# FACTORS AFFECTING THE ALGAL ASSAY PROCEDURE

by

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Prepared for

OFFICE OF RESEARCH AND MONITORING
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

1975

## ABSTRACT

Evaluations of the Algal Assay Procedure (AAP) have demonstrated its value for determining the level of nutrients in water samples which is available for the growth of algae as contrasted to chemical analyses of total nutrient contents. The maximum specific growth rate, $\mu$ max, has been shown not to be affected by the supply of N, P, or carbon when tests are carried out using the suggested AAP. The maximum yield of algae is affected by the initial concentration of N and P (and Fe, Ca, Mg, K, and S in other nutrition studies). The maximum yield is not affected by the supply of carbon in normal AAP cultures (foam or cotton plugs), but increased incubation times might be required for the maximum yield to be attained if comparatively concentrated nutrient solutions are used. The inoculum levels of algae suggested in the AAP are sufficiently low to make use of fluorometry as a measurement of the growth of algae in relatively dilute culture media. If cell counts or absorbance measurements are to be used to follow the growth of cultures, higher initial cell densities may be employed. The $\mu$ max of algae cultures is influenced by the light intensity, but maximum yields merely require longer incubation periods if less than suggested light intensities are used. The applications of the AAP with the suggested algal species or in vivo algae have demonstrated its value in determining which algal nutrient will limit the growth of algae in water samples.

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## ACKNOWLEDGMENTS

The technical assistance of Mrs. S. L. Faust and Mrs. C. R. Nadler is gratefully acknowledged. This work was supported by a grant from the Water Quality Office of the Environmental Protection Agency.

# SECTION I

## INTRODUCTION

The general purpose of the Algal Assay Procedure (AAP) is to measure the biologically available fertility of a water sample, as contrasted with chemical analyses of the components of the sample. By the algal response to the addition of nutrients, alone or in combinations, one can also determine the nutrient or nutrients limiting algal growth and the potential changes in algal growth with changes in nutrient concentrations. In other words, this is a practical test to compare the fertilities of water samples and predict the algal responses to changes in the water. The value of the test lies in the fact that one can differentiate between available nutrients and total chemical composition of water samples. Many sources of nutrients, such as the nitrogen and phosphorus of aerobic lake muds, are relatively unavailable for the growth of algae although chemically present (3,4,5,6,7,9).

Of critical importance is the fact that the relative fertility of water samples is what is being measured with the AAP. Thus, the selection of the sampling site, depth, season, and other possible factors will have an influence on the value of the results. As much care and consideration must be given to the sample collecting as would be expected to be given to carrying out the AAP. By careful selection of sampling sites and times of sampling, very worthwhile information of ecological importance can be obtained from results with the AAP; the comparative fertility of surface lake waters during the different seasons will indicate which lakes become deficient in one or more algal nutrients in midsummer and the effects of storms, lake turnover, or other natural versus man-caused changes in the available nutrient content. Potential sources of algal nutrients can be evaluated and their quantitative effect on the fertility of the receiving waters predicted by proper sampling. Sources of nutrients that are relatively unimportant during winter and spring due to the relatively high nutrient levels in the receiving waters can become important sources of

limiting algal nutrients, such as phosphorus, during the summer period when algal nutrients in the receiving waters are at minimal levels. Thus, it must be emphasized that samples of water are being analyzed in the AAP, and the interpretation of the results will depend upon a logical approach to selection of the samples to be analyzed. If one wanted to obtain more direct information on the week-to-week level of available algal nutrients in a body of water, it would be proper to analyze the nutrients contained in in situ algae. The algae growing in the environment can be used as in situ continuous monitors of the available algal nutrients in the water (8,10,11). Thus, predictions of changes in levels of algal nutrients based upon results with the AAP and manipulations in the environment can be evaluated by nutritional changes in in situ algae with minimal work effort.

Water samples for algal assays sometimes must be preserved for more convenient analysis times. The treatment to be given water samples before evaluating their nutrient content will depend upon the test to be used. Usually nutrient sorption tests using nutrient-limited algae require so little time and space to carry out that tests can readily be carried out on samples as fast as they are received or even in the process of bringing them to the laboratory, if necessary (6). Since the usual handling procedures in harvesting sorption tests consists of removing the test algae with a forceps or coarse plankton net funnel, most planktonic in situ algae in the water sample would not be harvested and analyzed. However, the presence of in situ algae in the sample during the sorption incubation may have an effect on the results because of competition with the test algae (5,8). The majority of in situ algae can readily be removed from water samples by plankton nets or centrifugation, and samples can be stored for short periods in the dark or under refrigeration. Since growth tests require a relatively large amount of laboratory space for the number of flasks and long incubations involved, it is frequently necessary to preserve water samples for more appropriate times of analyses. In situ algae may grow in some samples and compete with the test algae of growth tests or cause confusing results, such as when nitrogen-fixing algae might grow in tests of the available N content of water samples. Therefore, it is convenient to remove most organisms by membrane filtration (0.45 μ) or kill all organisms by autoclaving. Both of these preservation techniques will affect the nutrient content of the water samples. Membrane filtration removes particles that are insoluble at the time of filtration. It has been shown (3,4,6) that algae can utilize some forms of P, N, and Fe that are relatively

insoluble (shark teeth, hair, and pyrite crystals). AAP tests of water samples from four lakes in the Madison, Wisconsin area (January, 1973) after membrane filtration of the samples, indicated all four of the waters were deficient in Fe (stimulation of growth when Fe-EDTA was added), whereas there was no response to added Fe when autoclaved samples were tested. Thus, these water samples must have originally contained adequate available Fe which was removed by filtration.

There are several effects that autoclaving can have on the nutrient status of water samples. The method used to preserve water samples for AAP tests by autoclaving has been to autoclave the samples, cool, gas with $CO_2$ for 1/4 to 1/2 hour to resolubilize some of the precipitated materials, and aerate for 8 to 12 hours to remove excess $CO_2$. As has been mentioned, available P, N, and Fe can be released from in situ algae in autoclaved samples. During July, 1972, 24 lake water samples were tested for soluble $PO_4$-P, total P, and available P (sorption tests with Cladophora). Only two of the 24 raw or filtered (Whatman #2) samples had more than 0.02 mg P/l as soluble $PO_4$-P or available P. Six autoclaved samples had significantly higher soluble $PO_4$-P than the raw samples, and in all six autoclaved samples the available P was 2 to 4 times higher than the concentration of soluble $PO_4$-P. Thus, both soluble $PO_4$-P and other forms of available P were released from the in situ algae by autoclaving.

In contrast to the increase of soluble and available P caused by autoclaving lake water samples containing in situ algae, autoclaving muddy river water samples caused a decrease of soluble $PO_4$-P in 11 of 12 samples. This loss in soluble $PO_4$-P in muddy samples is believed to be due to sorption of P by the mud (3,9).

The general conclusions that can be drawn about water sample treatments are:

Raw samples - The nutrient content of untreated stored samples may change because of the growth of algae. Also, in situ algae can compete with test organisms in either sorption or growth tests. For sorption tests the effects of in situ algae can be removed by reducing their numbers to below competitive status by crude filtration through plankton nets or coarse filter papers. Minimal changes appear to take place in samples stored refrigerated in the dark.

Preserved samples - Membrane filtration will remove competitive organisms to nutrition tests as well as insoluble nutrients which might or might not be available nutrients.

Such treatments allow the measurement of nutrients soluble at the time of treatment. The filtration of samples of more than a few hundred ml, however, requires very special equipment not normal to routine laboratories. Autoclaving causes a release of nutrients from in situ algae and an increase in the sorption of nutrients by muds. However, the total available nutrient content of water samples (the availability of nutrients in the water and contributed by the death of in situ algae) can be measured after autoclaving. Samples to be stored for long periods could be crudely filtered to remove the majority of in situ algae or muds and then autoclaved as a compromise of the effort required and the integrity of results.

This report will demonstrate some useful techniques for carrying out the AAP, evaluations of the measurements that have been suggested for use, and some of the factors which might affect the results of the AAP as well as suggestions for short-cut modifications and expansions using in situ algae as the test organisms for the AAP.

## SECTION II

## COMPARISON OF RESULTS AND INTERPRETATIONS USING DIFFERENT METHODS OF MEASUREMENTS OF ALGAL GROWTH

### MEASUREMENTS

Dry weight - The amount of dry weight (suspended solids) in a culture of algae is probably the most reproducible measurement of growth when comparing different nutritional levels or species of algae. However, dry weight measurements require the greatest waste of cultures and are not suitable for low density cultures. Consequently, other techniques are usually used and the results calculated to dry weights.

Absorbance - The absorbance of algal cultures is preferably measured at 750 mμ in order to correlate results with suspended solids and not be affected by chlorophyll changes in the cultures. Absorbance measurements are well correlated to dry weights, and they can be followed without waste in cultures grown in tubes or in flasks with side arms fitting a colorimeter. However, the sensitivity of this method using 1 cm cells is less than 1/10 that of fluorescence measurements when green algae are used.

Cell counts - The use of haemocytometer slides and a microscope has advantages over other measurements in that one sees what is being measured, and contaminating algae can be detected, as well as changes in cell size or shape under different environments. The cells of algae do vary in size in young versus old cultures, and there are variations in shapes in Selenastrum capricornutum (AAP); N-limited cells are truly "capricorn"-shaped (long, curled ends) whereas P-limited cells are very stubby. In order to have reasonable accuracy in cell counts one should count at least 100 cells (95% confidence equals ± 5 cells). When using a 430 magnification with the microscope and a haemocytometer 25 fields would be required to be scanned for 100 cells if the culture contained 1,000,000 cells/ml. If the culture contained only 100,000 cells/ml, 250 fields would have to be searched to count 100 cells. Thus, unless one has the

use of a properly calibrated electronic particle counter, considerable effort would be required to attain 95% confidence in cell counts of cultures containing less than 1,000,000 cells/ml.

