SODIUM: A FACTOR IN GROWTH OF BLUE-GREEN ALGAE

By

Amelia Kay Ward

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

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The response of heterocystous blue-green algae to
varying concentrations of sodium was examined in axenic
culture as well as in situ studies. Laboratory cultures of
Anabaena cylindrica were treated with four concentrations
of sodium and three concentrations of nitrate to determine
the response in terms of rates of acetylene reduction,
^{14}C assimilation, excretion of organic carbon, concentration
of chlorophyll a, particulate organic carbon, particulate
organic nitrogen, and heterocyst and filament numbers.
Natural populations of blue-green algae, predominantly
Aphanizomenon sp., were used for sodium enrichment studies
in which carbon-14 assimilation and acetylene reduction
provided a measurement of comparative photosynthetic carbon
fixation and elemental nitrogen fixation, respectively.

Cultures of Anabaena cylindrica with no Na^+ added
suffered from decreased rates of acetylene reduction, ^{14}C
assimilation, excretion of organic carbon as well as lower

concentrations of chlorophyll a and particulate organic carbon compared to cultures supplied with 5, 10, and 50 mg Na^+ $liter^{-1}$. Sodium deficient cultures released a higher percentage of previously fixed carbon as organic carbon. No differences in any area measured were demonstrable among cultures grown with 5, 10, and 50 mg Na^+ $liter^{-1}$. High nitrate concentrations (20 mg NO_3 $liter^{-1}$) resulted in decreased rates of acetylene reduction and heterocyst numbers in sodium sufficient and sodium deficient cultures; however, decreased particulate organic nitrogen content at high nitrate levels occurred only in sodium deficient cultures. Higher percentages of excreted organic carbon occurred with increasing nitrate concentrations in sodium deficient cultures. Sodium enrichment of natural phytoplankton populations indicated increased photosynthetic carbon fixation with small additions of sodium (5 mg Na^+ $liter^{-1}$), whereas higher concentrations (50, 100, and 200 mg Na^+ $liter^{-1}$) elicited neither a stimulatory nor an inhibitory response. No increase in in situ acetylene reduction rates occurred with additions of sodium.

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INTRODUCTION

Several investigators have reported positive
growth responses in blue-green algal cultures to the pres-
ence of relatively high concentrations of sodium (Allen,
1952; Batterton & Van Baalan, 1971; Benecke, 1898; Emerson &
Lewis, 1942; Gerloff, et al., 1952; and Kratz & Myers,
1955). Maximum growth was obtained at various levels
ranging from one mg Na^+ liter^{-1} and higher for Anacystis
nidulans (Batterton & Van Baalan, 1971), 5 mg Na^+ liter^{-1}
and higher for Anabaena cylindrica (Allen & Arnon, 1955) to
40 mg Na^+ liter^{-1} for Anabaena variabilis and Anacystis
nidulans (Kratz & Myers, 1955). Blue-green algae are among
the few plants which have an absolute sodium requirement
(Allen & Arnon, 1955). In studies with Anabaena cylindrica
the criteria for an essential nutrient were defined as
follows: (1) normal plant growth is absent without the
element; (2) the deficiency symptoms caused by the absence
of the element are removed on its addition to the medium;
(3) the requirement is specific (the substitution of
another nutrient for the element cannot be made); and

(4) the function of the element or its direct effect on the metabolism of the plant is identified.

The physiological function of sodium in blue-green algae has never been elucidated completely. Sodium has been shown to inhibit nitrate reductase activity in cultures of <u>Anabaena</u> <u>cylindrica</u> grown with nitrate (Brownell & Nicholas, 1967). Sodium deficient cultures resulted in enhanced nitrate reductase activity and accumulation of toxic levels of nitrate. Higher levels of sodium were required to avoid deficiency symptoms in cultures grown with nitrate (0.4 meq/l NaCl) as compared with cultures grown without nitrate (0.004 meq/l NaCl).

