II: Nitrogen Available in Lake Ontario Tributary Water Samples and Urban Runoff from Madison, Wisconsin

ABSTRACT

This study was conducted on the potential significance of nitrogen, phosphorus and micronutrients in limiting planktonic algal growth in Lake Ontario and its major tributaries. Standard algal assay procedures were used. Samples of the open waters of Lake Ontario and Niagara River waters collected during the spring showed phosphorus limitation. By late summer these waters showed both nitrogen and phosphorus limitation. Genesee and Oswego Rivers showed, in general, nitrogen limitation. Samples of the Black River waters showed both nitrogen and phosphorus limitation.

This report was submitted in fulfillment of Contract No. R-800537-02 under the sponsorship of the Environmental Protection Agency. Work was completed as of June, 1975.

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ACKNOWLEDGMENTS

This investigation was conducted primarily at the University of Wisconsin, Madison. In addition to support given for this investigation by the U.S. Environmental Protection Agency, support was given by the Department of Civil and Environmental Engineering at the University of Wisconsin, Madison, and the Institute for Environmental Sciences at the University of Texas at Dallas. This investigation was conducted as part of the International Field Year for the Great Lakes.

Special recognition should be given to the assistance of personnel from the Grosse Ile laboratory of the U.S. EPA, especially N. Thomas and T. Davies; also, the advice and assistance of G.P. Fitzgerald is greatly appreciated.

SECTION I
INTRODUCTION

The objective of this study was to determine the limiting nutrients for the algal growth in Lake Ontario. The study included the measurement of the growth response of laboratory grown and natural algae in nutrient spiked Lake Ontario water.

During the summer and fall of 1972 and spring, 1973, water samples were collected from open lake at five locations and from the mouths of the Niagara, Genesee, Oswego and Black Rivers.

One or two gallon water samples from the surface of Lake Ontario were collected in pre-washed polyethylene containers and transported to Madison, Wisconsin, via commercial airliner or by car, depending upon when the samples were collected. The day of arrival at Madison ranged from three days to two weeks after collection, depending upon the mode of transportation. Upon arrival, the water samples were stored at $4^{\circ}C$. Figure 1 identifies the sampling stations in the open lake and its tributaries. Table 1 presents the sampling dates and the initial phosphorus and nitrogen concentrations of the samples collected.

Table 1. SAMPLING DATES AND INITIAL PHOSPHORUS AND NITROGEN CONCENTRATIONS FOR LAKE ONTARIO AND TRIBUTARY WATER

Location	Station/ Sample No.	Date Collected	TP mg/l P	"TN mg/l N
Lake Ontario	10	26 June 72	18	*0.07
Lake Ontario	10	5 Mar 73	+3	--
Lake Ontario	10	1 Apr 73	5	--
Lake Ontario	10	2 May 73	+19	0.31
Lake Ontario	10	15 Jun 73	9	*0.05
Lake Ontario	93	24 Aug 72	15	--
Lake Ontario	64	23 Aug 72	17	--
Lake Ontario	75	19 Jul 72	36	*0.05
Lake Ontario	75	5 Mar 73	+3	0.39
Lake Ontario	75	1 Apr 73	3	0.35
Lake Ontario	75	2 May 73	13	0.28
Lake Ontario	75	15 Jun 73	10	*0.05
Lake Ontario	45	5 Mar 73	+6	0.40
Lake Ontario	45	1 Apr 73	4	0.35
Lake Ontario	45	2 May 73	14	0.27
Lake Ontario	45	15 Jun 73	10	*0.05
Lake Ontario	19	2 Aug 72	25	--
Lake Ontario	Near Niagara River 7	23 Jul 72	14	*0.05
Lake Ontario	Near Niagara River 9	19 Jul 72	100	*0.10
Lake Ontario	Near Rochester NY 17	2 Aug 72	43	--
Lake Ontario	Near Rochester NY 20	14 Aug 72	37	--

(continued)

Table 1. SAMPLING DATES AND INITIAL PHOSPHORUS AND NITROGEN CONCENTRATIONS FOR LAKE ONTARIO AND TRIBUTARY WATER

Location	Station/ Sample No.	Date Collected	TP mg/l P	"TN mg/l N
Oswego River	30	20 Mar 73	306	--
Oswego River	31	28 Mar 73	95	1.27
Oswego River	35	29 Mar 73	105	1.16
Oswego River	43	1 May 73	96	1.42
Oswego River	52	28 May 73	104	1.49
Oswego River	54	31 May 73	87	--
Oswego River	55	4 Jun 73	96	--
Oswego River	59	17 Jun 73	147	2.30
Oswego River	11	18 Jul 72	--	--
Oswego River	28	2 Mar 73	80	1.34
Oswego River	29	12 Mar 73	106	--
Black River	12	19 Jul 72	--	--
Black River	25	28 Aug 72	53	--
Black River	36	29 Mar 73	34	--
Black River	44	1 May 73	34	0.59
Black River	53	28 May 73	41	0.75
Black River	60	17 Jun 73	99	0.25
Fort Niagara				
Niagara River	6	10 Jul 72	8	*0.05
Niagara River	18	2 Aug 72	22	--
Niagara River	27	26 Feb 73	18	--
Niagara River	33	28 Mar 73	34	--
Niagara River	41	30 Apr 73	22	0.45
Niagara River	50	27 May 73	26	0.82
Niagara River	56	15 Jun 73	59	1.13

(continued)

Table 1. SAMPLING DATES AND INITIAL PHOSPHORUS AND NITROGEN CONCENTRATIONS FOR LAKE ONTARIO AND TRIBUTARY WATER

Location	Station/ Sample No.	Date Collected	TP mg/l P	"TN mg/l N
Beaver Island				
Niagara River	32	28 Mar 73	30	--
Niagara River	40	30 Apr 73	15	--
Niagara River	49	27 May 73	51	--
Niagara River	57	15 Jun 73	86	--
Genesee River	14	20 Jul 72	--	0.69
Genesee River	16	2 Aug 72	167	--
Genesee River	34	29 Mar 73	386	2.21
Genesee River	42	30 Apr 73	105	1.52
Genesee River	51	28 May 73	173	1.26
Genesee River	58	16 Jun 73	204	2.26

"TN = NO_3^- -N + total Kjeldahl-N; + = Dissolved reactive phosphorus only;

* = NO_3^--N only

Phosphorus and nitrogen values were analyzed by William Cowen, University of Wisconsin, Madison.

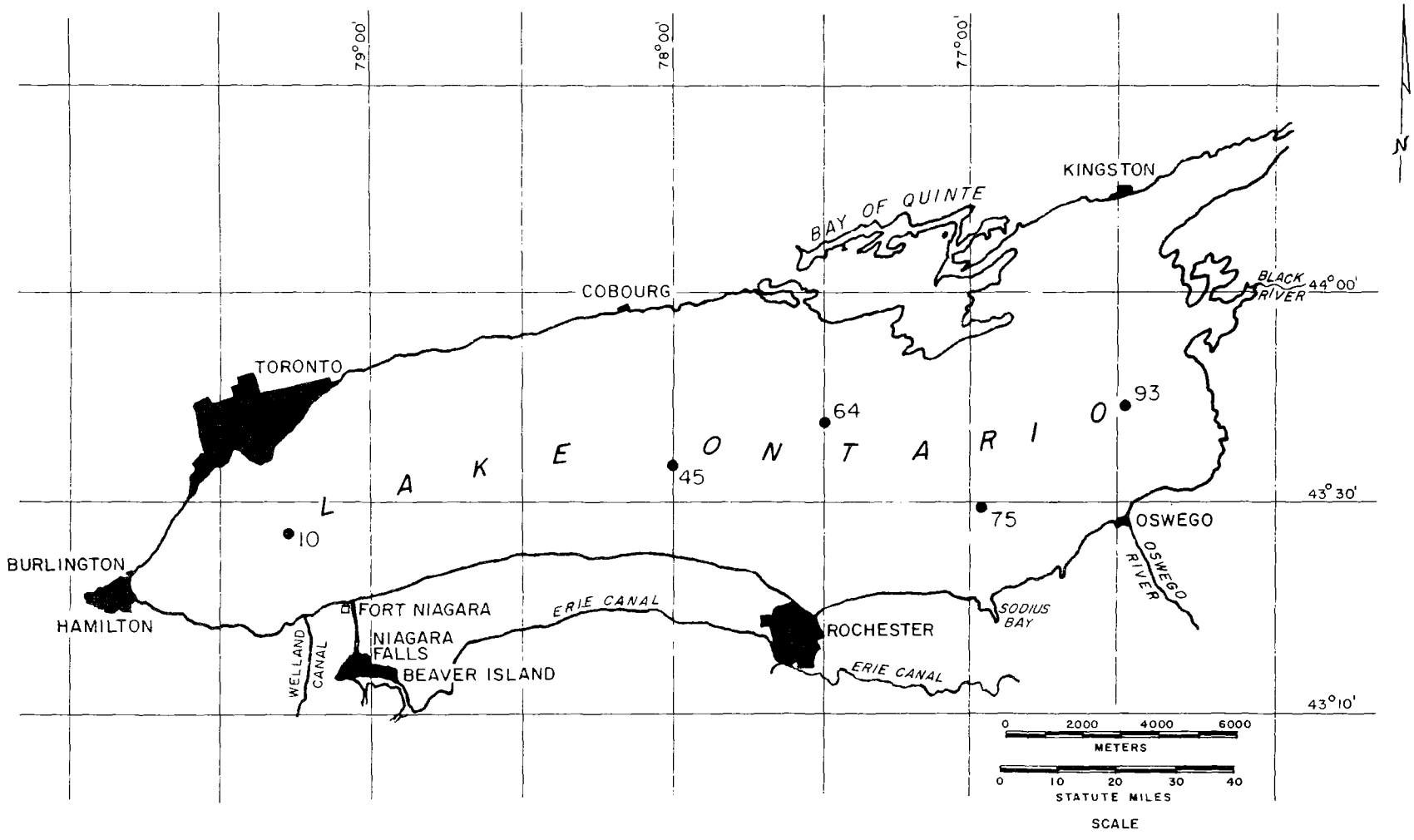

Figure 1. Lake Ontario and tributary sampling sites

SECTION II

CONCLUSIONS

It is concluded for Lake Ontario waters and the Niagara and Black Rivers that phosphorus is the key limiting element controlling excessive algal growth. At certain times of the year, especially in late summer, nitrogen also limits algal growth in these waters. The Genesee River water samples showed nitrogen to be the element most likely controlling algal growth. Similar results showing nitrogen controlling growth were obtained for the Oswego River; however, some Oswego River samples showed algal growth limitation possibly due to a lack of micronutrients or more probably, the presence of a toxic metal whose toxicity is eliminated by the addition of EDTA.

The overall conclusion derived from this study is that steps should immediately be taken to reduce the phosphorus input to Lake Ontario to the maximum extent economically feasible which includes treatment of all domestic wastewaters entering Lake Ontario or its tributaries for 90 percent phosphorus removal.

SECTION III

RECOMMENDATIONS

Based on the results of this study, it is recommended that all municipalities of more than 1,000 people which contrubute domestic wastewaters to Lake Ontario and its tributaries immediately initiate 90 percent phosphorus removal from its domestic wastewaters.

The following additional studies are recommended:

1. One of the major water quality problems of Lake Ontario is the excessive attached algal growth near the shore caused primarily by Cladophora. It is recommended that studies be made on the factors limiting Cladophora growth on the near shore waters of Lake Ontario.
2. Additional studies of the factors limiting algal growth in the Genesee and Oswego Rivers should be initiated with particular attention given to determining whether phosphorus input reduction to these waters can be of sufficient magnitude to make algal growth limited by phosphorus within the rivers and river mouths. Also, attention should be given to the possible presence of toxicants in these rivers which is currently limiting algal growth.

SECTION IV

EXPERIMENTAL PROCEDURES

REAGENTS

A stock phosphate solution was prepared by dissolving KH_2PO_4 in glass-distilled water. Nitrogen added to the Lake Ontario cultures was in the form of nitrate. A stock nitrate solution was prepared by dissolving $NaNO_3$ in glass-distilled water.

The stock micronutrient solution (EPA 1971) contained 185.52 mg/l H_3BO_3, 265.26 mg/l $MnCl_2$, 32.709 mg/l $ZnCl_2$, 0.780 mg/l $CoCl_2$, 0.009 mg/l $CuCl_2$, 7.26 mg/l $Na_2MoO_4 \cdot 2H_2O$, 96.0 mg/l $FeCl_3$, and 300 mg/l $Na_2EDTA \cdot 2H_2O$.