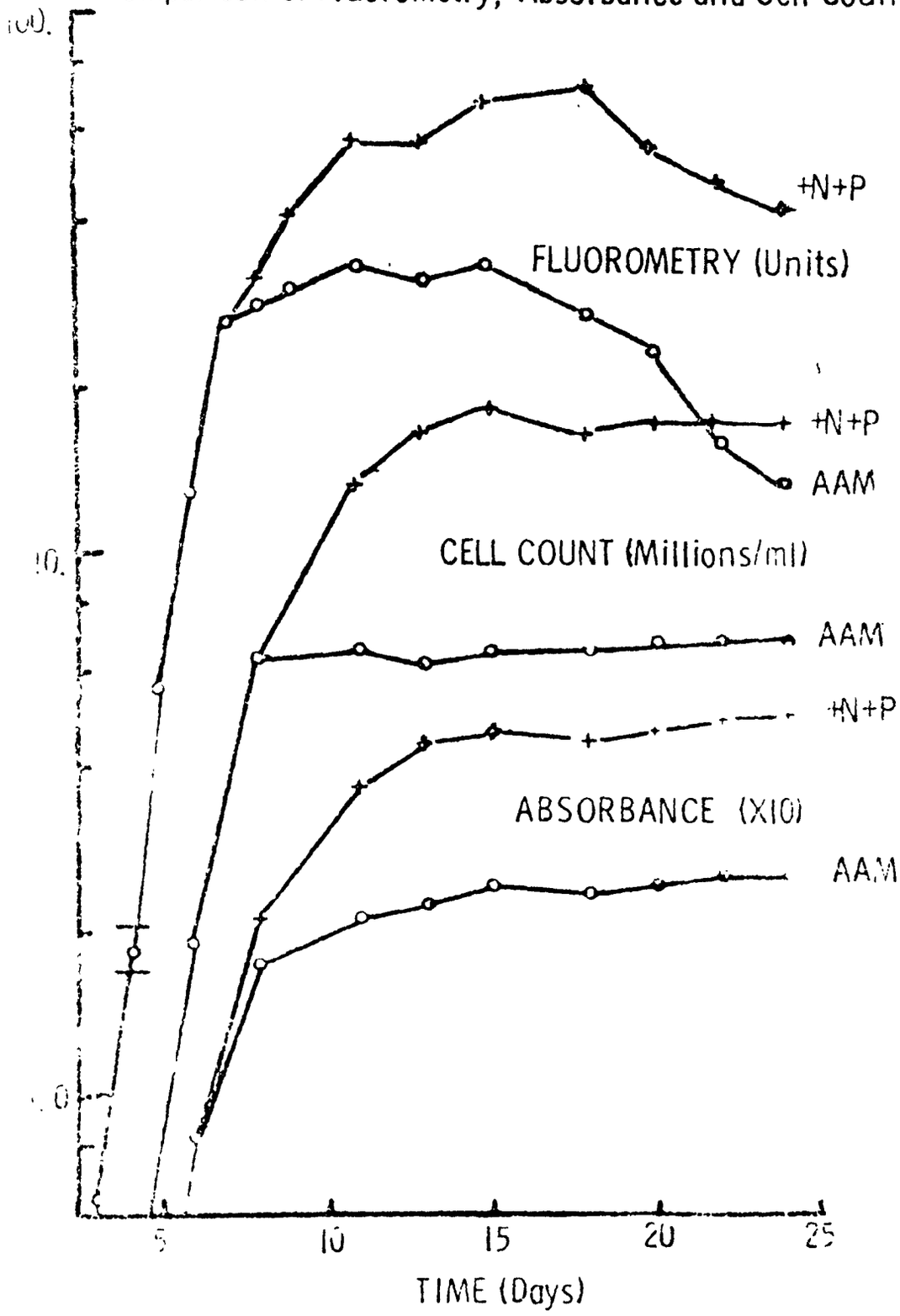
Fluorometry - The fluorescence of the chlorophyll a of algae is easily measured without extractions or much waste of culture volume. Direct measurements with live algae can use as little as 2 ml, and some fluorometers can be adapted to very simple flow-through systems with minimal waste. In our measurements we used an Aminco fluoromicrophotometer with the standard photomultiplier tube (4-6250) and filters of 360 nm and 415 nm. The filters used in a fluorometer must be selected carefully since different algal species will require different filters for best results. With this instrument one Aminco fluorescent unit was equivalent to 0.045 mg chlorophyll a/l. The in vivo fluorescence measurement of Selenastrum was the most sensitive and reproducible technique: as few as 500 cells/ml could be readily detected. However, the fluorescence of Selenastrum cells in different media does not necessarily correlate with the absorbance of the culture. Selenastrum growing in the Algal Assay Medium (AAM) with a culture absorbance of 0.10 would have about 20 fluorometry units, whereas Selenastrum growing in Gorham's Medium (12) with an absorbance of 0.10 would have 40 fluorometry units. In a later section it will be pointed out that the fluorometry of cultures decreases after they pass their peak growth and become senescent (less chlorophyll a per mg dry weight). Thus, fluorometry is well suited to follow the growth of cultures up to their maximal level and compare different nutritional and environmental factors on these stages of growth, but is suitable for only gross comparisons with cultures that have passed their peak.

Correlation of measurements - Using Selenastrum growing in AAM, a culture with 20 fluorometry units would have an absorbance (1 cm, 750 mμ) of 0.10, a dry weight of 40 mg/l, and have 2,000,000 cells/ml.

## WHEN AND WHAT TO MEASURE

The results of a typical experiment following the growth of Selenastrum in AAM and AAM supplemented with N and P (final concentration 2 x normal) are presented in Figure 1 as the fluorometry (Aminco units), cell counts, or absorbance (1 cm, 750 mμ) of the cultures at different incu-

Fig. 1. Rate of growth of Selenastrum:
Comparison of Fluorometry, Absorbance and Cell Counts



bation times. The 95% confidence limits of the fluorometry data are presented for Day 3.

It can be seen that the maximum rate of growth in AAM is the same as that in AAM supplemented with more N and P. It must be emphasized that the period of maximal rate of growth in Selenastrum cultures takes place at very low cell densities. In actual fact, once Selenastrum cultures take on a definite green color, they have passed their period of maximal rates of growth used to calculate μ max. At about 7 days the AAM cultures stop growing, but the supplemented AAM cultures continue to grow, at reduced rates, until about 15 days. All three measurements indicate the maximum yield of these cultures, but only fluorometry measurements could be used for calculating the maximum specific growth rate, μ max. However, the fluorescence of the chlorophyll a of these cultures decreased after the maximum yield had been attained and a definite yellowing of the cultures took place. Thus, the importance of selecting the proper time for yield measurements if one is using fluorometry cannot be understated. When cultures with different nutritional characteristics are to be compared it is sometimes necessary to harvest them at different times in order to detect the maximum yield by fluorometric measurements. Cell counts and absorbance measurements do not decrease with the age of cultures under these conditions, but it must be remembered that they are not very sensitive methods at low cell densities.

Spikes of AAM level of P, P + N, and N + Fe were added to AAM and the rate of growth and final yield of Selenastrum measured using fluorometry (Figure 2) in order to demonstrate which nutrient in AAM first becomes limiting to Selenastrum and what effect spikes of nutrients have on the rates of growth as compared to final yield of algae. (All cultures had the same growth through Day 4 (see 95% confidence limits), indicating that the maximum specific growth rate was not affected by the different levels of nutrients being tested.) The maximum yield in AAM was reached at Day 7, but cultures with added P or P + N continued to grow for 2 or 3 more days. Thus, P is the first nutrient to limit the growth of Selenastrum in AAM, but the cultures with added P soon run out of N also. The effect of added N + Fe was to stimulate the depletion of P in the cultures, and these cultures turned yellow before those in normal AAM. Therefore, without added P, the addition of extra N + Fe does not help the growth of Selenastrum in AAM and might be slightly inhibitory.