The paucity of information regarding the physiological function of sodium is matched by the lack of information on the possible ecological effects of sodium on natural populations of blue-green algae. Provasoli (1969) conjectured that increases in salinity in Lake Washington could have been a contributory factor to the ensuing blue-green algal bloom (Edmondson, <u>et</u> <u>al</u>., 1956). The presence of both high levels of sodium (50 mg Na^+ liter^{-1}) and dense populations of blue-green algae have been noted in hypereutrophic Sylvan Lake in contrast to low levels of sodium (3 mg liter^{-1}) and a lack of blue-green dominant populations in Goose Lake and hard-water lakes in general (Wetzel, 1966). The occurrence of the two phenomena may be unrelated, but the possibility of sodium contributing

as one of several interrelated causal factors appears worthy of further investigation.

Ecologically, nitrogen-fixing blue-green algae are of particular interest because of their potential to contribute simultaneously to the carbon and nitrogen input of certain water strata. Large populations of heterocystous blue-green algae and maximum nitrogen-fixing rates are generally associated, spatially and temporally, with minimal concentrations of combined inorganic nitrogen forms (Goering & Neess, 1964; Stewart, et al., 1970; Duong, 1972; Horne & Goldman, 1972; Horne, et al., 1972). However, the presence of inorganic forms does not necessarily preclude elemental nitrogen fixation (Dugdale & Dugdale, 1965; Goering & Dugdale, 1966; Horne & Fogg, 1970). The understanding of the effects of any factor which has the potential to influence directly the nitrogen metabolism of heterocystous blue-green algae would seem of major importance in further clarifying the many complex interactions which result in the presence of natural populations of heterocystous blue-green algae. Sodium has been implicated as such a factor.

The primary objective of the following study was to investigate the response of blue-green algae to various levels of sodium concentration. It was restricted to nitrogen-fixing members of the Cyanophyta with major emphasis on facets of carbon and nitrogen metabolism. The research followed two directions:

1. Assays of the response of <u>Anabaena</u> <u>cylindrica</u> in axenic culture to varying concentrations of sodium and nitrate.

2. <u>In</u> <u>situ</u> studies designed to describe the response of natural populations of heterocystous blue-green algae to sodium enrichment by means of carbon-14 radioassay as well as acetylene reduction techniques.

MATERIALS AND METHODS

Laboratory

Culturing Procedures

A culture of <u>Anabaena</u> <u>cylindrica</u>, generously supplied by Professor Akihiko Hattori, University of Tokyo, Tokyo, Japan, was maintained in axenic culture for laboratory experiments. The medium used for experimental purposes, G4 (a modification of Gorham's medium), was of the following composition (mg liter^{-1}): K_2CO_3, 26; $K_3C_6H_5O_7 \cdot H_2O$, 235; K_2HPO_4, 39; $MgSO_4 \cdot 7H_2O$, 75; $CaCl_2$, 27; ferric citrate, 6; nitrilotriacetic acid, 40. Potassium nitrate was used in medium containing nitrate. Sodium was variable and added as NaCl. Micronutrients were supplied as follows (mg liter^{-1}): $ZnSO_4 \cdot 7H_2O$, 1; $MnSO_4 \cdot H_2O$, 1; LiCl, 0.5; $CoCl_2 \cdot 6H_2O$, 0.5; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.1. The pH was adjusted to 7.0 with 0.5 N KOH before autoclaving; final pH after autoclaving was 7.9. The medium was dispensed in 300 ml aliquots to 500 ml polycarbonate flasks. Polycarbonate was used to prevent Na^+ contamination from glass flasks. G4 medium with no NaCl added, autoclaved once in a polycarbonate flask and stored for 26 days was

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found to have an absolute Na^+ concentration of 0.236 mg Na^+ liter^{-1} compared with 0.661 mg Na^+ liter^{-1} in medium autoclaved once in a glass flask and stored for the same period of time. Repeated autoclaving also increased the sodium concentration of the medium in a glass flask, whereas no increase in sodium concentration was observed in medium repeatedly autoclaved in a polycarbonate flask. Sodium concentrations in the text and figures refer to sodium added as NaCl, not the absolute sodium concentration of the medium.