For the experiments where ^{14}C-technique was used, carbon-14 was added to the samples as a basic carbonate solution. Sealed ampules of ^{14}C-bicarbonate were purchased and diluted to the appropriate volume to produce 1 μCi ^{14}C/ml solution. Sodium hydroxide solution was added to the ^{14}C-bicarbonate solution to raise the pH to 10.4 to prevent losses of ^{14}C-CO_2.

The scintillation cocktail used in the counting procedure contained 75.0 g napthalene, 10.5 g 2,5-diphenyl oxazole (PPO) and 0.45 g 1,4-bis-{2-C4-methyl-5-phenyl-oxazolyl} - benzene (dimethyl PoPoP) diluted to 1 liter with 300 ml ethylene glycol monoethyl ether (cellusolve) and 1,4-dioxane. All reagents used in the preparation of this cocktail were scintillation grade.

Sample activity was determined by liquid scintillation counting using a Packard 3320 Tri-Carb Scintillation Spectrometer. All samples were counted for 10 minutes and the activity of each sample was reported in counts per minute (CPM) after correcting the observed data for counting time and quenching.

ASSAY METHODS

Filtered Lake Water

In filtered lake and tributary waters, the Algal Assay Procedure (AAP) (National Eutrophication Research Program, 1971) was used and the growth response of a laboratory algal culture Selenastrum capricornutum was studied using absorbance (light scattering) measurements.

One liter of the sample was autoclaved at 15 psi for 15 minutes, cooled to room temperature, and let stand for a few hours. The sample was then filtered through 0.45 μ pore size membrane filter. The ph of the test water was then measured. If the pH of the filtered sample was not between 7.0 and 8.5, an adjustment of pH was made by passing air or CO_2 through the sample. The bioassays were run on 40 ml volumes of the autoclaved filtered samples in 125 ml flasks. The preparation of the glasswares followed the recommended procedure in "Algal Assay Procedure, Bottle Test," (National Eutrophication Research Program, 1971). Selenastrum capricornutum was the test algae grown in a synthetic algal nutrient medium (Table 2) with 3X phosphorus and 3X nitrogen. One-to-two-weeks' old culture was used as a source of inoculum. The cells from the Selenastrum culture were centrifuged for 30 minutes at 1500 rpm and the supernatant was discarded. The sedimented cells were resuspended in sodium bicarbonate solution (15 mg $NaHCO_3$/1) and centrifuged again for

Table 2. SYNTHETIC ALGAL NUTRIENT MEDIUM, NAAM

Compound	Concentration (mg/l)	Element	Concentration (mg/l)
$NaNO_3$	25.50	N	4.20
K_2HPO_4	1.04	P	0.186
$MgCl_2$	5.70	Mg	2.90
$MgSO_4 \cdot 7H_2O$	14.70	S	1.91
$CaCl_2 \cdot 2H_2O$	4.41	C	2.14
$NaHCO_3$	15.00	Ca	1.20
		Na	11.00
		K	0.47
H_3BO_3	185.52	B	32.46
$MnCl_2$	264.26	Mn	115.37
$ZnCl_2$	32.7	Zn	15.69
$CoCl_2$	0.78	Co	0.35
$CuCl_2$	0.009	Cu	0.004
$Na_2MoO_4 \cdot 2H_2O$	7.26	Mo	2.88
$FeCl_3$	96.00	Fe	33.05
$Na_2EDTA \cdot 2H_2O$	300.00		

30 minutes at 1500 rpm. The sedimented algal cells were resuspended in the bicarbonate solution and the number of cells in suspension was counted using a hemocytometer. The suspension was then diluted with bicarbonate solution to give the final cell concentration of 2×10^5 cells/ml. An initial cell concentration of 10^3 cells/ml of the sample was used.

The water samples were spiked with phosphorus, nitrogen or micronutrients to give the final concentration of 100 µg P/l, 1000 µg N/l, and the concentration equivalent of AAP micronutrients, respectively. The flasks with water samples were spiked with phosphate, nitrate, and micronutrients, individually and in combination, to identify the growth-limiting nutrient(s). Table 3 is an outline of the procedure which was employed to assess the nutrient status of Lake Ontario water. The flasks with the test samples were incubated at $24^\circ \pm 3^\circ$C under "cool-white" fluorescent lighting - 400 ft c ± 10 percent illumination. Measurement of *in vivo* absorbance was made on days 8 to 16 of the incubation, using 10 cm cells and Beckman DU Spectrophotometer. Aliquots taken for absorbance measurements were returned to their flasks after taking the reading.

The absorbance reading at 750 nm for an algal culture was calibrated with the dry weight of algae. A known aliquot of a dense culture of *Selenastrum capricornutum* was filtered through a glass fiber filter, oven-dried at 110°C, cooled, and weighed. The same culture was diluted by different volumes of water and the absorbance measured for each dilution. A calibration curve is presented in Figure 2.

To be sure that the absorbance measurements were sensitive enough for the purpose of the present study, fluorescence measurements were made on some of the AAP tests and the results were compared with the results from the

Table 3. ALGAL BIOASSAY EXPERIMENTAL DESIGN

Treatment	No. Flasks
Lake water control - 40 ml lake water	3
Phosphorus spikes - Lake water + 100 µg P/l	3
Nitrogen spikes - Lake water + 1000 µg N/l	3
Combined spikes - Lake water + 100 µg P/l + 1000 µg N/l	3
Micronutrient spikes - Lake water + micronutrients	3
Combined Micronutrient spikes - Lake water + Micronutrients + 1000 µg N/l	3
Growth References - Phosphorus	
*NAAM-P medium	3
NAAM-P + 100 µg P/l	3
Growth References - Nitrogen	
*NAAM-N medium	3
NAAM-N + 1000 µg N/l	3
TOTAL	30

*synthetic algal assay medium (Table 2)

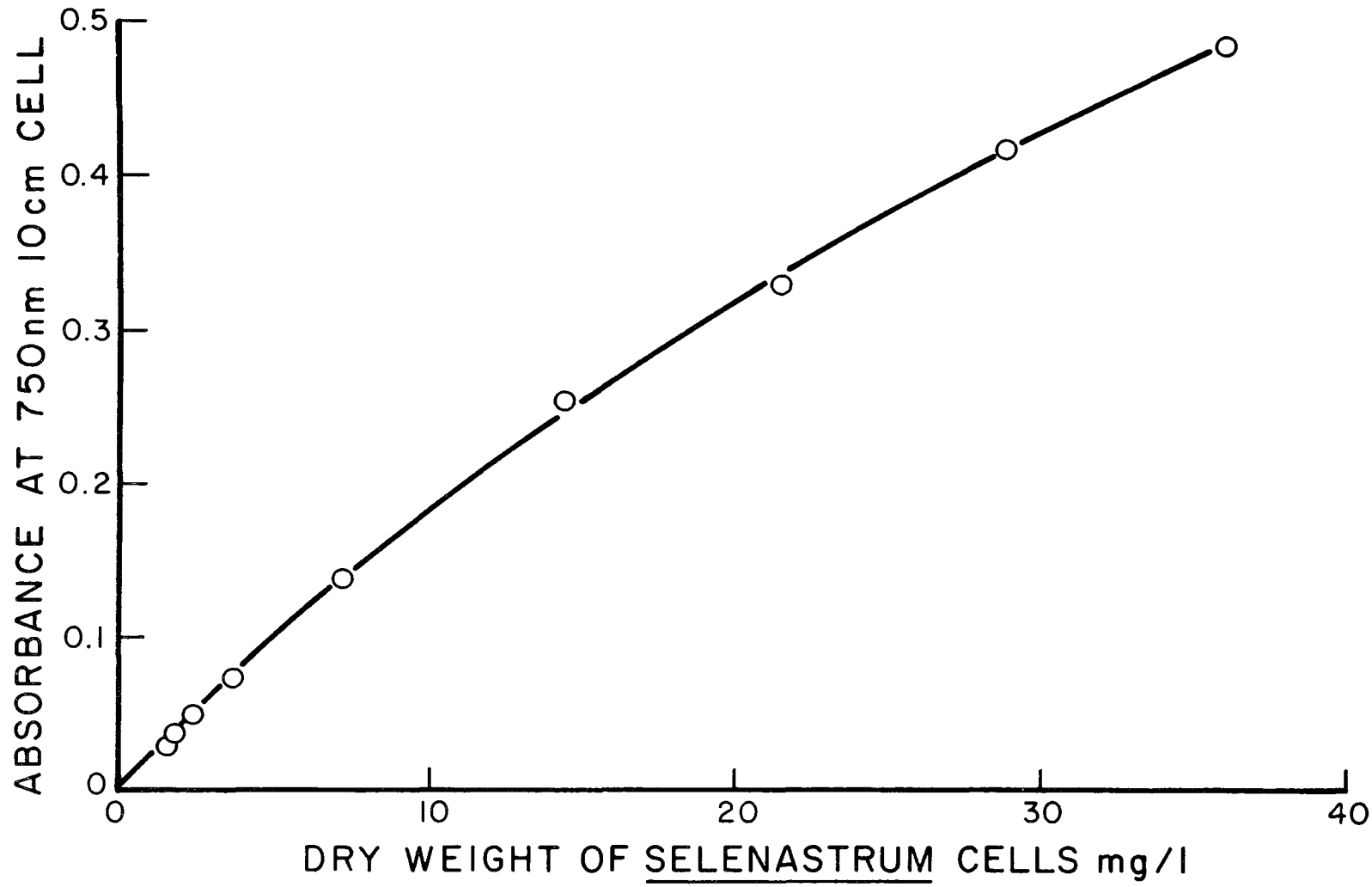

Figure 2. Relationship between absorbance and dry weight of Selenastrum capricornutum cells.

absorbance measurements. Fluorescence can be measured at early stages of growth increases, due to the sensitivity of the technique. Figure 3 is a plot of fluorescence vs time for Sample 14 of Genesee River water sampled on July 20, 1972. The measurements of increase in fluorescence were made from days 3 through 10. The results indicate an increased growth response of Selenastrum in samples with the addition of nitrogen plus micronutrients. This is similar to the curve presented in Figure 3, which is a plot of absorbance vs time for Genesse River water sample. The absorbance measurements were made from days 9 through 13 and the results as seen from the Figure 4 indicate an increased growth with nitrogen plus micronutrients addition in the sample. Since both absorbance and fluoresence measurements served the same purpose in the present study, due to the easy accessibility of the instrument, absorbance measurements were used in the present study to follow the growth response of Selenastrum.

Unfiltered Lake Water

In the present nutrient spiking study, ^{14}C-technique was used to assess the growth of the "natural" algae present in unfiltered lake water. The first step in processing the unfiltered samples was to determine the initial productivity of the sample. Six 25 ml aliquots of the sample were spiked with 0.5 μCi ^{14}C-$CO_3^=$ each and incubated for four hours. Three of the replicates were incubated under 400 ft c of light supplied by fluorescent lights. The remaining three samples were incubated at the same temperature as the first three samples but in the dark. After a four-hour incubation period, the samples were filtered through 0.45 μ pore size Millipore filters at a pressure differential equivalent to 15 cm of mercury. During the filtration step, the sample