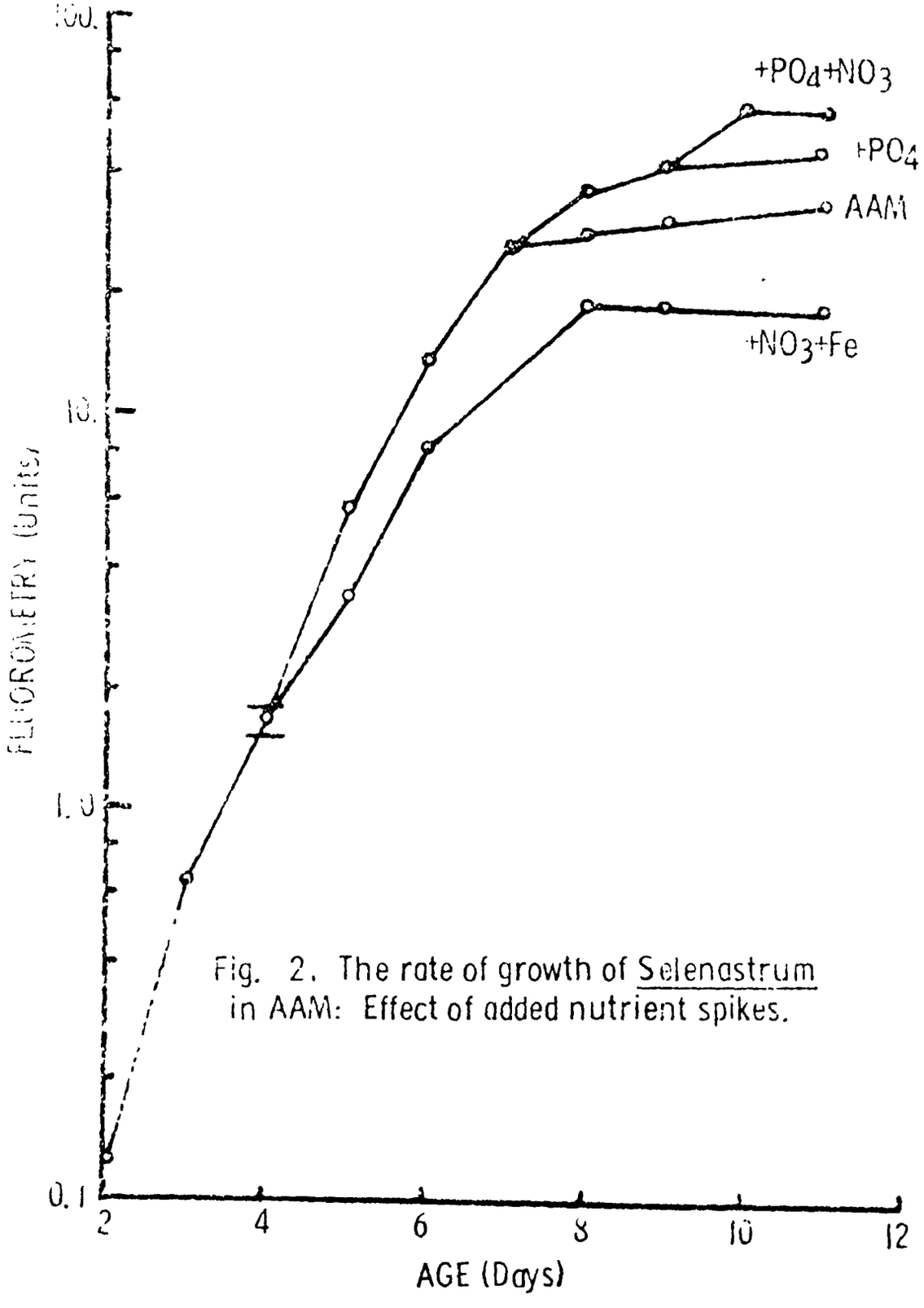



Fig. 2. The rate of growth of Selenastrum in AAM: Effect of added nutrient spikes.

* A series of studies have been made to demonstrate how different levels of nutrients in a culture medium will affect the yield of algae, but will not affect the maximum specific growth rate, μ max. This is in contradiction to the supposition (1,2) that μ max can be correlated with limiting nutrients when one is dealing with P or N. In the first test different concentrations of P in P-free AAM were incubated with 1,000 cells/ml of Selenastrum, and the growth of the alga was followed for 9 days (Figure 3). The second test was with different concentrations of N (Figure 4).

* The results of these tests indicate that whereas the maximum yield of Selenastrum depends upon the concentration of P or N in the media, the rate of growth of the cultures is the same up to the point when the cultures become deficient in P or N. This latter point takes place considerably past the time of the maximum growth rate. A summary of the final yields and maximum specific growth rates for the different concentrations of P and N is presented in Table 1. Thus, it can be seen that the same rate of growth was attained in 0.025 mg P/l as in 0.3 mg P/l, and a lower μ max was attained in 8 mg N/l than in the medium with 0.5 mg N/l. The relationship between the fluorometry, absorbance, and cell counts of these cultures is also presented.

Further evidence that the maximum rate of growth in bottle tests of the AAP is not related to the composition of the culture medium is presented in Figure 5, which follows the growth of Selenastrum in AAM and Gorham's medium, a considerably more concentrated algal culture medium (737 versus 66 mg/l dissolved solids). The growth of Selenastrum in the two media is identical until Day 5 when growth in AAM slows, while growth continues, but at a slower rate, in Gorham's medium. The average μ max for all cultures in AAM was 1.80 ± .18, and for cultures in Gorham's medium it was 1.66 ± .14. Thus, media with such different compositions support the growth of Selenastrum at comparable rates of growth up to the stage when the maximum rate no longer is supported. There is further growth in more concentrated media, but the rate of growth is not at maximal levels.

As a test of whether natural waters would produce similar results, samples of water from Lakes Wingra and Kegonsa (Madison, Wis), collected 4/11/73, were used in AAP tests

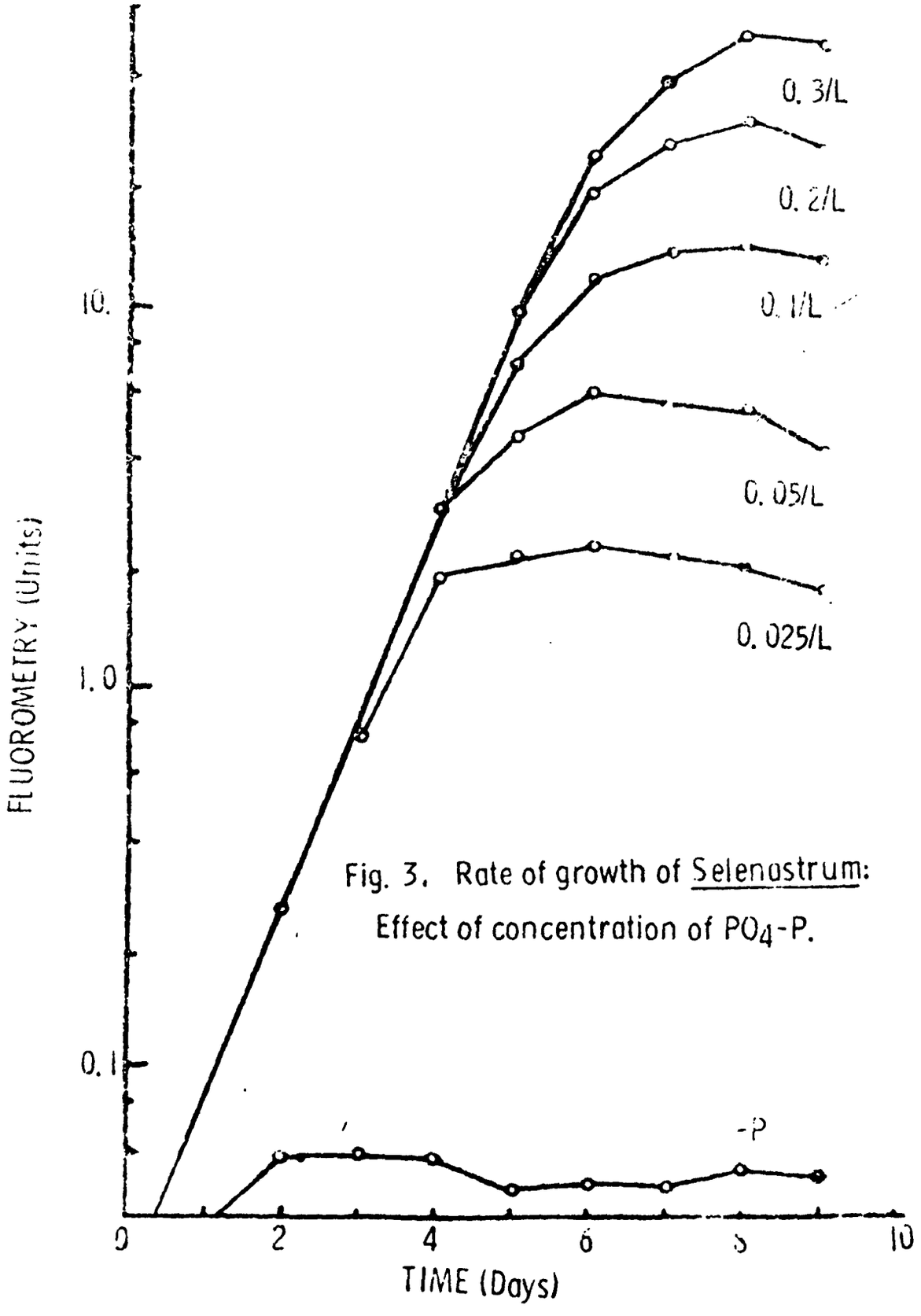



Fig. 3. Rate of growth of Selenastrum: Effect of concentration of $PO_4$-P.

Fig. 4, Rate of growth of Selenastrum:

Effect of concentration of $NO_3$-N.