An aliquot from a fresh stock culture was used as an inoculum for experimental flasks. Twenty ml of stock culture were transferred aseptically to 100 ml G4 medium without sodium in a glass flask; twenty ml from this flask were then transferred to 100 ml G4 medium without sodium in a polycarbonate flask. A two ml aliquot from the poly-carbonate flask was used as an inoculum for each experi-mental flask, thus, sufficiently diluting the sodium from the stock culture. Duplicate flasks of all treatments were prepared in each experiment. Experimental cultures were maintained in a Sherer growth chamber on a rotating platform at $23^{\circ}C$ and at a light intensity of 1936 lux (Weston Illumination Meter, Model 756) supplied from fluorescent and incandescent light sources on a light regime of 16 hours light and 8 hours dark.

Acetylene Reduction

The acetylene reduction technique for determination of elemental nitrogen fixation was first developed (Dilworth, 1966; Schöllhorn & Burris, 1966) and applied to blue-green algae in the mid-1960s (Stewart, et al., 1967, 1968). Since then it has been used extensively in both laboratory and in situ studies. However, it is an indirect technique and not without problems (see discussion in Fogg, et al., 1973, and Stewart, 1973). In this study it was used as a reasonable estimate of the comparative N_2-fixing ability of heterocystous blue-green algae.

Aliquots (5 ml) from each culture flask were transferred aseptically to a 15 ml serum vial and sealed with serum bottle caps. Vials were flushed for two minutes with a nitrogen-free gas: CO_2, 0.0395%; O_2, 23.60%, argon balance (Matheson Gas Products, Joliet, Illinois), injected with 1.0 ml purified acetylene (Matheson Gas Products), and incubated two hours under the same conditions as the culture flasks. The algae were killed with 0.2 ml 2% $HgCl_2$. Control vials were injected with $HgCl_2$ immediately after injection of acetylene and allowed to incubate under the same conditions as the experimental vials. Samples (1.0 ml) from the headspace were injected into a Packard 409 gas chromatograph equipped with a H_2-flame ionization detector. A stainless steel column (3 mm X 2 meters) packed with Porapak-N (80-100 Mesh) was used for all analyses. Oven temperature was 65°C. Flow rates of gases

were as follows: He, 11 ml minute^{-1}; H_2, 18 ml minute^{-1}; air, 240 ml minute^{-1}. Areas of ethylene peaks (height of peak X width at 1/2 peak height) were quantified by relating the areas of the peaks to a known series of standards of purified ethylene (Matheson Gas Products).

Chlorophyll a

Aliquots (5 or 10 ml) were withdrawn aseptically from each culture flask and filtered onto a Millipore AA filter. Filters were homogenized (Teflon homogenizer) in 90% basic aqueous acetone, centrifuged, and the absorption of the supernatant measured by a Hitachi-Perkin Elmer spectrophotometer (Model UV-VIS 139). Calculations were those from Parsons and Strickland (1963) modified by Westlake (1969). Corrected chlorophyll a and pheopigments were also measured using the equations of Parsons and Strickland (1963) modified by Wetzel and Westlake (1969).

Carbon Assimilation and Excreted Organic Carbon

The day an experiment was terminated, a 35 ml aliquot was withdrawn from each experimental flask and placed in a sterile 100 ml erlenmeyer flask. Either 400 µl or 100 µl of $KH^{14}CO_3$ (4.96 µCi ml^{-1}) were injected into each flask. After a two hour incubation period under the same conditions as the experimental flasks, duplicate 5.0 ml aliquots were filtered onto Millipore HA filters for Geiger-Müller radioassay (Nuclear-Chicago D-47 of known

counting efficiency). Excreted organic carbon was measured from the same flasks. Ten ml of culture sample were filtered onto a Millipore HA filter; the filtrate was acidified with 3% H_3PO_4, purged with N_2 for two minutes, and two ml pipetted onto planchets for radioassay as above.

Particulate Organic Carbon

The day an experiment was terminated, duplicate 25 ml samples from each experimental flask were filtered onto pre-combusted (45 minutes, $525^{\circ}C$) glass fiber filters (Reeve Angel, 984H). Filters were analyzed for carbon by the procedures of Strickland and Parsons (1968) in which wet oxidation by acid dichromate is used with dextrose (D-glucose) as a standard.