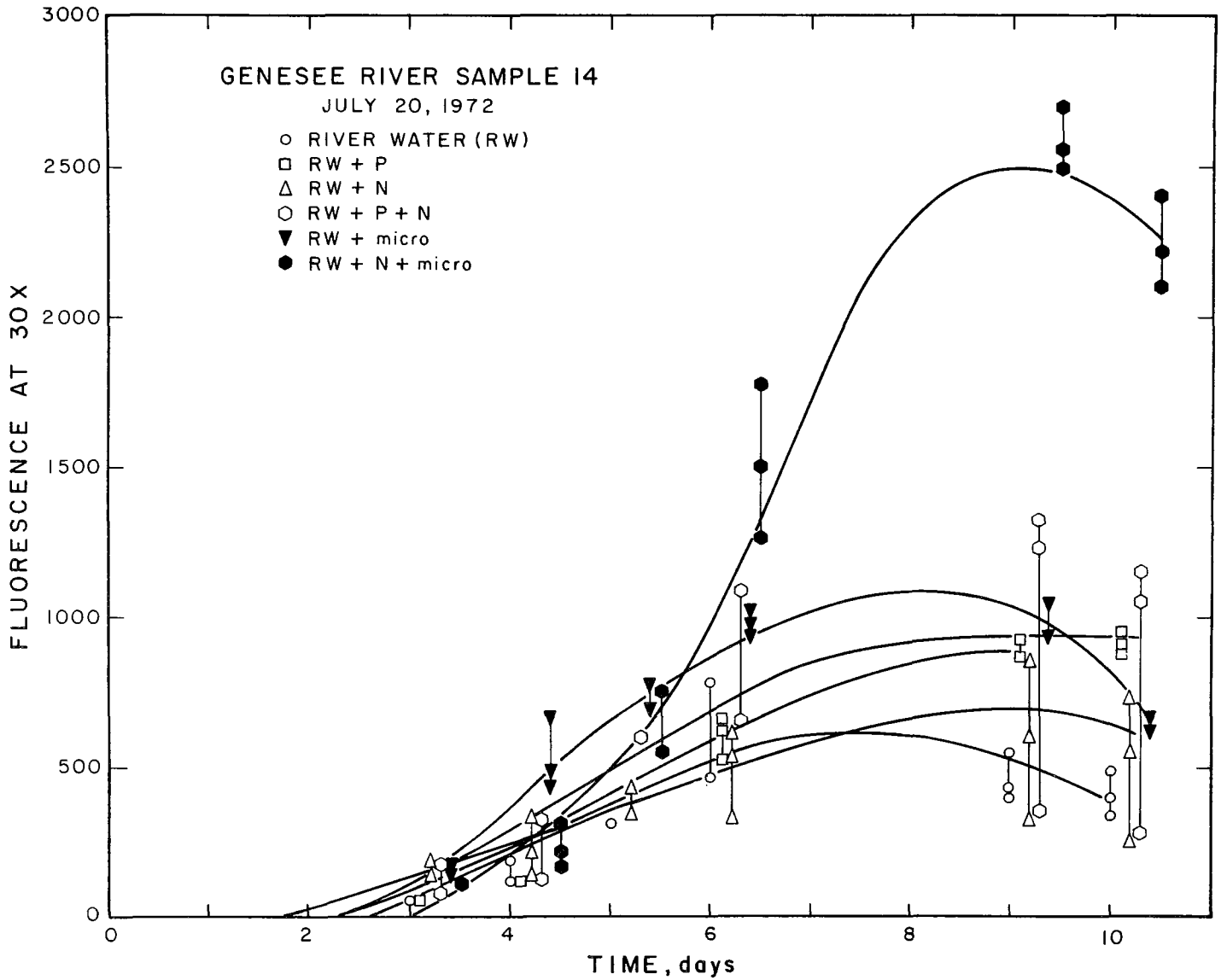

Figure 3. The growth of Selenastrum in Genesee River waters.

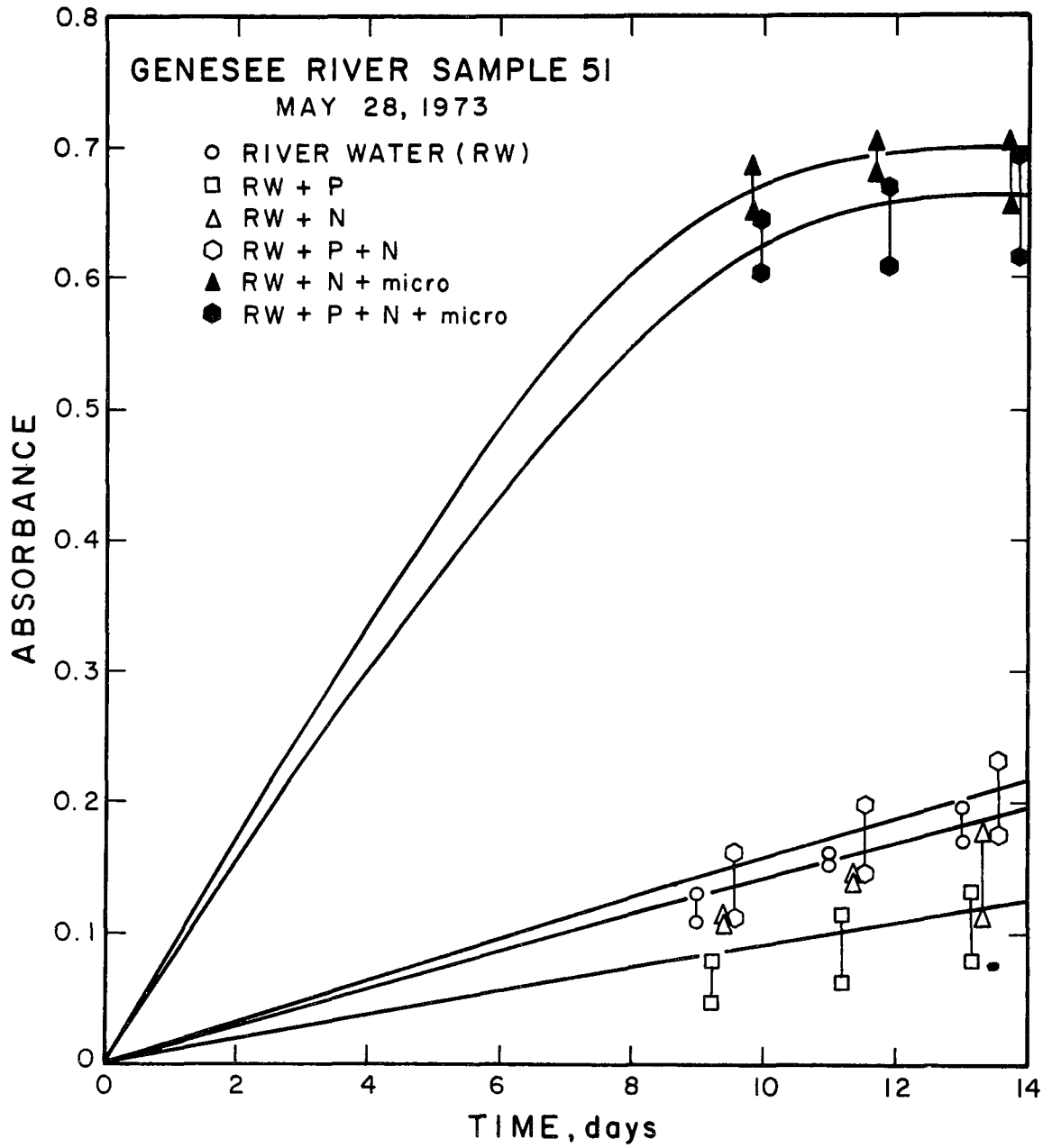

Figure 4. The growth of Selenastrum in Genesee River water.

bottles were rinsed twice with distilled water and the filters were rinsed five times with distilled water. The total wash volume was approximately 50 ml.

Filters were placed in a desiccator containing concentrated HCl for one-half hour and then dried overnight in a second desiccator containing silica gel. The filters were dissolved in a 10 ml scintillation cocktail and 5 ml dioxane. The samples were counted for ten minutes to determine the amount of carbon-14 incorporation. Net carbon fixation for the sample was calculated by subtracting the dark bottle fixation from the light bottle fixation.

Once the initial productivity of the sample was determined, the experiment was continued to determine the effect of nutrient additions. Fifty ml aliquots of the lake water samples were placed in 125 ml culture flasks. The phosphate, nitrate, and micronutrient solutions were then added singularly and in combination to the lake water sample. Triplicate samples were prepared for each experimental condition. The flasks were then sealed with parafilm wax paper and incubated for one week at 22 $\pm 2^{\circ}$C under 400 ft c of light.

A set of screw-capped, four-oz jars was prepared for dark incubation by painting the glass walls completely black. A set of unpainted four-oz jars was used for the incubation of the samples in light.

Following the incubation period of one week, two 25 ml samples were taken from each of the triplicate flasks. One sample was placed in a four-oz jar painted black for dark incubation and the other sample was placed in the unpainted jar. To each of the jars, one-half μCi ^{14}C-$CO_3^=$ was added, and the jars were incubated for an additional four-hour period.

The short four-hour incubation period was completed under the same conditions of temperature and light intensity

as the longer one-week incubation period except for keeping the designated dark bottle samples out of the light. The samples were filtered and the filters were processed as described earlier. The net carbon assimilation was calculated as the difference between the light and dark bottle fixation and compared to the initial and incubated lake water samples.

SECTION V
EXPERIMENTAL RESULTS AND DISCUSSION

RESULTS

The results of the algal bioassay on Lake Ontario and its tributary waters will be dealt with individually in this section.

Lake Ontario

The bioassay response of Selenastrum capricornutum, with and without the addition of nutrients, was measured using absorbance. Five samples from Station 10, situated north of Welland Canal, four samples from Station 45 located north of Sodius Bay and one sample each from Stations 64 and 93 were received and used in the algal bioassay studies. In addition, two samples from near the mouth of Niagara River, two samples from near the mouth of Genesee River and one sample from near Toronto, Ontario, were collected and AAP tests were run. The data are tabulated in Appendix A.

The water samples had total phosphorus concentrations in the range of 5 to 43 µg P/l and the total nitrogen 0.25 to 0.40 mg N/l. All 21 samples collected from Lake Ontario showed phosphorus or phosphorus and nitrogen limitation. Typical growth response curves are presented in Figure 5. These figures are a plot of absorbance vs time for Lake Ontario water sample collected from Station 45 on March 5, 1973. The vertical lines indicate the absorbance range obtained for triplicate samples. An increase in algal

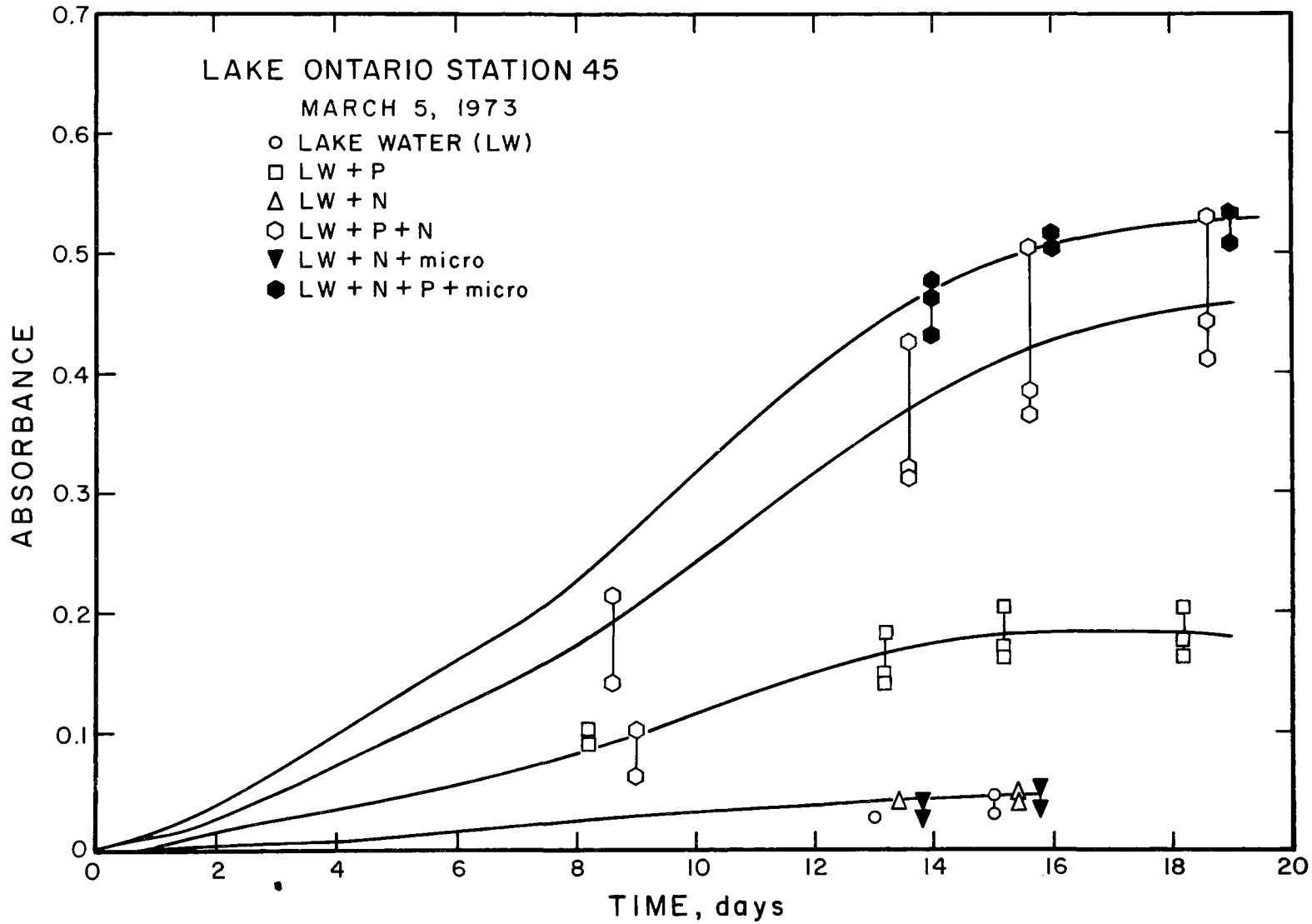

Figure 5. The growth of Selenastrum in Lake Ontario water.

growth in water samples with phosphorus (100 µg P/l final concentration) was observed. The addition of phosphorus (100 µg P/l) and nitrogen (1000 µg N/l) increased the growth to a greater extent. It is possible that the addition of phosphorus alone would have altered the nitrogen:phosphorus atomic ratio in the sample to create an artificial nitrogen limited condition which is alleviated in the samples with phosphorus plus nitrogen additions. This could be one reason for the increased growth in samples with phosphorus plus nitrogen spikes. A second possible reason is that the biologically available phosphorus in the lake water may be below the minimum required concentration levels and the biologically available nitrogen concentration is just above the minimum required level for the algal growth. Under these conditions, an addition of phosphorus would increase the growth while the addition of nitrogen would not increase the growth of algae since the initial phosphorus concentration in water is below the minimum required level. The addition of phosphorus and nitrogen would increase the growth of algae more than the sample with the addition of phosphorus alone.