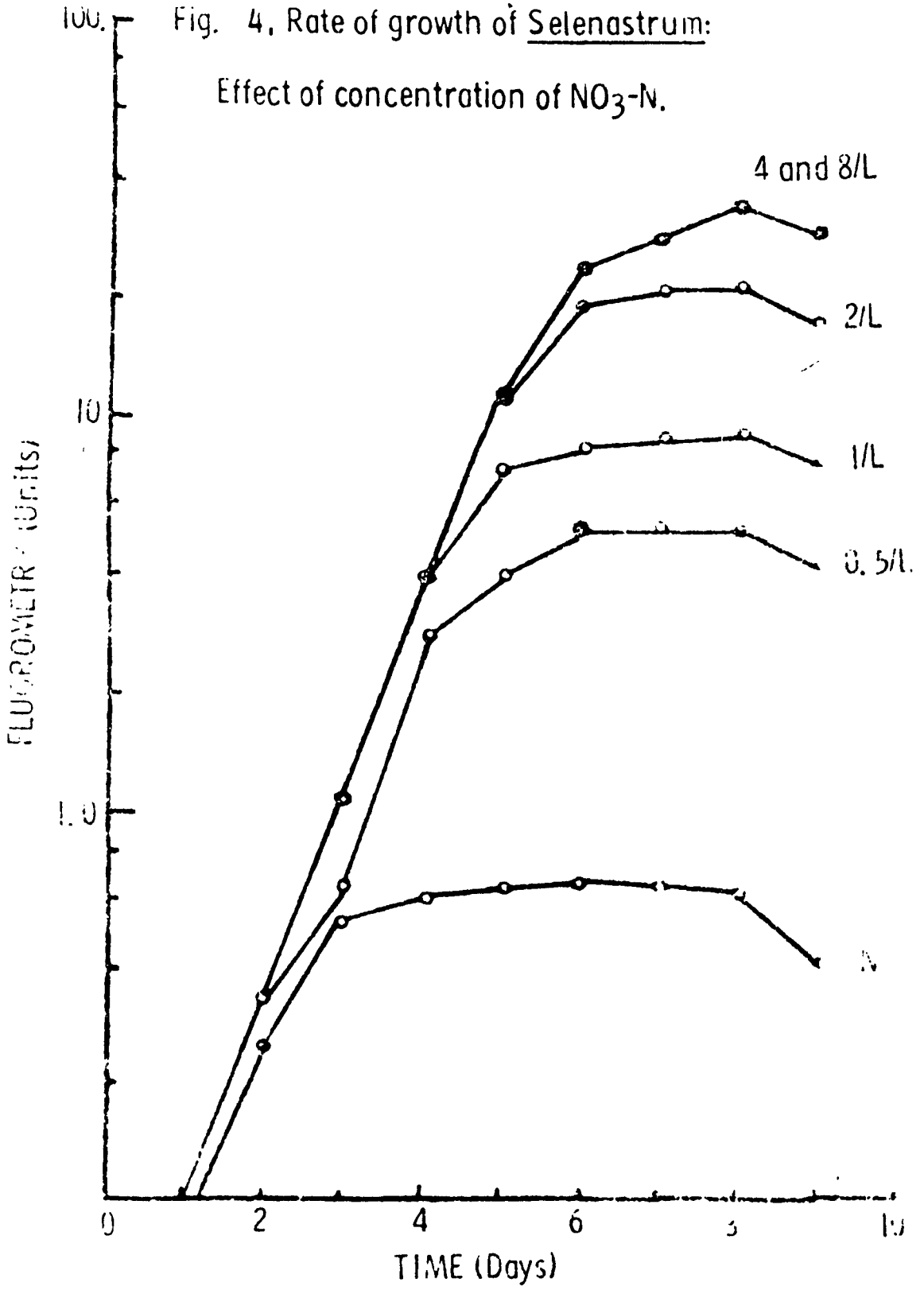
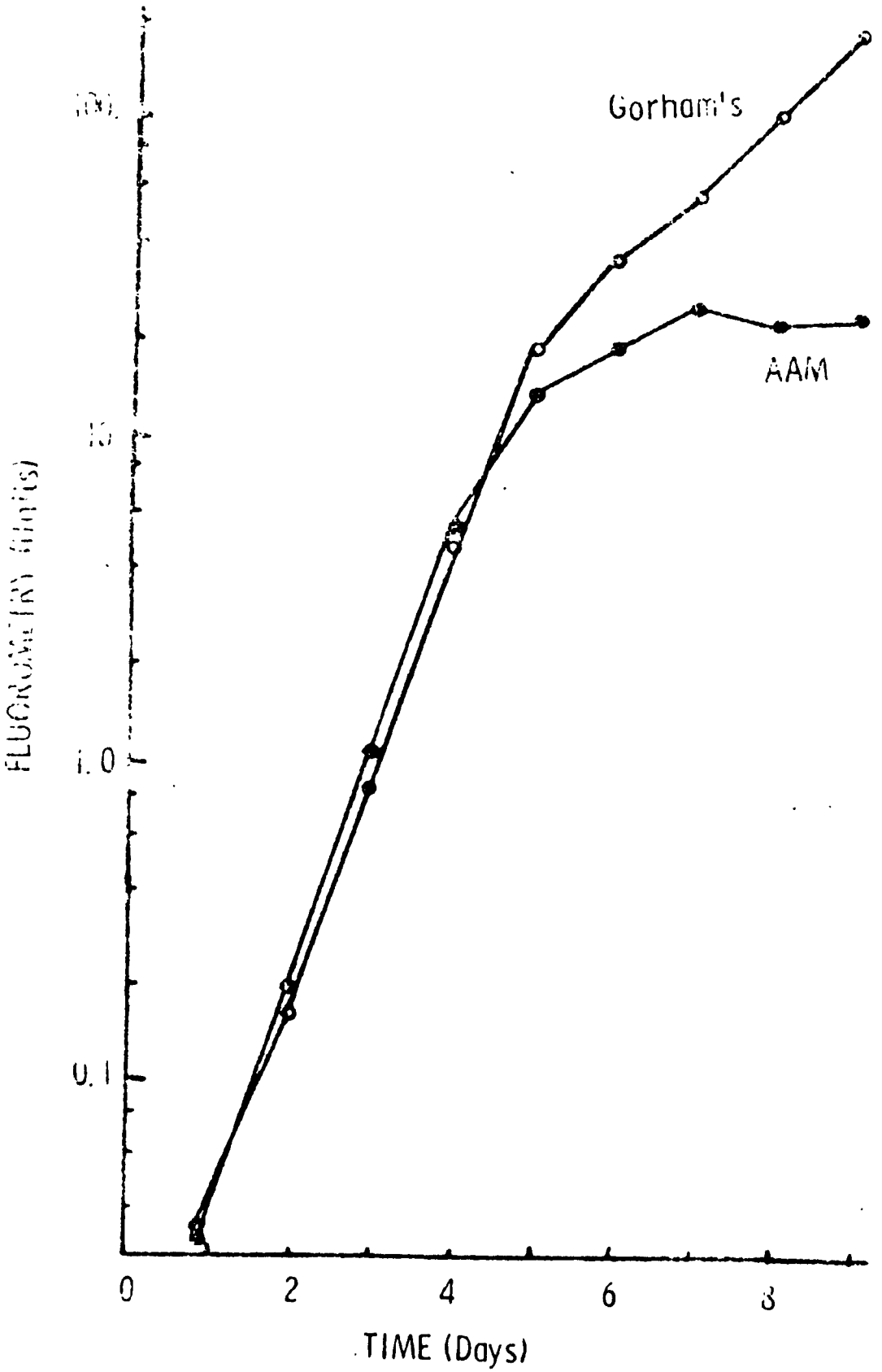


Table 1. COMPARISONS OF YIELDS AND μ MAX OF SELENASTRUM GROWN IN ALGAL ASSAY MEDIUM WITH DIFFERENT CONCENTRATIONS OF PHOSPHORUS AND NITROGEN. FINAL YIELDS AFTER 9 DAYS OF INCUBATION.

| Nutrient concentration (mg/l) | Maximum yield | | | μ max |
|---|---|---|---|---|
| | Fluorometry (Aminco units) | Absorbance (1 cm, 750 mμ) | Cell count (cells/ml) | |
| -P | 0.05 | 0.0 | - | - |
| 0.025 | 2.2 | .03 | 230,000 | 1.22 ± .18 |
| 0.05 | 6.0 | .07 | 1,100,000 | 1.23 ± .08 |
| 0.1 | 14. | .15 | 2,100,000 | 1.29 ± .02 |
| 0.2 | 30. | .17 | 2,800,000 | 1.32 ± .10 |
| 0.5 | 54. | .21 | 6,700,000 | 1.24 ± .023 |
| -N | 0.65 | 0.01 | - | - |
| 0.5 | 5.0 | .06 | 1,600,000 | 1.43 ± .08 |
| 1.0 | 9.0 | .10 | 2,800,000 | 1.37 ± .03 |
| 2.0 | 22. | .16 | 4,000,000 | 1.35 ± .11 |
| 4.0 | 36. | .16 | 4,700,000 | 1.32 ± .053 |
| 8.0 | 36. | .16 | 5,300,000 | 1.29 ± .087 |