In some of the Lake Ontario samples, the addition of phosphorus alone did not increase the growth response of the laboratory algae. A markedly increased growth was observed in the samples with phosphorus and nitrogen spikes. Such a condition, where phosphorus and nitrogen are limiting the algal growth, is possible in Lake Ontario waters where the total phosphorus concentration is in the lower range (<10 µg P/l) and the total nitrogen concentration is also low. Either phosphorus or nitrogen spiking individually will still keep the other nutrient below the required level for the algal growth. The addition of both phosphorus and nitrogen in the same sample will thus alleviate the phosphorus and

nitrogen limited conditions and increase the growth of algal cells. A typical example of such a situation is seen in Figure 6 which is a plot of absorbance vs time for the water sample from Station 75 collected on (July 19, 1972.)

Based on variations on the absorbance values which correlate with the algal cell counts, nine of 21 samples receiving phosphate showed greater growth than the incubated lake water controls. Seventeen of 21 samples showed increased growth in samples with phosphate plus nitrate additions.

The Lake Ontario water samples collected from near the mouth of tributaries showed a growth response similar to the deeper lake water samples. Figures 7 and 8 show the growth of Selenastrum in lake water collected near the mouth of Niagara and Genesee Rivers, respectively. While the sample collected near Niagara River showed a phosphorus limited condition, the water sample from near Genesee River showed increase in growth in flasks with phosphorus spiked samples and flasks with nitrogen plus micronutrients added samples.

To assess the growth of "natural" lake algae in Lake Ontario water, absorbance measurement was replaced by ^{14}C technique. The unfiltered lake water was processed as described in the experimental procedure. The first sample was collected from Station 64 in August, 1972. The lake water sample was spiked with 100 µg P/l or 1000 µg N/l or 100 µg P/l + 1000 µg N/l or 1000 µg N/l + micronutrients. All additions were made as 1.25 ml from working solutions to a 50 ml unfiltered lake water sample. A set of lake water samples without any nutrient additions was also included in each experiment.

Net carbon fixation results for water samples from Station 64 are presented in Figure 9. Incubation of lake water alone and lake water with nutrients for one week did

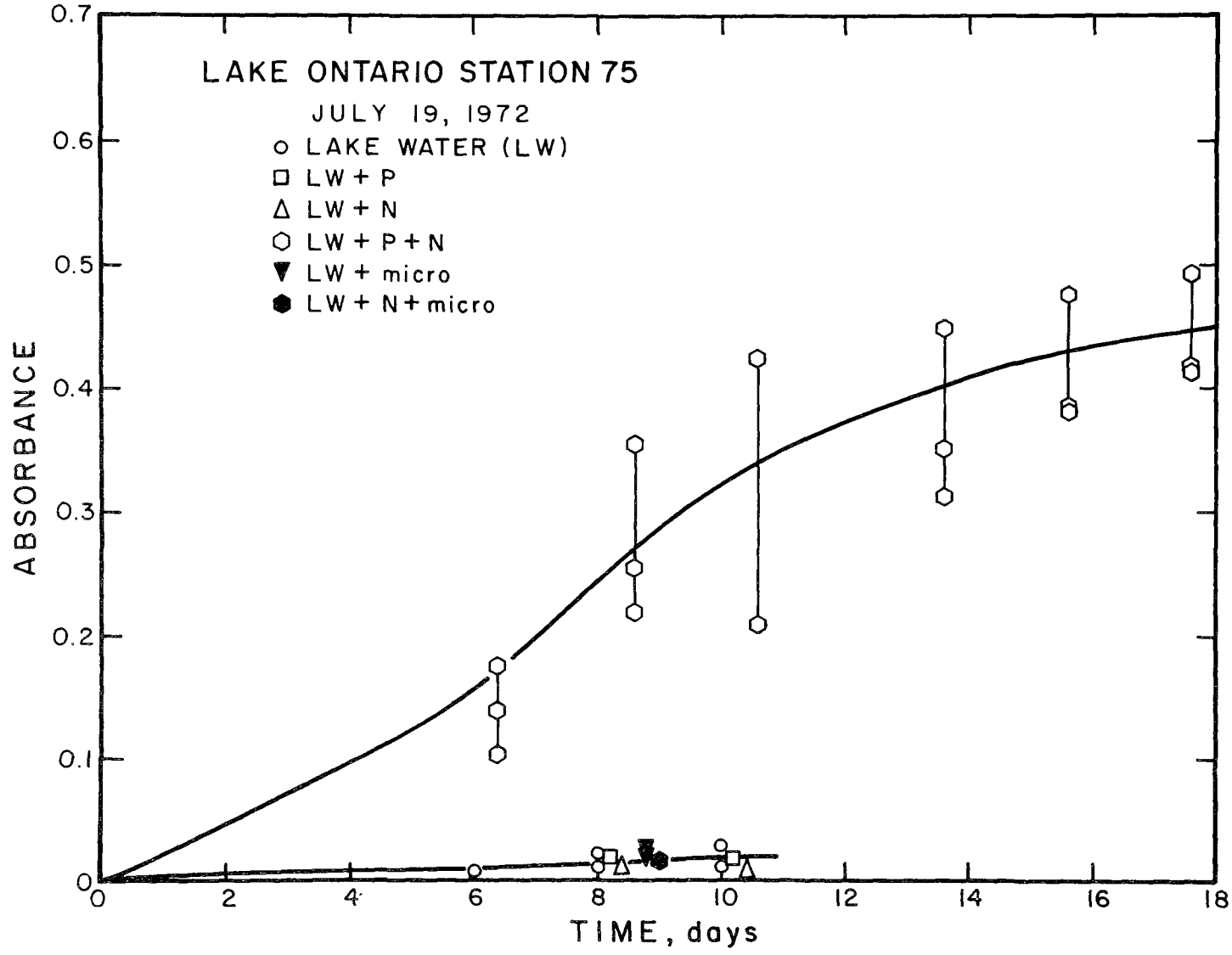

Figure 6. The growth of Selenastrum in Lake Ontario water.

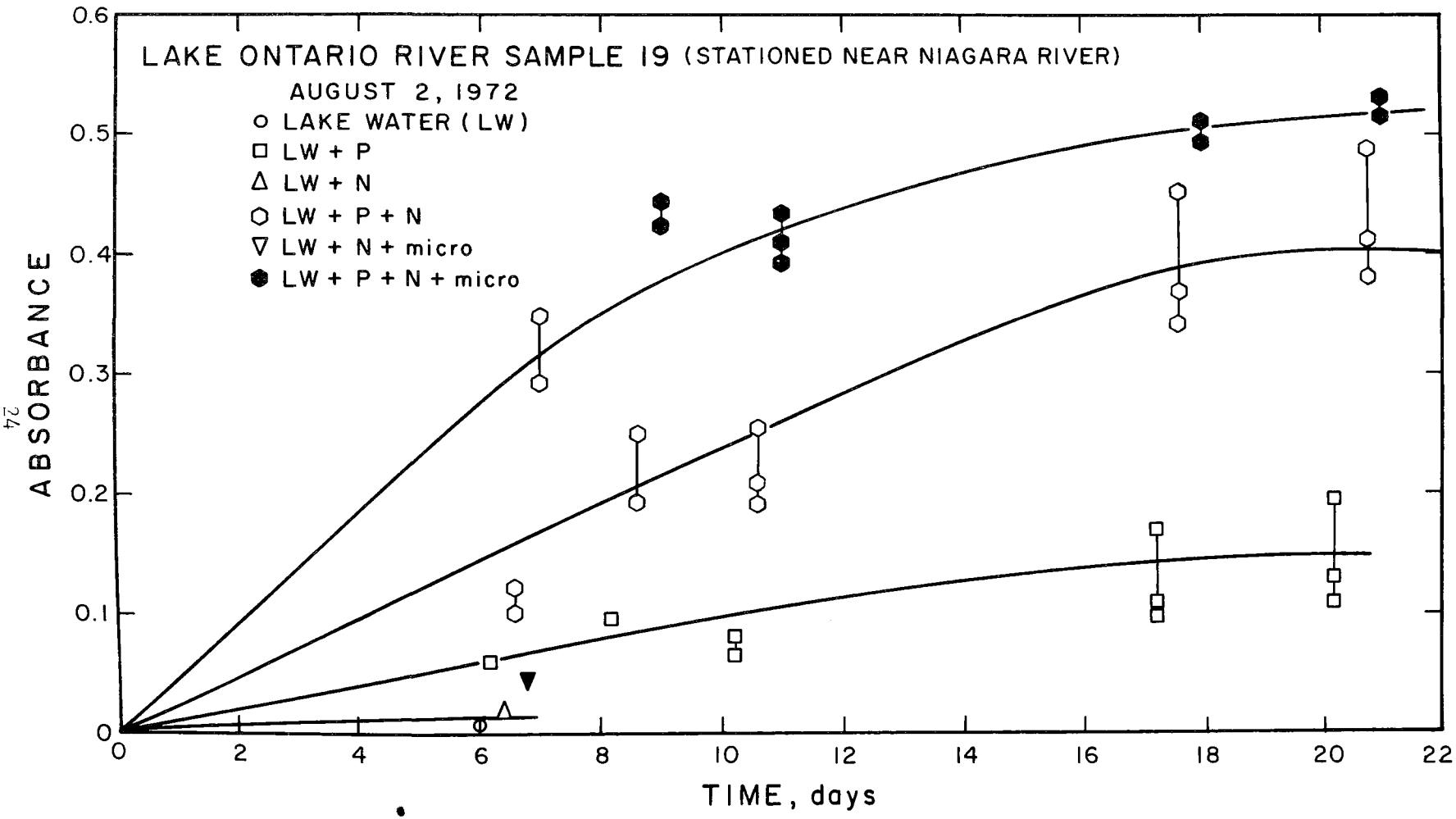

Figure 7. The growth of Selenastrum in Lake Ontario water.

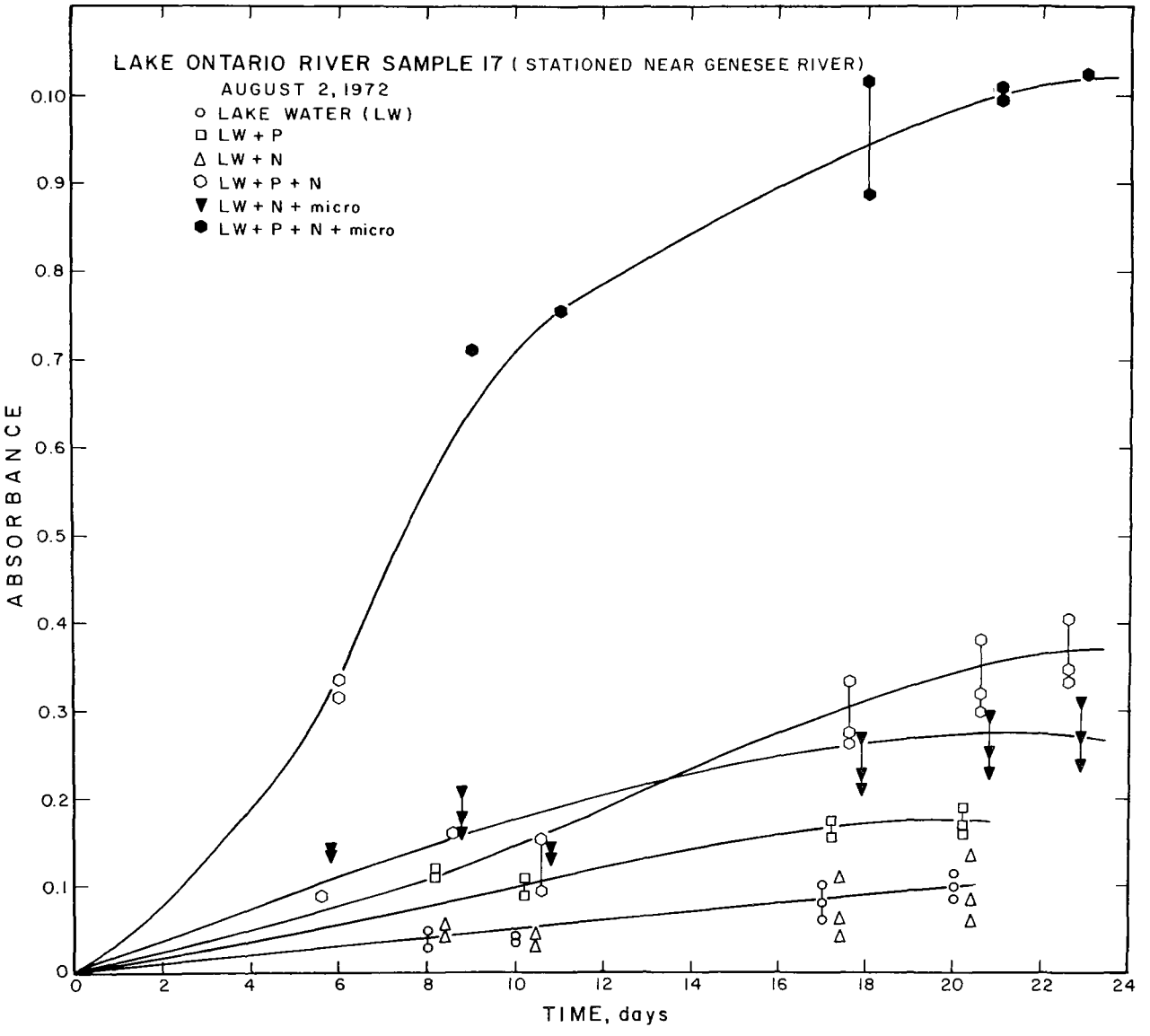

Figure 8. The growth of Selenastrum in Lake Ontario water.

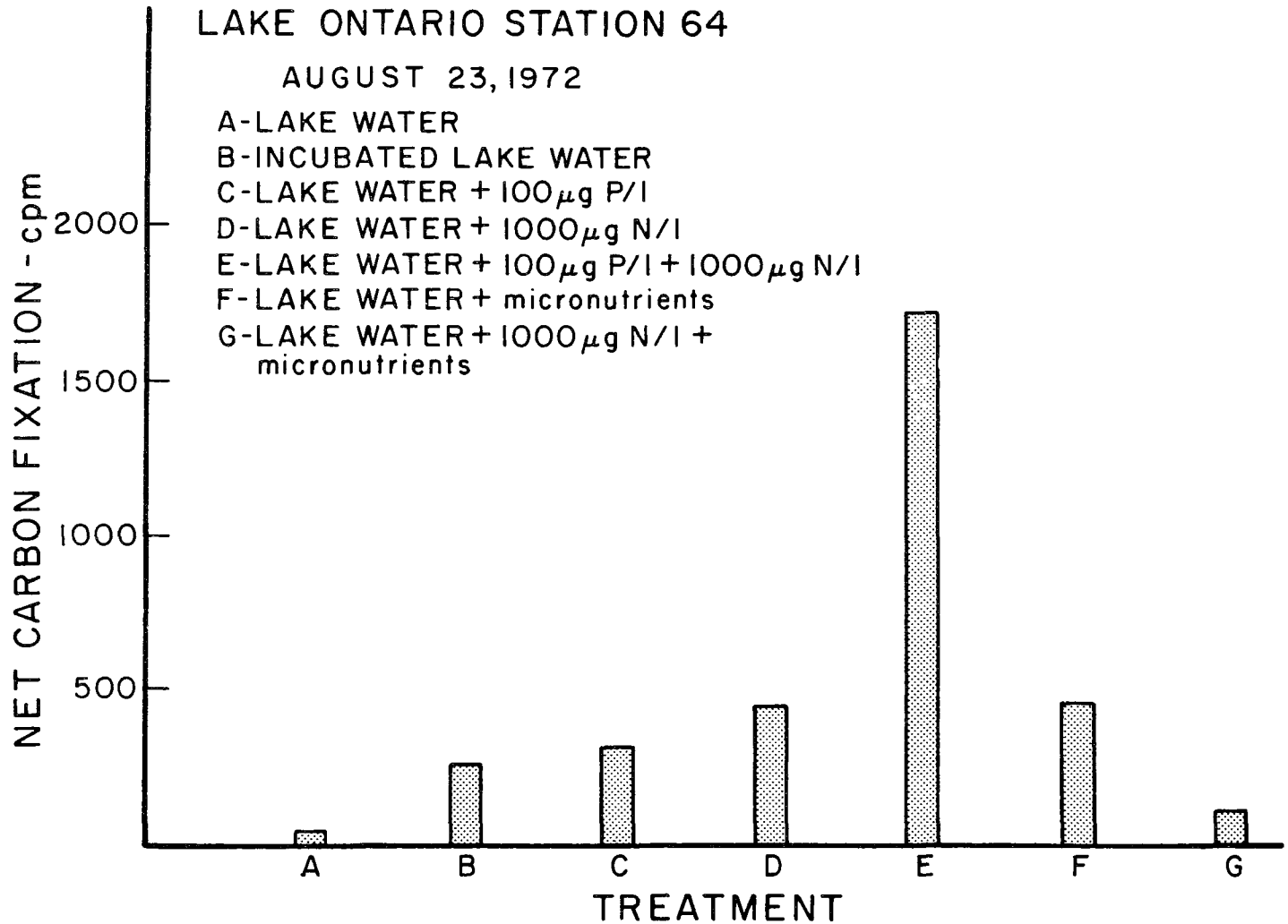

Figure 9. Effect of nutrient spiking on the growth of "natural" Lake Ontario algae in Lake Ontario water.

result in increased carbon fixation; however, based on variations in the C-14 activity values in the light minus dark bottles, eight of 26 samples receiving phosphate + nitrate showed significantly greater growth than the incubated lake water controls at 0.05 confidence level. (95 percent probability that the response is actually greater than the incubated lake water controls.) For samples with phosphate + nitrate + micronutrients, seven of seven samples showed significantly greater growth than the incubated lake water controls at the 0.05 confidence level. Table 4 gives the response of growth of lake water culture to different treatments for water samples collected from various locations.

Alum Treatment --

The potential benefit that may be derived from a reduction of phosphorus present in Lake Ontario water on the growth of algae was assessed by studying the algal response in alum-treated lake water. One-liter water samples in Imhoff Cones were treated with 5 or 10 ml of alum (10 g/l of $Al_2(SO_4)_3 14\ H_2O$), stirred continuously for 30 seconds, and allowed to settle. After 24 hours, the supernatant was filtered through 0.45 μ pore size filters. The phosphorus content on the filtered sample was measured to be sure that phosphorus had been removed by alum treatment. Fifty ml samples of filtrate were taken in 120 ml Erlenmeyer flasks and treated with 100 μg P/l or 100 μg P/l + micronutrients. A set of controls of filtered alum-treated lake water was used in each experiment. All treatments and controls were run in triplicates. One ml of untreated lake water was added as "seed" to all the test flasks. After seven days of incubation, the samples were spiked with $^{14}C\text{-}CO_3^=$ and incubated in light and dark environments for four hours. The difference in the ^{14}C counts taken between the light and dark bottles in each set of treatment was used to assess the algal growth.

Table 4. RESPONSE OF INCUBATED TREATED SAMPLES RELATIVE TO INCUBATED LAKE WATER SAMPLES

Station	Response	* Treatment									
		10 P	25 P	100 P	150 N	375 N	1000 N	10 P + 150 N	25 P + 375 N	100 P + 1000 N	100 P + 1000 N + MicroNu.
10	No effect	3	3	3	3	2	3	2	2	3	0
	Stimulation	0	0	0	0	1	1	1	1	0	4
45	No effect	2	2	2	2	2	2	1	2	2	0
	Stimulation	0	0	0	0	0	0	1	0	0	1
75	No effect	3	3	3	3	3	2	2	2	2	0
	Stimulation	0	0	0	0	0	1	1	1	1	2
64	No effect	-	-	1	-	-	1	-	-	0	-
	Stimulation	-	-	0	-	-	0	-	-	1	-
93	No effect	-	-	1	-	-	1	-	1	0	-
	Stimulation	-	-	0	-	-	0	-	-	1	-

* All treatments are given in µg/l.

- = No Analysis.

The results of alum-treated experiments indicated a lack of growth stimulation of the "natural" phytoplankton in the alum-treated Lake Ontario water. A stimulatory effect was observed on alum-treated lake water samples spiked with phosphorus or phosphorus + micronutrients. The results are summarized in Table 5. For the water samples collected in March, April, and May of 1973, from Stations 10, 45, and 75, stimulatory effect on the growth of natural algae was seen in both phosphorus or phosphorus + micronutrients spiked samples. Except in two cases, all were significant at 0.05 confidence level.

Tributaries

Niagara River--

A total of 11 water samples was collected from Niagara River, seven of which were from near Fort Niagara and four from near Beaver Island. The total phosphorus concentrations were in the range of 8 to 86 µg P/l. Most of the samples had concentrations between 20 and 30 µg P/l of total phosphorus. Algal Assay Procedure (AAP) tests were run on these samples and the results showed an increase in algal growth in phosphorus-spiked samples. These results are tabulated in Appendix B. A typical relationship of absorbance vs time for Sample 57 collected on June 15, 1973, is given in Figure 10. Figures 11 and 12 present typical results obtained on other days for the Niagara River samples.

Sample 41 from Niagara River had low total phosphorus (22 µg P/l) and total nitrogen concentration (0.45 mg N/l). The AAP results presented in Figure 12 show an increase in growth response with phosphorus addition to the sample. A greater increase in growth was observed in samples with phosphorus plus nitrogen spikes. Sample 50 (Figure 12) had low total phosphorus concentration (26 µg P/l) and higher total nitrogen concentration (0.82 mg N/l). The AAP results show an increase in growth in samples with phosphorus spikes.

Table 5. EFFECT OF ALUM TREATMENT ON THE GROWTH OF "NATURAL" LAKE ALGAE IN LAKE ONTARIO WATER

Station	Date Collected	Treatment	
		Alum + 100 µg P/l	Alum + 100 µg P/l + Micronutrients
10	1 April 73	ST*	ST
45	1 April 73	ST	ST
75a	1 April 73	ST	ST
75	1 April 73	NE**	ST
10	2 May 73	ST	NE
75	2 May 73	ST	ST
45	6 May 73	ST	ST
75	6 May 73	ST	ST

[a]All but one sample received 100 mg of alum ($Al_2[SO_4]_3 \cdot 14\ H_2O$)/l of sample. One water sample from Station 75 (1 April 73) was treated with 50 mg/l of alum

* ST = Stimulation

**NE = No effect in comparison to the alum treated sample.

The sample with phosphorus plus nitrogen did not show greater increase in growth when compared with the results from the flasks with phosphorus spikes only.