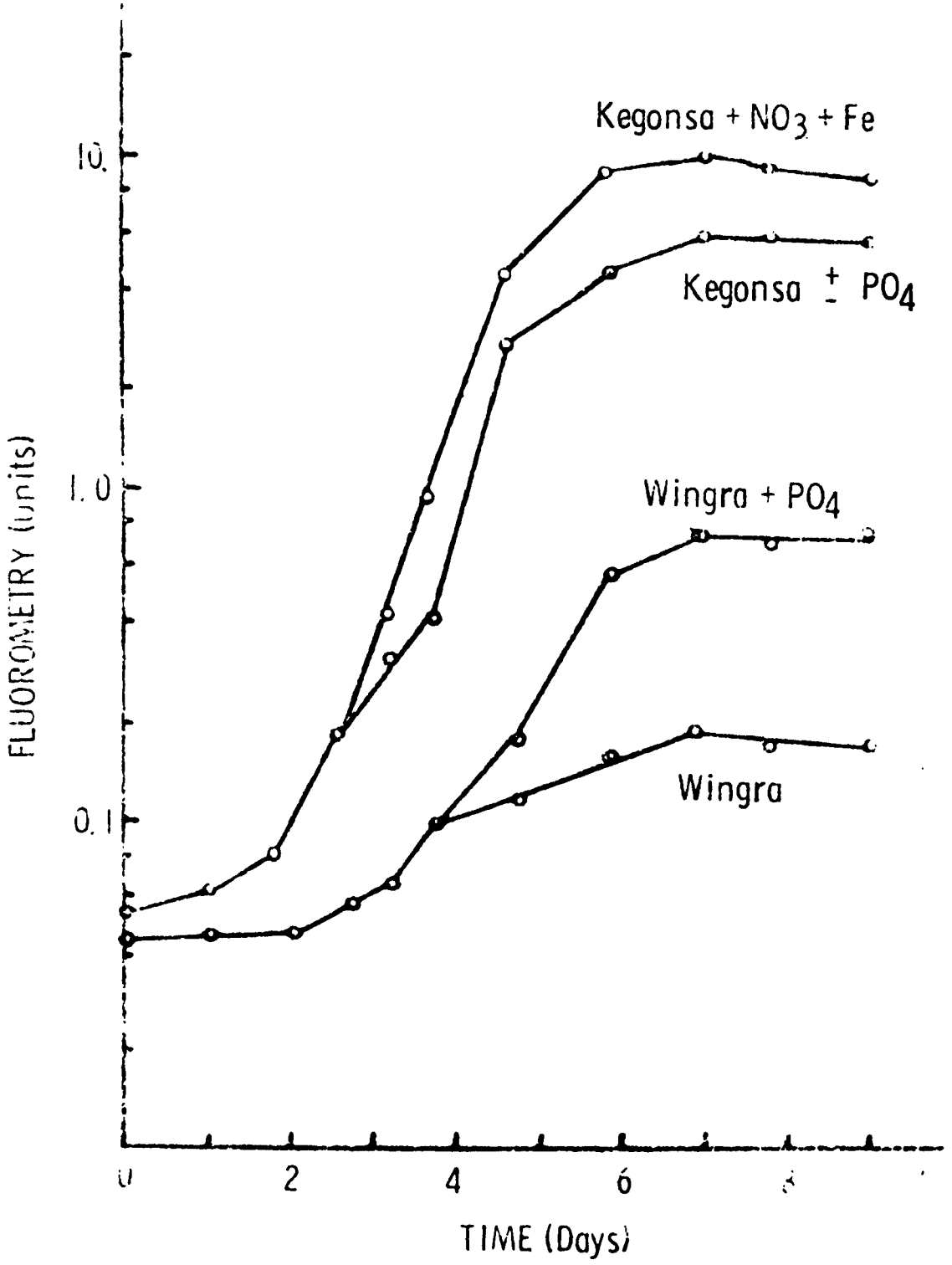
Fig. 5. Rate of growth of Selenastrum: Gorham's vs. AAM.



with and without various spikes. The data summarized in Figure 6 indicate that so little growth took place in Lake Wingra water that no definite μ max could be calculated (0.48 ± .44), the growth going from 0.04 to 0.2 Aminco units. When the Lake Wingra water was supplemented with P (AAM level) there was increased growth (μ max 1.03 ± .62) to 0.7 units, thus indicating that Lake Wingra at this time was relatively low in P. The rate of growth of Selenastrum in Lake Kegonsa was similar to that in AAM. When P was added to Lake Kegonsa waters there was no stimulation of growth of the algae, but increased yields did result with the addition of N + Fe. The rate of growth (μ max 1.48 ± .75), however, was not increased with this latter spike even though it contained the nutrient which limited growth in Lake Kegonsa water. Other tests indicated that spikes of only N would result in the same increase in yield of algae in Lake Kegonsa waters, collected at this time of year, as spikes of N + Fe or N + P + Fe.

The general conclusions from the results presented thus far are that the yield of algal cultures in the AAP-bottle test is dependent upon the nutrient content of the media being tested, whereas the maximum specific growth rate is independent of the media. The maximal yield of algae can be readily measured by fluorometry, cell counts, or absorbance, and the final yield can be either calculated as dry weight (suspended solids) from these data or measured directly in those cultures containing at least 5 mg of algae.

Fig. 6. Rate of growth of Selenastrum: Effect of nutrients added to lake waters.



## SECTION III

## EFFECTS OF SOME PHYSICAL FACTORS

### SIZE OF INOCULUM

The results in the previous section when Selenastrum was grown in Lake Wingra water (Figure 6) point out the necessity of using relatively low inoculum densities when dealing with oligotrophic waters. In that test, an initial concentration of 1,000 cells/ml was used and there was about a 5-fold increase in growth of the algae. It can be seen that if 10,000 cells/ml had been the initial cell concentration there would probably not have been enough nutrients in the lake water sample to bring about even a doubling of the cell density. Thus, there is need for very sensitive measuring methods even though they may not be perfect for all uses. If one was interested in the nutrition of oxidation ponds fed with different sewages, the use of absorbance measurements and relatively high initial cell densities would be appropriate.

The sensitivity of the available means for algal measurement will determine how low the nutrient levels can be which will be detected and differentiated in lake waters. By using in vivo chlorophyll a fluorescence measurements one can detect as low as 500 Selenastrum cells/ml. Thus measurements of growth of Selenastrum can be followed from this level upwards. Without modification of the method to make it more sensitive the use of less dense initial cell concentrations would be of little value. However, except for measurements of the growth of algae in extremely oligotrophic waters there is no real necessity to start with such low cell densities. The fact that the initial density of cells does not affect either the $\mu$ max of algae cultures nor the final yield is demonstrated in Figures 7 and 8. Cell densities of from 500/ml to 8,000/ml were tested in AAM in the first test, and the results indicate that all the cultures reached the relatively same concentration in the AAM after 8 days of incubation. The $\mu$ max for the different inocula ranged from 1.44 $\pm$ .25 with

Fig. 7. Rate of growth of Selenastrum: Effect of size of inoculum.

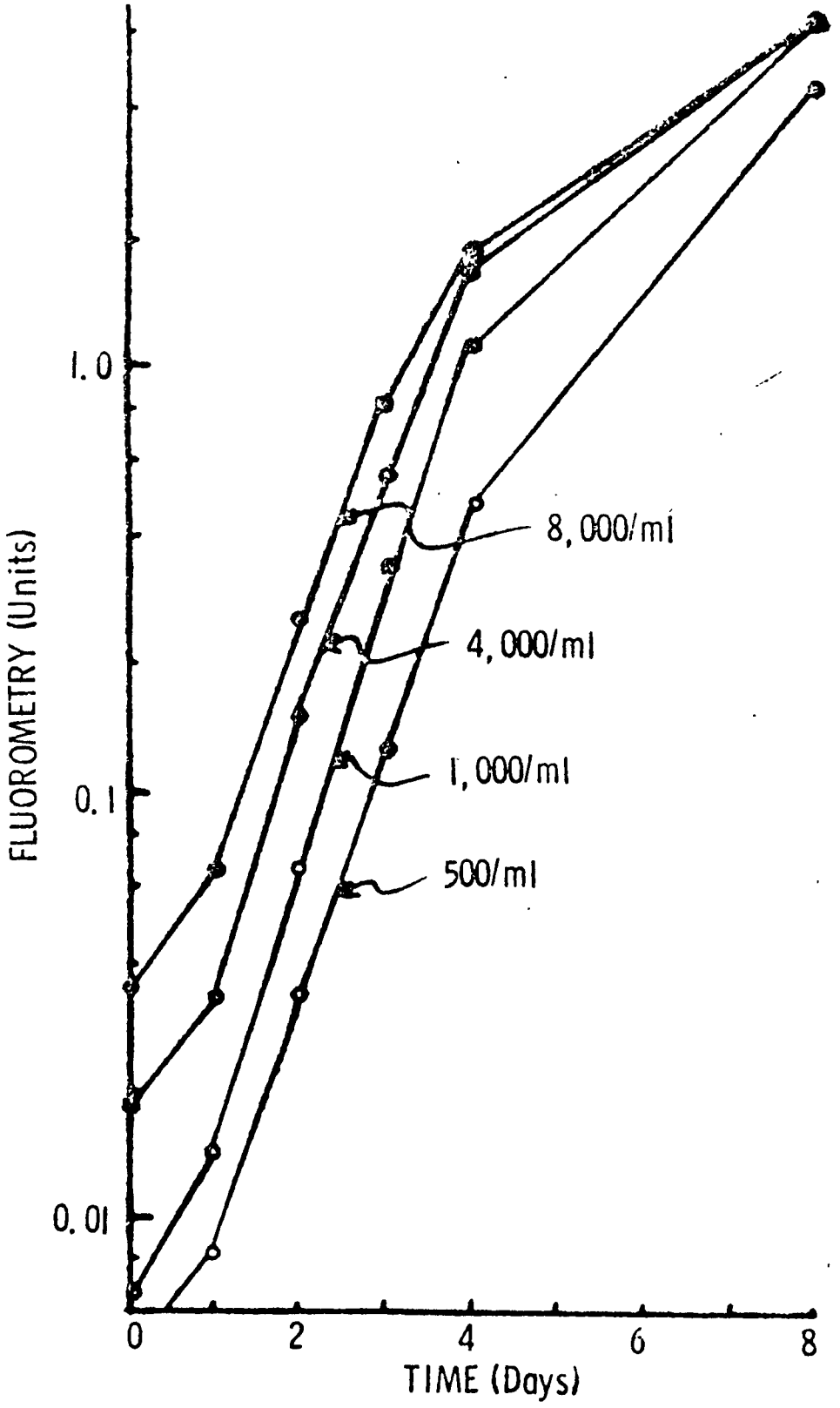
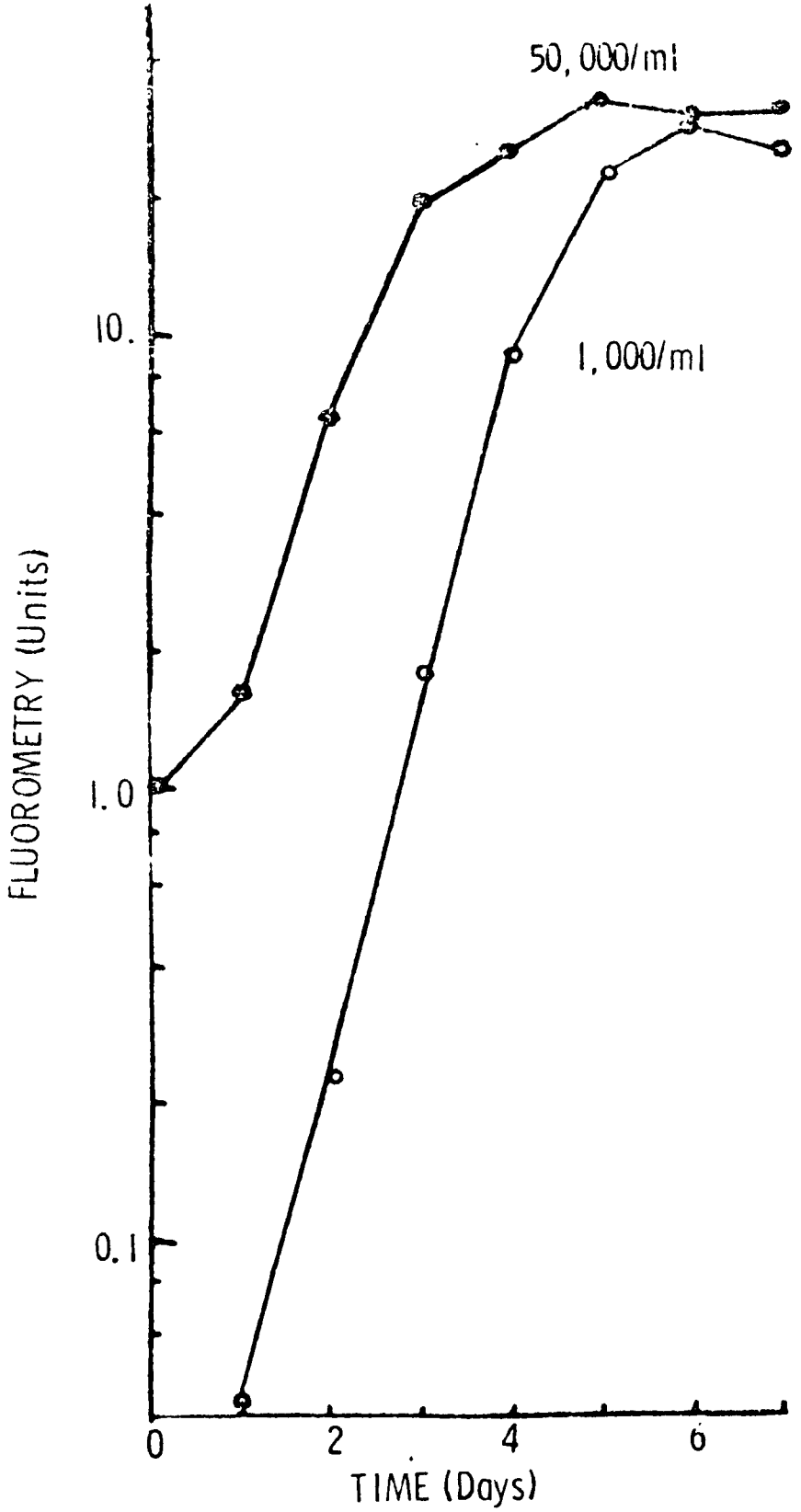