In Sample 50, the nitrogen concentration was high enough not to limit the algal growth. In Sample 41, the available nitrogen concentration could possibly be at the borderline of minimum required concentration. Therefore, when nitrogen was added to the sample along with phosphorus, an increased

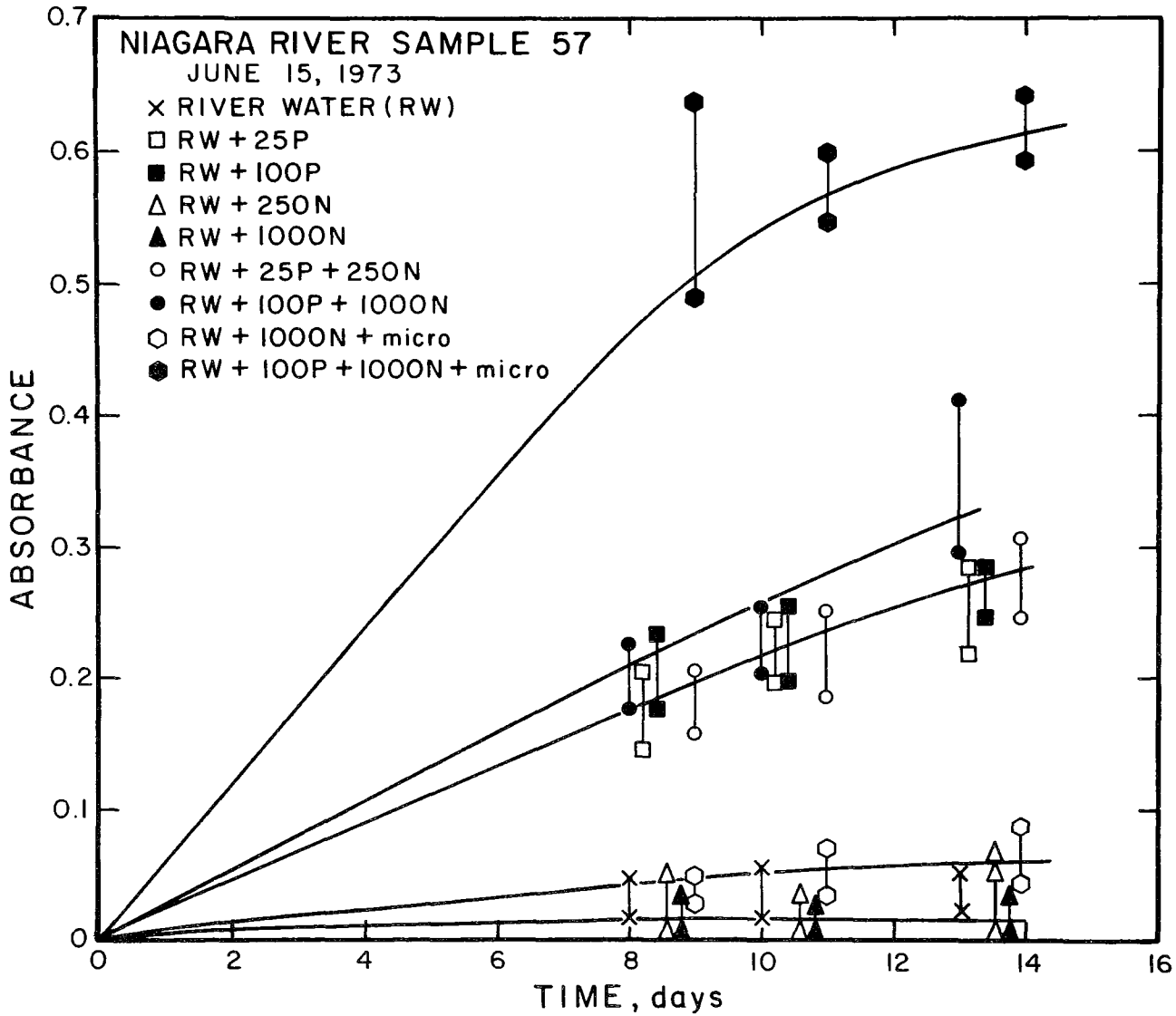

Figure 10. The growth of Selenastrum in Niagara River water.

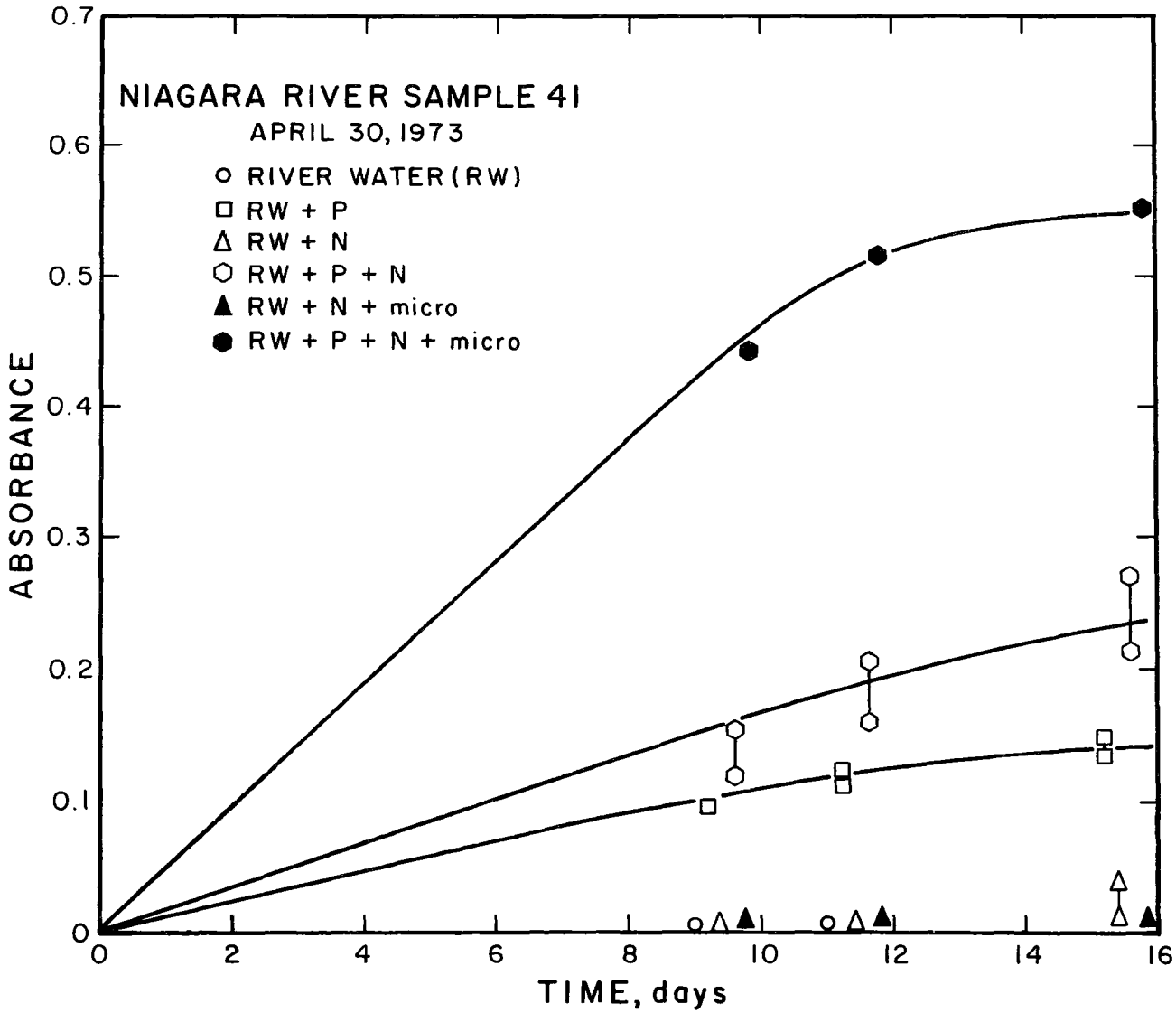

Figure 11. The growth of Selenastrum in Niagara River water.

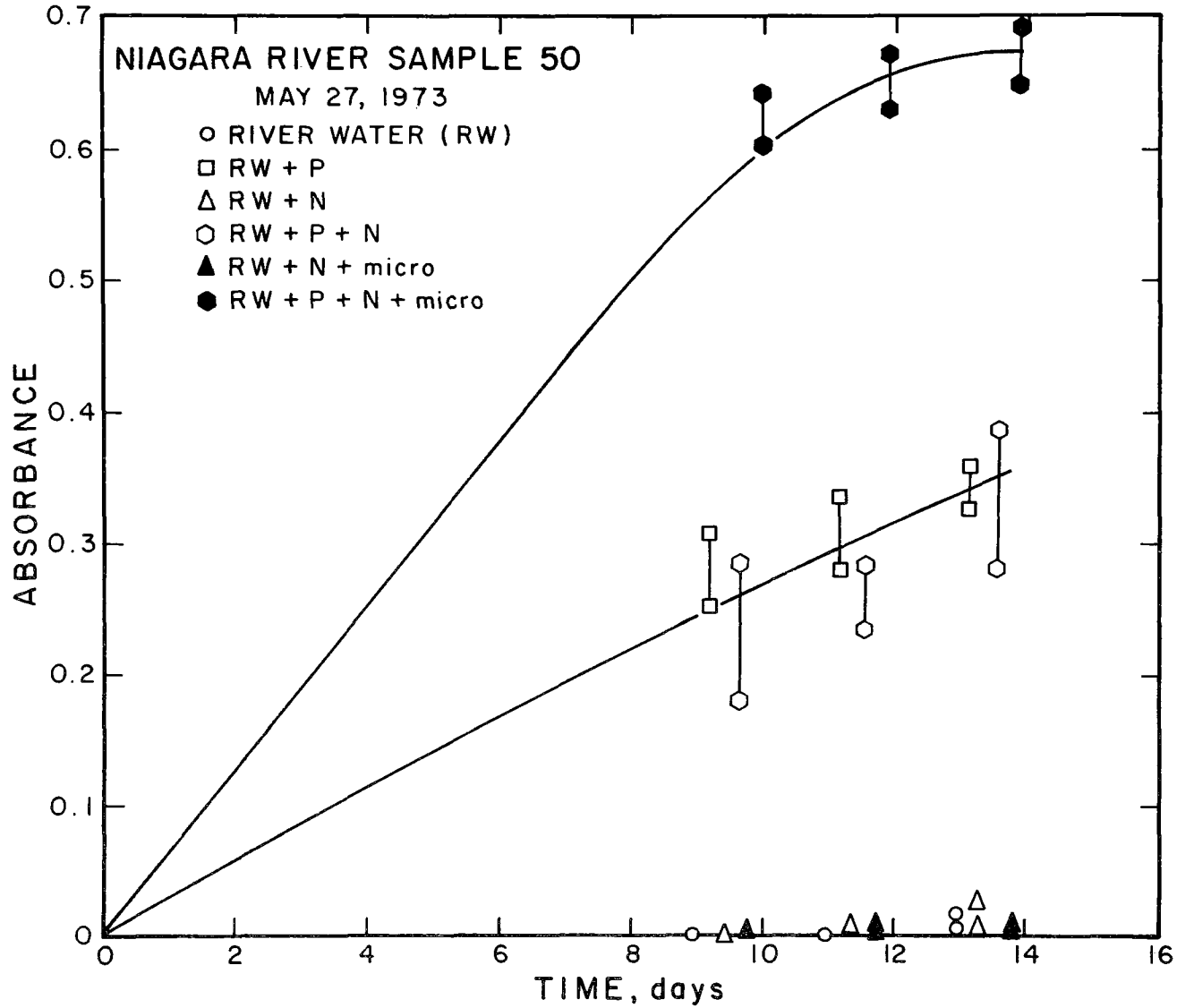

Figure 12. The growth of Selenastrum in Niagara River water.

growth was observed in Sample 41.

All 11 samples from Niagara River showed phosphorus-limited condition as determined by the growth of Selenastrum. Some water samples which had low total nitrogen concentration showed an increased algal growth response in flasks with phosphate + nitrate spikes. These results are similar to the results obtained with Lake Ontario water samples.

Genesee River --

Six samples were collected from Genesee River near Rochester and the total phosphorus concentrations were in the range of 105 to 386 µg P/l and the total nitrogen concentrations were in the range of 0.69 to 2.26 mg N/l. AAP tests were run on all these samples and the results are tabulated in Appendix C. With the exception of one sample (34), the Genesee River water samples showed increased growth response with the addition of nitrogen in the presence of micronutrients. A typical growth response curve is presented in Figure 13. The growth of Selenastrum as measured by absorbance did not show any change when phosphorus was added to the river water samples, but there was an increased growth when nitrogen plus micronutrients were added to the river water samples.

These results indicate a possible nitrogen limitation in the samples collected from Genesee River. It should be noted, however, that only six samples were obtained from Genesee River over a period of one year, four of them collected during March - June 1973 and more samples should be assayed before any conclusion can be drawn about the annual cycle of nitrogen and phosphorus limitation in these waters.