Fig. 8. Rate of growth of Selenastrum: Effect of size of inoculum.

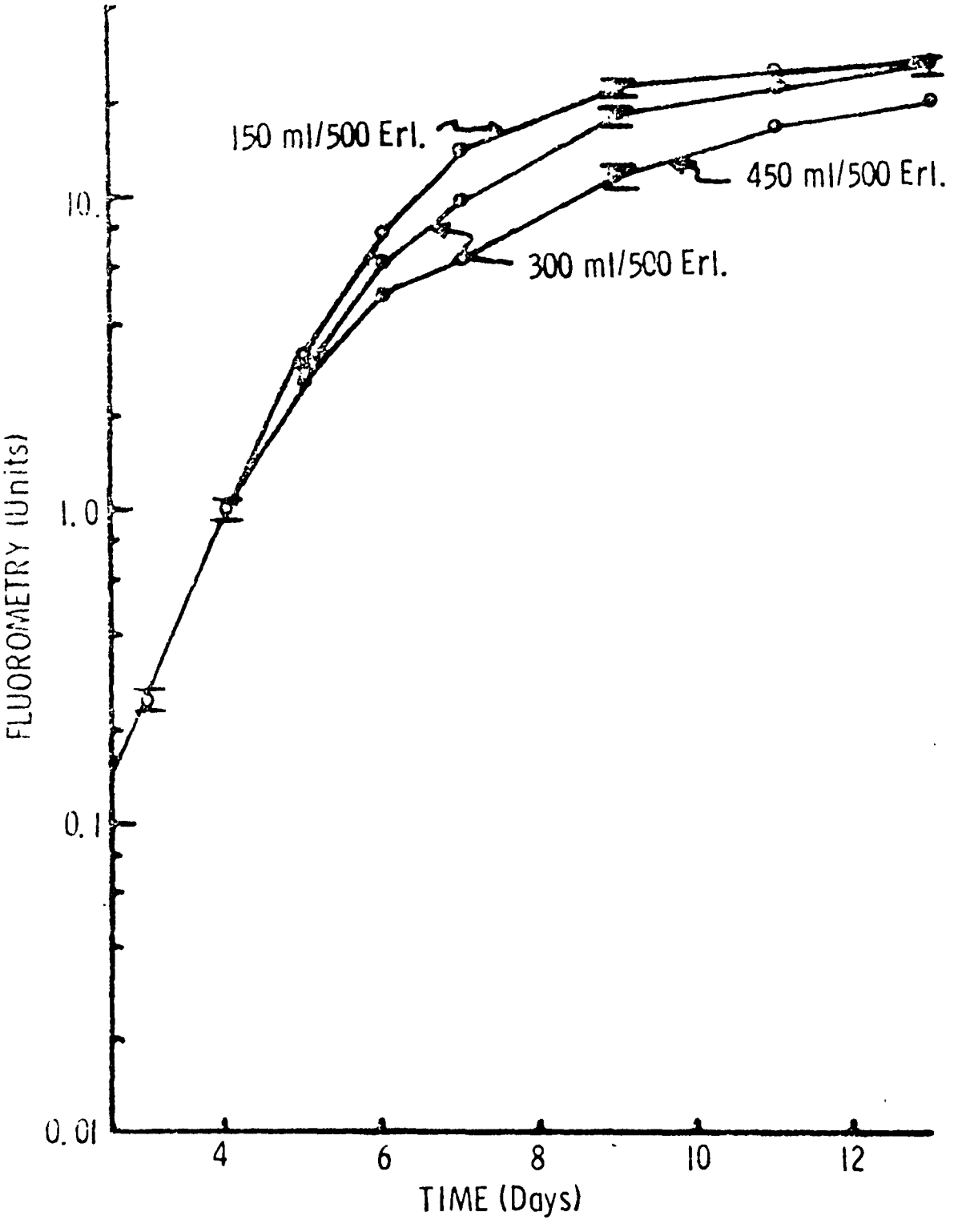


500 cells/ml to 1.71 ± .20 for 1,000 cells/ml; the μ max with 4,000 cells/ml was intermediate at 1.56 ± .04. When 1,000 and 50,000 cells/ml were compared in AAM, the same yield was attained at 6 days and the μ max were 1.92 ± .091 and 1.41 ± .11, respectively. Thus, the initial cell density affects only the sensitivity of differentiation that can be made between algal cultures, and the sensitivity of the available means of measuring the algae will determine how low an initial cell density is practical. Fluorometry allows us to start with 1,000 Selenastrum cells/ml, but at least 10 times this concentration would be appropriate if absorbance measurements were to be used.

## FLASK SIZE AND SHAKING

Under the assumption that available carbon ($CO_2$ or $HCO_3^-$-$CO_3^=$) would limit the growth of algae in the AAP, various relatively low volumes of liquid per flask are suggested for use. This is to allow the atmosphere to replenish the carbon used by the algae. At relatively low nutrient levels, and consequently low concentrations of algae, the rate of supply of available carbon from the atmosphere is sufficient to keep up with the rate of growth of the algae. Also, lower volumes require relatively lower amounts of carbon. If the algal mass increases sufficiently to become carbon-limited there will be an increase in the pH of the algal culture. This usually takes place after the cultures have taken on a definite green color and consequently occurs after the period of maximal rate of growth. Thus, the rate of maximal growth of algae will not be affected if the suggested culture volumes per flask size are used. The fact that later growth periods may be carbon-limited has no effect on the final yield of the cultures, merely more time is required to reach the maximum yield. This is pointed out by data on the growth of Selenastrum cultures in AAM in which volumes of 150, 300, and 450 ml per 500 ml Erlenmeyer flask were compared (Figure 9). The 95% confidence limits on all cultures at Days 3 and 4 indicate that all the cultures were growing at the same rate up to Day 5. After six days of incubation the effect of the different volumes on the growth of Selenastrum became apparent. The 95% confidence limits at Day 9 indicate there were significant differences in the yields of the cultures at that time. However, at Day 13 there was no difference between the growths in 150 and 300 ml, so the final yield of cultures can be assumed to be equal regardless of the culture volume if one waits long enough, in contrast to results with different initial concentrations

Fig. 9. Rate of growth of Selenastrum: Effect of culture volume.



of N, P, or Fe, since the atmosphere is a constant supply of $CO_2$ to loosely plugged cultures.

In order to demonstrate that the atmosphere is the source of $CO_2$ for these cultures, a series of flasks were compared which had been plugged with the usually-used plastic foam plugs (or plugging cotton in some tests) or which were plugged with solid rubber stoppers (Figure 10). The rate of growth with either type of flask closure was the same up to Day 4. Apparently, after this time the cultures plugged with the rubber stoppers ran out of available carbon since growth ceased at Day 5 while the growth in foam-plugged cultures continued to Day 7 and resulted in a final yield of nearly 10 times that in the rubber-stoppered flasks. As further evidence that the exclusion of atmospheric $CO_2$ caused the cessation of growth in the rubber-stoppered cultures, the rubber stoppers of two cultures were replaced with foam plugs. Within one day these cultures had started growing again, and by Day 11 their growth had nearly equaled that in the original foam-plugged cultures.

When cultures become carbon-limited, the pH of the cultures increases. This was shown in the previous cultures using different volumes; the pH of the 150, 300 and 450 ml cultures at Day 13 were 8.3, 8.9, and 9.4, respectively. However, if similar cultures were aerated (200 ml per minute) similar yields were obtained with all volumes at Day 9 (20-26 fluorometry units), and the pH of the cultures were only 8.1-8.2. Therefore, aeration of cultures containing larger liquid volumes than the recommended levels would serve to prevent the pH of the cultures from rising significantly as long as relatively dilute culture media were used. In more concentrated media, such as Gorham's medium or sewage effluents, the mass of algae grown is so great that aeration cannot supply the carbon-demand of the cultures and the air must be supplemented with $CO_2$. A concentration of 0.5% $CO_2$ in air is sufficient to maintain the pH of cultures of algae in Gorham's medium at pH 7.0-7.5 up to culture densities of at least 1,000 mg/l.

Shaking of cultures has been suggested as an alternative to merely leaving the cultures quiescent in a culture room. In order to test if shaking improved the rate of growth of Selenastrum in AAM, tests were carried out with different volumes and with or without shaking (100 oscillations per minute). Quiescent cultures with 25 ml/50 ml Erlenmeyer flasks had $\mu$ max of 1.53; 50 ml/125 ml Erlenmeyer flasks had $\mu$ max of 1.76 ± .099; and 150 ml/500 ml Erlenmeyer flasks had $\mu$ max of 1.82 ± .17. Cultures of

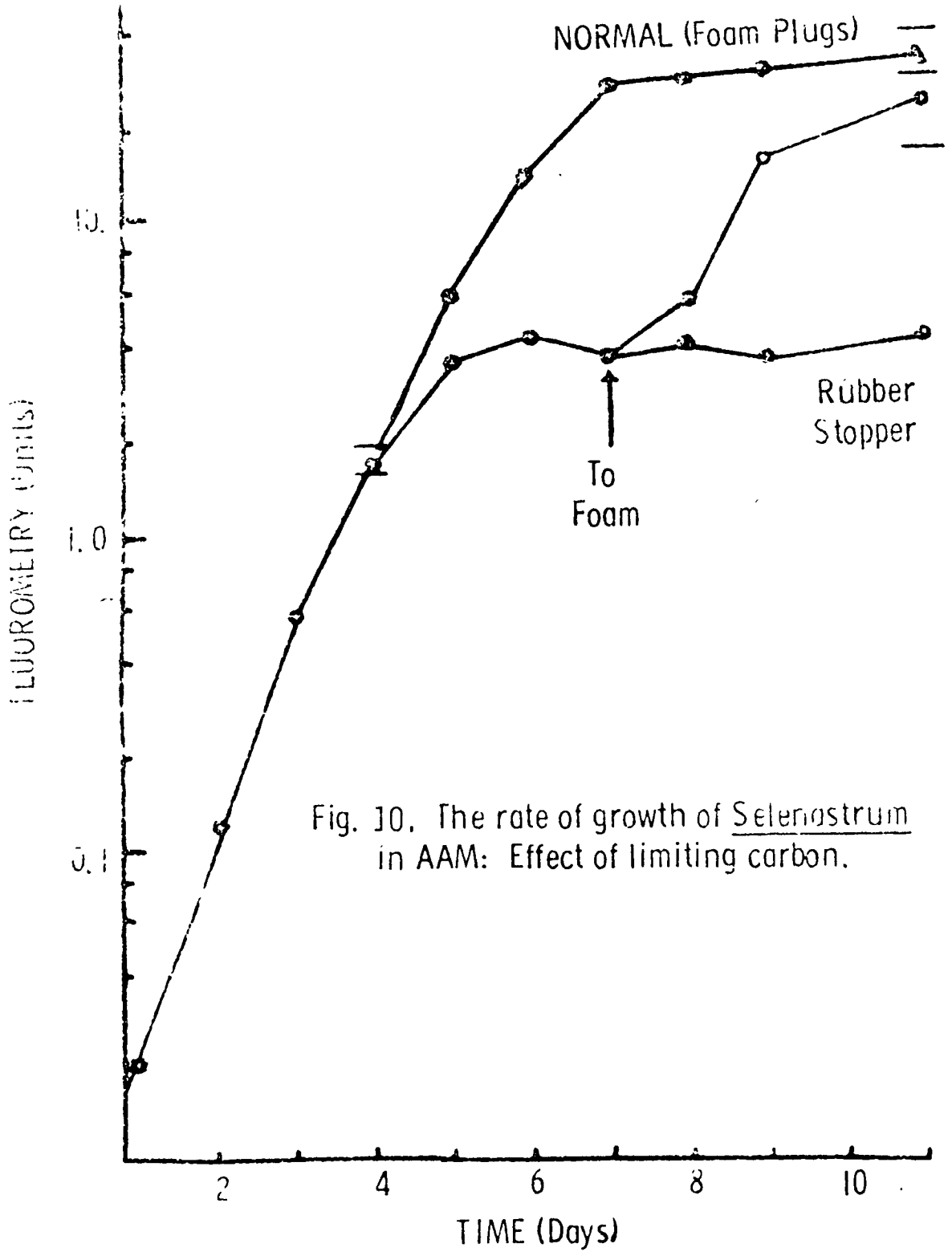


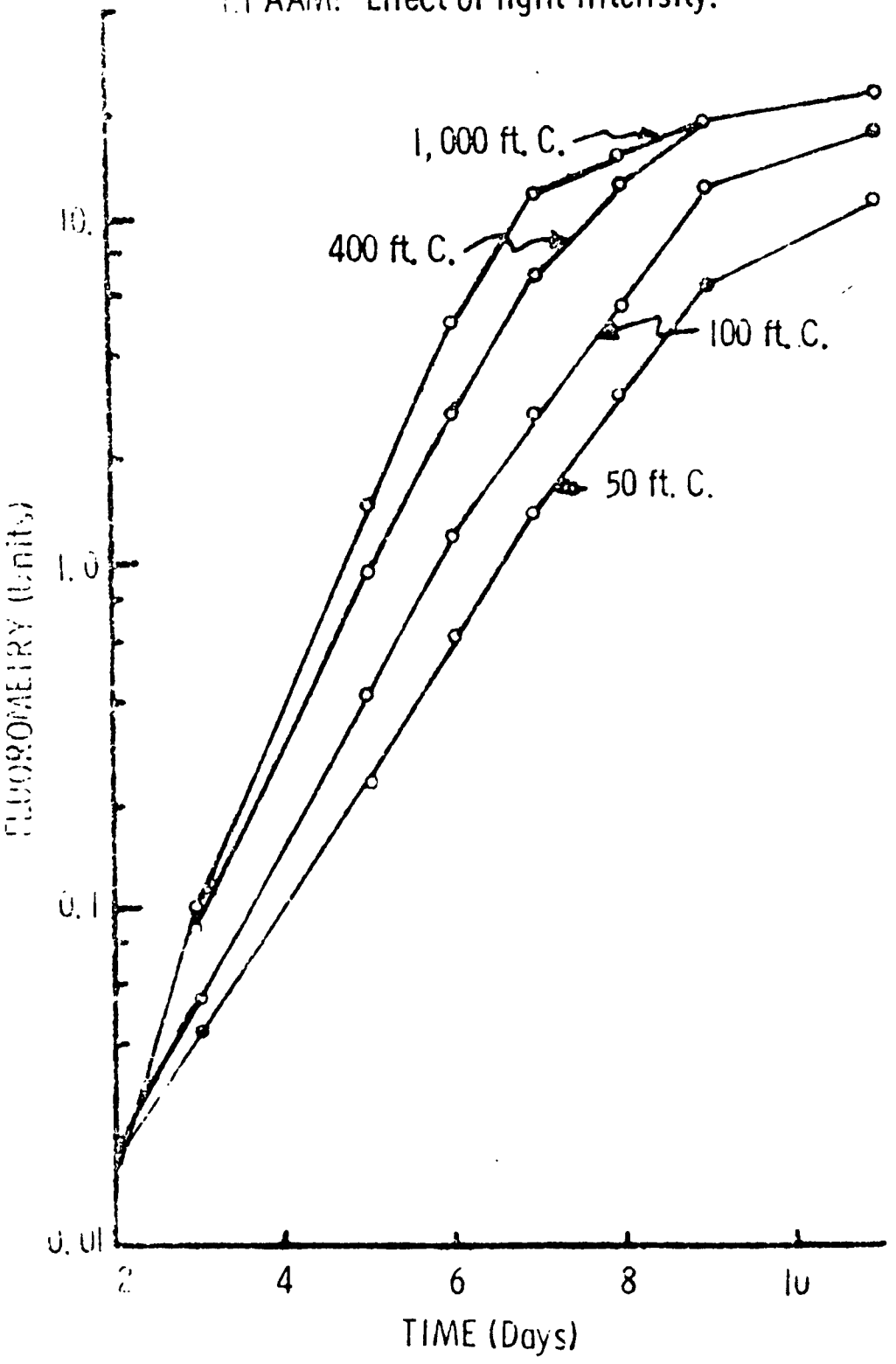

Fig. 10. The rate of growth of Selenastrum in AAM: Effect of limiting carbon.

150 ml/500 ml Erlenmeyer flasks which were shaken had μ max of 1.83 ± .091. Thus, shaking of cultures results in no improved maximum rates of growth as long as relatively low volumes of liquid per flask are used.