Oswego River --

During July 1972 through June 1973, eleven water samples were collected from the Oswego River. The total phosphorus concentrations were in the range of 80 to 306 µg P/l and

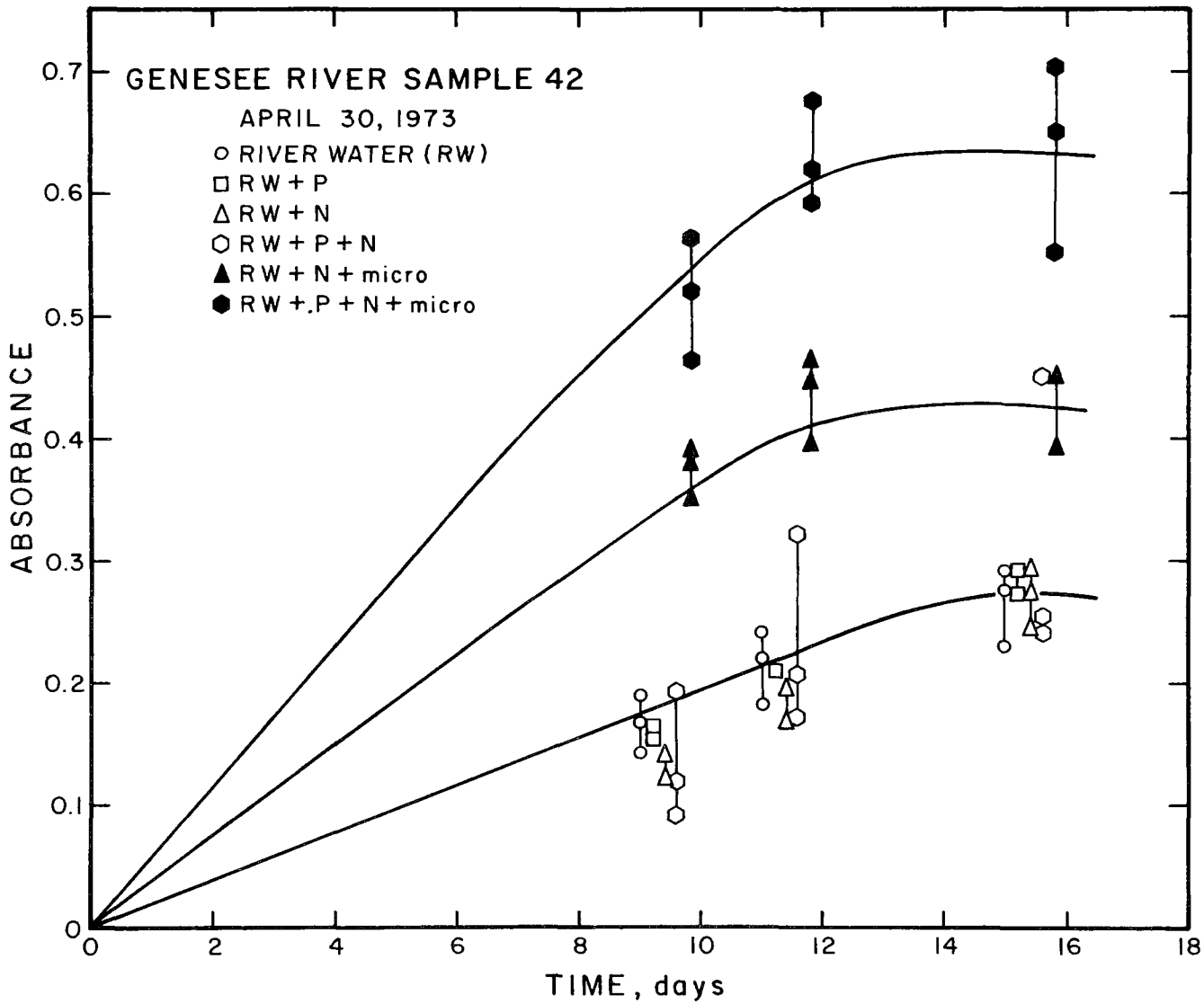

Figure 13. The growth of Selenastrum in Genesee River water.

the total nitrogen concentrations were in the range of 1.16 to 2.30 mg N/l. AAP tests were run on all of these samples and the results are tabulated in Appendix D.

The results from the AAP tests on Oswego can be separated into two sets. Seven samples showed no increase in growth of Selenastrum with any of the added nutrients individually or in combinations. The algae showed good growth response in all the samples including the control river water samples. This indicates nutrient enriched condition in these water samples. A typical growth curve is presented in Figure 14. Although a slight change in the growth is noted for different treatments as seen by the family of curves, the overlap of absorbance values of one treatment on the other indicates no significant change in growth responses of various treatments. On the other hand, four of the Oswego River samples showed a different type of growth response with various treatments in AAP tests. One of the test results is plotted in Figures 15 and 16 For Sample 55, an increased growth response was observed in flasks with nitrogen with micronutrients. The other treatments which included two concentrations of phosphorus and two concentrations of nitrogen spiked individually or in combination did not increase the growth of Selenastrum. This figure is typical of the other three sets of data obtained with the Oswego River water samples.

Black River --

Six Black River water samples were collected during July 1972 to June 1973. The total phosphorus concentrations were in the range of 34 to 99 µg P/l and the total nitrogen concentrations were in the range of 0.59 to 1.25 mg N/l. AAP tests were run on all these samples and the results are tabulated in Appendix E.

All the six samples showed an increase in algal growth

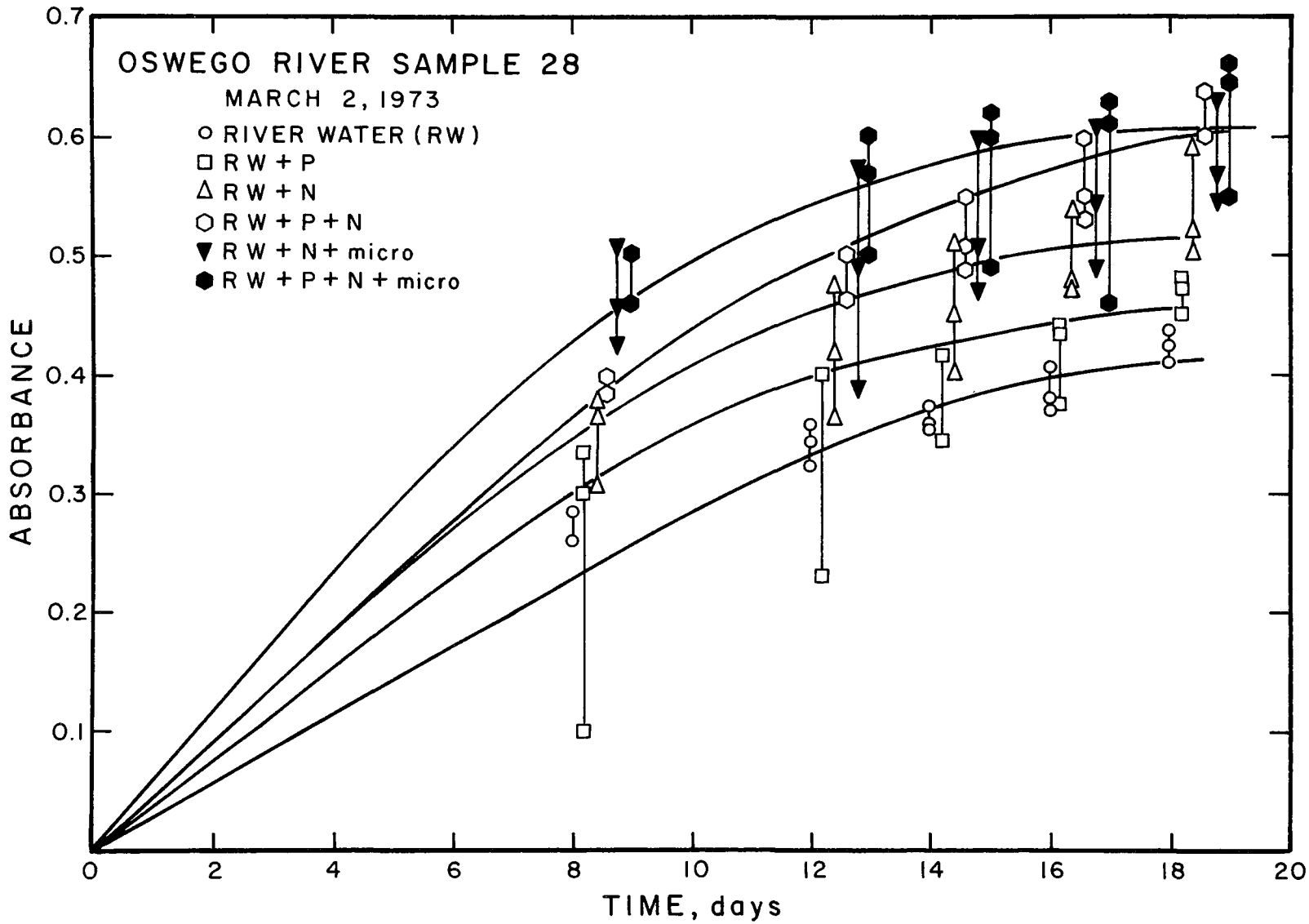

Figure 14. The growth of Selenastrum in Oswego River water.

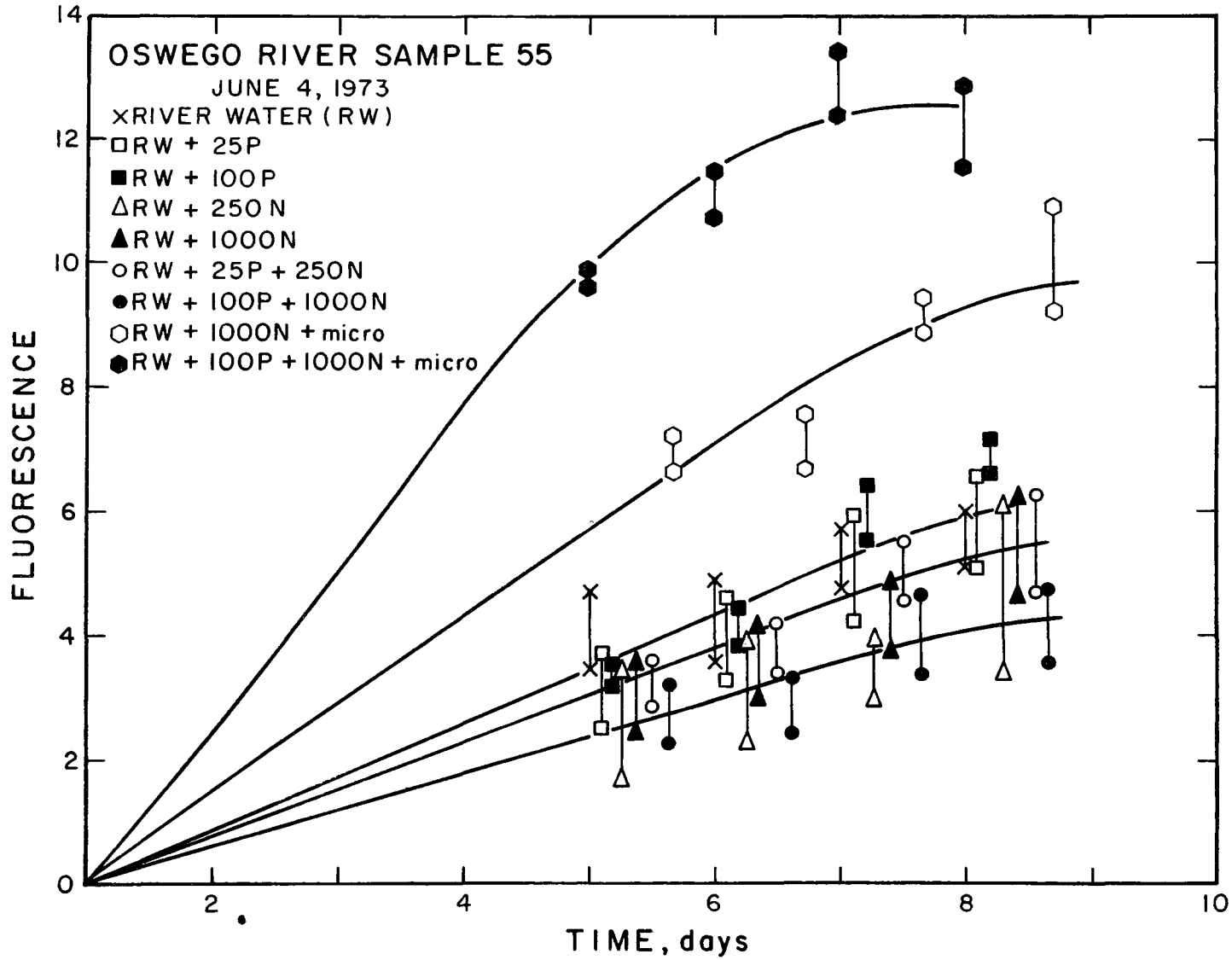

Figure 15. The growth of Selenastrum in Oswego River water.