## EFFECT OF LIGHT INTENSITY

Thus far, different concentrations of sources of nutrients have been shown to not affect the maximum rates of growth of the green alga Selenastrum. One factor not evaluated thus far has been the source of energy to the cultures, light. When cultures of Selenastrum in AAM were incubated at different light intensities we found that within 3 days there was an effect of light intensity on the rates of growth (Figure 11). By Day 7, cultures in 50 ft C of light had yields of approximately 15% of those in the 400 ft C suggested for AAP tests. Cultures at 100 ft C were intermediate with about 25% growth. Higher yields were attained in cultures with 1,000 ft C up until Day 9 when cultures from 200, 400 and 1,000 ft C had similar cell densities. By Day 11 the algal growth of cultures in 100 ft C had also reached those with the higher light intensities and cultures in 50 ft C were within 50%. Thus, light intensity will affect the rate of growth of algae in nutrition studies, but with increased incubation the algal growth in cultures with less than the recommended 400 ft C will catch up.

Fig. 11. The rate of growth of Selenastrum in AAM: Effect of light intensity.



## SECTION IV

## THE APPLICATION OF AAP

### COMPARISONS USING THE THREE AAP ALGAE

As a demonstration of the utility of the AAP for measuring the available nutrients in different lake waters, results of tests using Selenastrum (1,000 cells/ml), Microcystis aeruginosa (50,000 cells/ml), and Anabaena flos aquae (50,000 cells/ml) are summarized in Table 2 as the calculated concentrations of available P from growth tests as compared to the soluble $PO_4$-P of the lake waters. There is some variability in the results using the different algae, but it is evident that reasonably good results were obtained with any of the three algae. More detailed studies of this nature have been published elsewhere (7,8).

### USE OF IN SITU ALGAE IN THE AAP

The suggested algae for use in the AAP were selected to represent the green algae, non-nitrogen-fixing blue-green algae, and nitrogen-fixing blue-green algae. These representative algae can be maintained in laboratories as stock cultures and are thus available for use at any time. However, other algae can also be used in AAP tests, and they do not necessarily have to be laboratory cultures. Mixtures of algae of ecological importance can be readily used to compare results obtained with the AAP algae. For instance, a series of tests of the growth of algae from Lake Kegonsa (May, 1973) (mostly Centrales Diatoms) have been carried out. The first test compared the rates of growth of this mixture of diatoms versus Selenastrum in Gorham's medium. Ten ml of Lake Kegonsa water were added to 150 ml of medium whereas an initial Selenastrum concentration of 1,000 cells/ml was used. Either type of algae grew very well in this medium. At Day 4 the Lake Kegonsa algae consisted mostly of diatoms (Centrales and Pennales) with some green algae. The $\mu$ max of this mixture of algae

Table 2. COMPARISONS OF SOLUBLE $PO_4$-P AND CALCULATED AVAILABLE PHOSPHORUS FROM AAP GROWTH TESTS OF WATER SAMPLES FROM THE OUTLETS OF MADISON, WIS AREA LAKES

| Date | Lake sampled | Chemical analyses | Available phosphorus by growth tests (mg P/1) | | |
|---|---|---|---|---|---|
| | | Soluble $PO_4$-P (mg P/1) | Selenastrum[a] | Microcystis[b] | Anabaena[c] |
| 1/30/73[d] | Mendota | 0.12 | 0.14 | 0.082 | 0.12 |
| | Monona | .076 | .080 | .050 | .12 |
| | Wingra | .010 | 0 | .005 | .005 |
| | Waubesa | .060 | .062 | .035 | .050 |
| | Kegonsa | .020 | .010 | .025 | .025 |
| 1/18/73[e] | Mendota | .14 | .16 | .12 | .13 |
| | Monona | .10 | .11 | .080 | .070 |
| | Wingra | .020 | .010 | .010 | .005 |
| | Waubesa | .066 | .062 | .05 | .035 |
| | Kegonsa | .042 | .020 | .025 | .010 |

[a] *Selenastrum capricornutum* (AAP), 1,000 cells/ml

[b] *Microcystis aeruginosa* (AAP), 50,000 cells/ml

[c] *Anabaena flos aquae* (AAP), 50,000 cells/ml

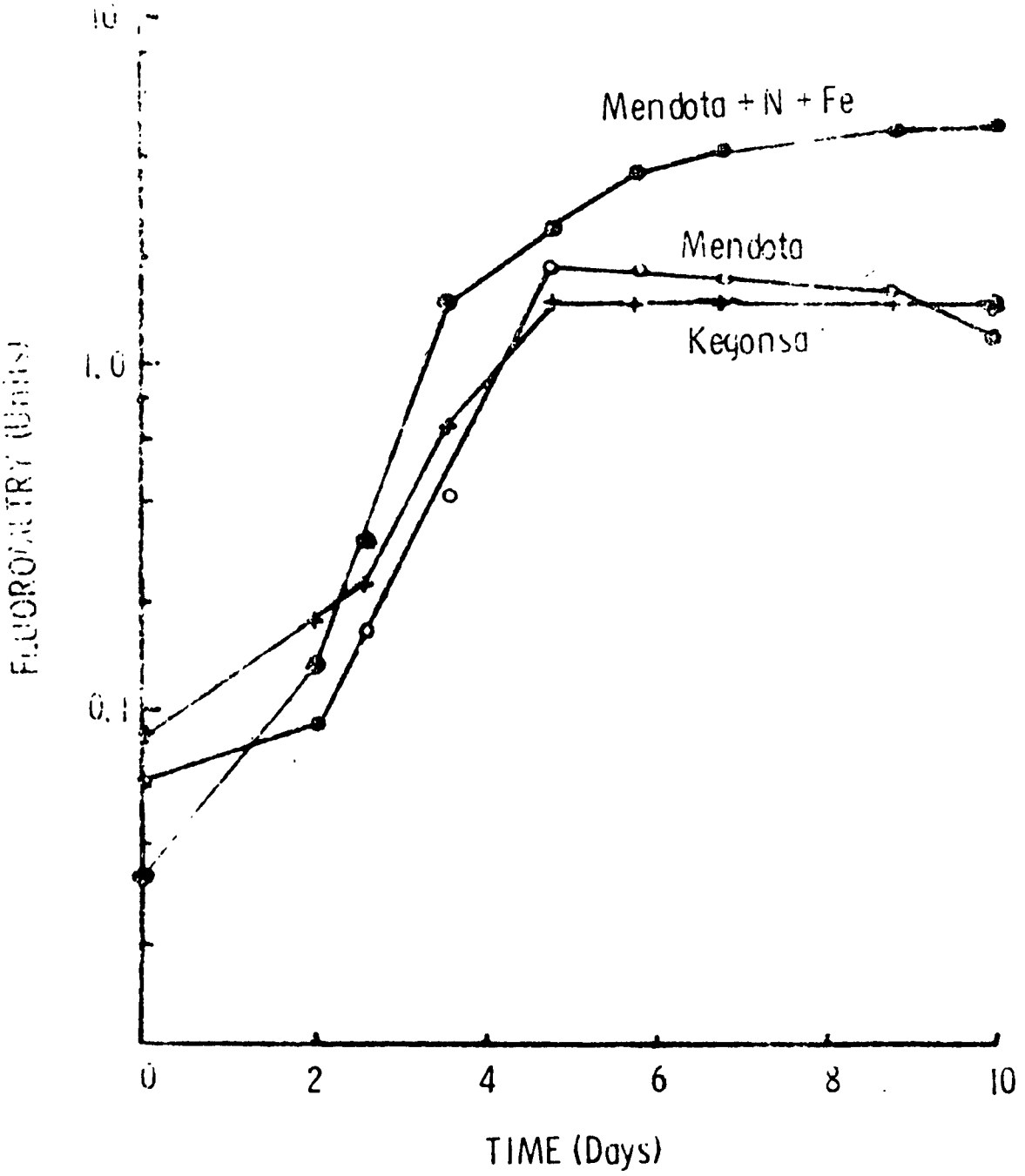
[d] Low flow period

[e] High flow period

was 1.92 ± .35 whereas the μ max of the Selenastrum was 1.72 ± .21. Similar results were also obtained when tests were carried out in AAM.

Mixtures of algae from Lake Kegonsa were used in AAP tests with different lake waters with and without spikes of nutrients. When raw lake waters (150 ml) were inoculated with 10 ml of Lake Kegonsa water the initial fluorescence of the cultures varied from 0.03 to 0.084 Aminco units depending upon the source of water (Figure 12). The maximum yield in Lakes Kegonsa and Mendota waters was reached by Day 5 (1.6 and 1.8 fluorometry units, respectively). Lake Mendota or Kegonsa waters supplemented with N or N + Fe (AAM levels) continued to grow until Day 7. There was little growth of algae in Lake Wingra waters unless $PO_4$-P was added. Since the waters of Lakes Kegonsa and Mendota at the time of sampling (May, 1973) contained 0.06 and 0.13 mg $PO_4$-P/l, respectively, there was no stimulation of growth over that in unspiked cultures when more $PO_4$-P was added. However, increased growth occurred in either lake water when N was added. Thus, tests with these mixtures of in situ algae indicated that algal growth in these lake waters would be limited by available N and available P in Lake Wingra water. These conclusions are the same as arrived at when Selenastrum was used (Figure 6). Therefore, one can obtain logical results using in situ algae in AAP tests, but the convenience and reproducibility of tests with the selected three species of laboratory algae make them the preferred organisms of use for most applications. Special tests, if warranted, using in situ algae could be used as checks on the results with Selenastrum.

Fig. 12. Rate of growth of Lake Kegonsa diatoms in lake waters.



## SECTION V

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