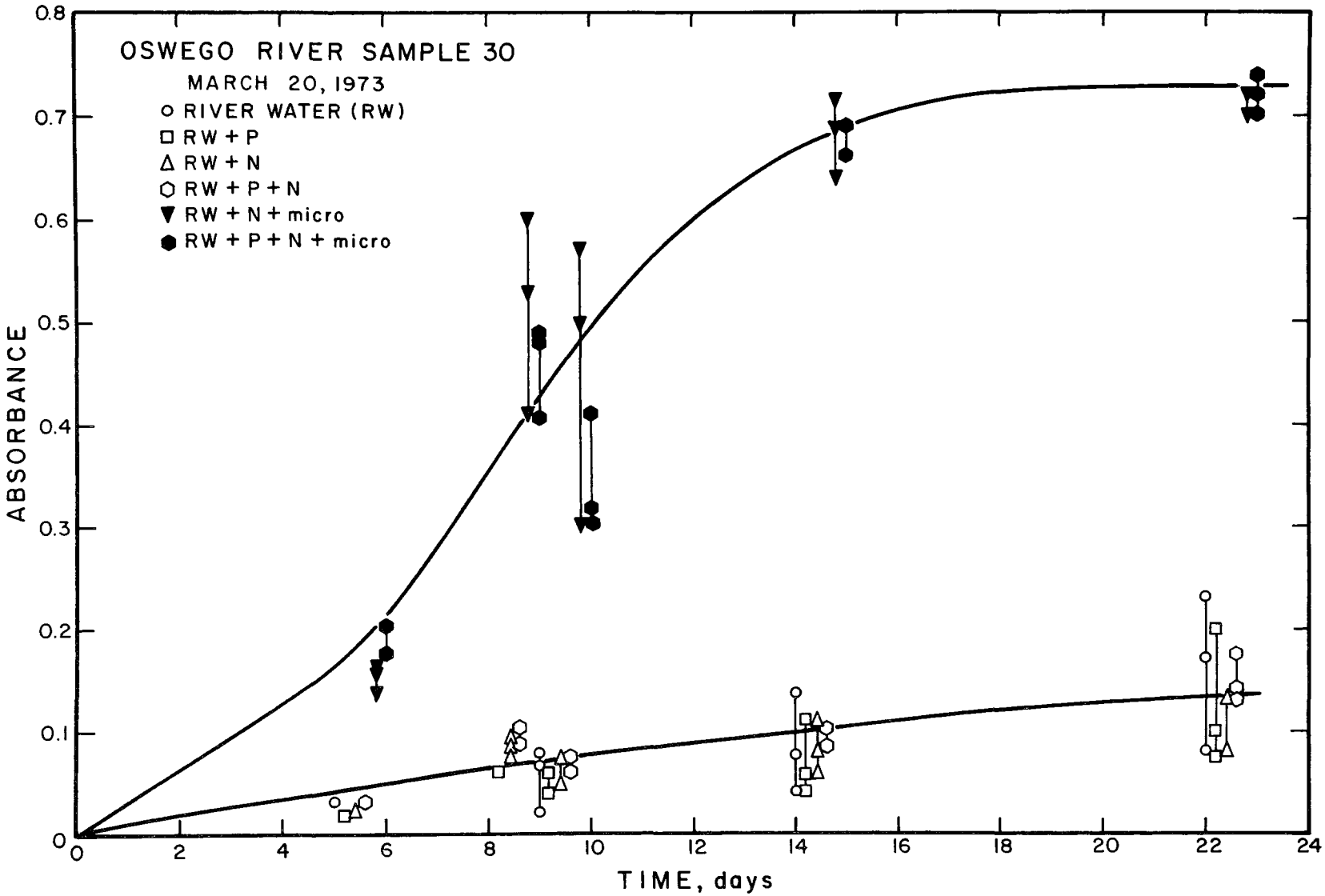

Figure 16. The growth of Selenastrum in Oswego River water.

when phosphorus plus nitrogen were added to the water samples. A typical growth curve is shown in Figure 17 for Black River water Sample 53. Neither phosphorus nor nitrogen added individually increased the growth of Selenastrum in Black River water samples. The river water clearly showed phosphorus and nitrogen limited conditions in all the six samples.

DISCUSSION

The nutrient-spiking studies of Lake Ontario and the Niagara River during the late summer required both nitrogen and phosphorus for stimulation of growth of the test algae and "natural" algae. Similar results were observed for the Black River. These results indicate both nitrogen and phosphorus are limiting planktonic algal growth in Lake Ontario and at these river mouths.

The samples of the Genesee and Oswego Rivers showed, in general, nitrogen stimulation. Also, many of the samples from these rivers demonstrated stimulation due to the addition of the micronutrients solution used in the NAA media for the AAP Procedure. Further, many of the samples studied in this investigation showed both N & P stimulation at a high level of N & P addition. Results of this type must be examined in light of the conditions of the test. In a bioassay of this type, it is possible to make any element limiting by adding large amounts of other essential elements and providing sufficient light for growth. Proper interpretation of the data for this type of bioassay requires that one consider the stimulation, or lack thereof, when small amounts of a potential-limiting element are added.

Care must also be exercised in interpreting the micronutrient stimulation data found for the Genesee and Oswego Rivers. The micronutrient solution also contains EDTA, a strong complexing agent. There is increasing evidence that

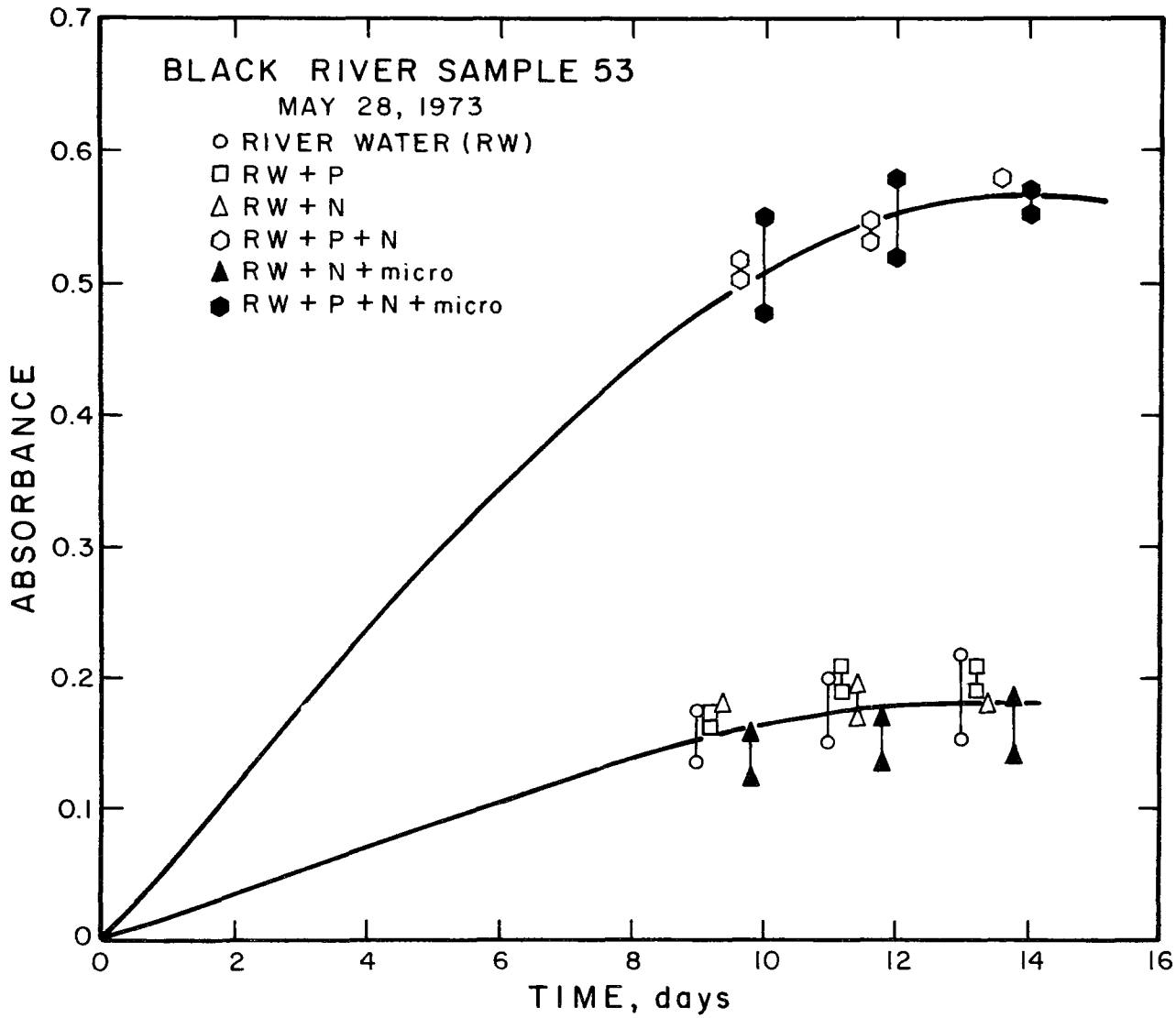

Figure 17. The growth of Selenastrum in Black River water.

algal growth in many waters near urban centers is inhibited by toxic elements in the water. Often, this toxicity can be eliminated by addition of a complexing agent. The stimulation noted in this study by the micronutrient solution may have been due to a removal of toxicity present in the water by the EDTA in the solution. Additional study of these waters in which the various components of the micronutrient solution are tested individually or in certain combinations must be conducted before it will be possible to ascertain what is the cause of the stimulation.

The results of this investigation provide valuable information on the approaches that should be used to reduce the excessive algal growth in Lake Ontario. Since essentially all lake water samples showed phosphorus limitation, efforts should be directed to limiting potentially available phosphorus inputs to the lake. The fact that the Lake Ontario open water samples taken during late summer showed both phosphorus and nitrogen limitation does not change the excessive fertilization control strategy since, in general, nitrogen control is considerably more difficult and expensive than phosphorus control.

Based on the results of this study, it is concluded that a substantial reduction of immediate potentially available phosphorus will tend to reduce planktonic algal growth in Lake Ontario. It is readily apparent, based on the estimated phosphorus sources, that immediate steps should be taken to provide advanced waste treatment removal of P from all major domestic wastewater sources entering Lake Ontario or its tributaries from the U.S. and Canada.

Phosphorus removal from domestic wastewaters by advanced waste treatment methods may not result in reduced algal growth in tributary rivers and nearshore regions or near urban centers of the lake such as Rochester and the Genesee and Oswego Rivers because phosphorus is not currently limiting

algal growth. It is possible that phosphorus removal at domestic wastewater treatment plants located in these areas could be sufficient to cause phosphorus to be limiting. It is also possible that advanced waste treatment could reduce the concentration of the apparent toxicant present in these rivers which would stimulate algal growth in the river and nearshore waters due to the excess phosphorus present. There is a need for a more detailed assessment of factors limiting algal growth in these waters and relative nutrient sources before reliable predictions can be made on the environmental impact of advanced waste treatment of domestic wastewaters in the Genesee and Oswego River basins. Even though the potential impact of phosphorus removal cannot be predicted for these rivers and nearshore waters of Lake Ontario, it is clear that such a practice will be of some benefit in reducing the rate of and possibly reversing the algal growth in Lake Ontario.

REFERENCES

National Eutrophication Research Program. 1971. Algal Assay Procedure Bottle Test. U.S. Environmental Protection Agency, Corvallis, Oregon. 82 p.

SECTION VII

APPENDICES*

*In order to reduce printing costs, appendices have not been included in this report. However, they may be purchased in paper or microfiche copy from the National Technical Information Service, U.S. Department of Commerce, Springfield VA 22151.

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16. ABSTRACT

This study was conducted on the potential significance of nitrogen, phosphorus and micronutrients in limiting planktonic algal growth in Lake Ontario and its major tributaries. Standard algal assay procedures were used. Samples of the open waters of Lake Ontario and Niagara River waters collected during the spring showed phosphorus limitation. By late summer these waters showed both nitrogen and phosphorus limitation. Genesee and Oswego Rivers showed, in general, nitrogen limitation. Samples of the Black River Waters showed both nitrogen and phosphorus limitation.

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