Particulate Organic Nitrogen

Samples to be analyzed for particulate organic nitrogen were filtered by the same procedure as those for particulate organic carbon. Filters were subjected to a modification of the Kjeldahl determination described by McKenzie and Wallace (1954). Filters were predigested overnight in four ml of concentrated H_2SO_4 with 150 mg salicylic acid. After the addition of 300 mg $Na_2S_2O_3 \cdot 5H_2O$, 3 g K_2SO_4, and 150 mg HgO, samples were digested over heat until clear and then refluxed for 45 minutes. Samples were steam distilled, using methyl red in dilute boric acid as an indicator, and titrated against 0.005 N HCl. An indicator blank was used for the determination of the endpoint.

Filament and Heterocyst Enumeration

Samples from each culture flask were preserved with Lugol's solution. One ml was transferred to a Sedgewick-Rafter chamber for counting at 150X.

Sodium Analysis

Sodium concentrations of culture media and filtered lakewater samples were measured with a Jarrell Ash atomic absorption spectrophotometer (Model 82-700).

Field Procedures

Carbon-14 Sodium Enrichment Bioassays

Water samples were collected with a Van Dorn water sampler and distributed to 500 ml erlenmeyer flasks (summer, 1973) or 125 ml bottles (summer, 1974). Samples were injected with NaCl from autoclaved stock solutions to yield a final known Na^+ concentration. Three replicate light bottles and one dark bottle were used for each concentration as well as for untreated lakewater controls. Bottles were injected with 1.0 ml $KH^{14}CO_3$ (4.96 $\mu Ci\ ml^{-1}$), suspended at the depth from which the water was collected and allowed to incubate. After incubation, 25 ml aliquots were filtered onto Millipore HA filters, fumed with HCl (Wetzel, 1965a) and analyzed by radioassay as described in Laboratory Procedures.

Acetylene Reduction Enrichment
Bioassays

Methods of in situ acetylene reduction followed closely those described by Duong (1972). Water samples were collected and the algae concentrated by gently filtering one liter of lakewater through 30 μm Nitex netting; algae were kept suspended in a small amount of filtrate during filtration to prevent disruption of cells and then rinsed from the netting into a beaker and resuspended in 100 ml of lakewater filtrate. Aliquots (7 ml) from this sample were distributed to 15 ml serum vials, injected with 0.5 ml of an appropriate NaCl solution, stoppered, flushed for two minutes with nitrogen-free gas (see Laboratory Procedures), injected with 1.0 ml acetylene, resuspended at the depth from which the water was collected, and incubated for two hours. Three replicate vials were used for each sodium concentration as well as for untreated lakewater samples. The algae were killed with 0.2 ml 2% $HgCl_2$. Controls were injected with $HgCl_2$ immediately after injection with acetylene and incubated with experimental vials. Vials were brought to the laboratory and 1.0 ml of headspace gas injected into a Varian Aerograph gas chromatograph (Model 500-D) with a flame ionization detector. A stainless steel column (3 mm X 2 meters) packed with Porapak-N (80-100 mesh) was used for all analyses. Flow rates of the gases were as follows: He, 18 ml $minute^{-1}$; H_2, 25 ml $minute^{-1}$; and air, 18 ml $minute^{-1}$.

Column temperature was 50°C. Ethylene was quantified as described in Laboratory Procedures.

Enumeration of Algal Cells and Heterocysts

Phytoplankton samples were preserved with Lugol's solution, 5 ml placed in a settling chamber and counted at 200 X using a Wild inverted microscope.

RESULTS AND DISCUSSION

Response of Anabaena
cylindrica to Sodium

The first three experiments were designed to determine if varying levels of sodium elicit different responses from the laboratory culture of Anabaena cylindrica. Four concentrations of sodium at one concentration of nitrate were used in each experiment, the nitrate level varying in each experiment. However, the exact values obtained in one experiment cannot be compared directly with values in other experiments because of variations in initial inoculum cell density. Therefore, only the responses to four concentrations of sodium at a given level of nitrate were compared.

The greatest difference in rates of acetylene reduction at each concentration of nitrate occurred between the levels of 0 and 5 mg Na^+ $liter^{-1}$ (Figure 1). Nested one-way analyses of variance revealed a significant difference in the acetylene reduction rates on day 14 among sodium treatments at the 1% level ($P \leq 0.01$). Little difference resulted from concentrations of sodium at 5, 10, and 50 mg Na^+ $liter^{-1}$. Increases in concentrations of
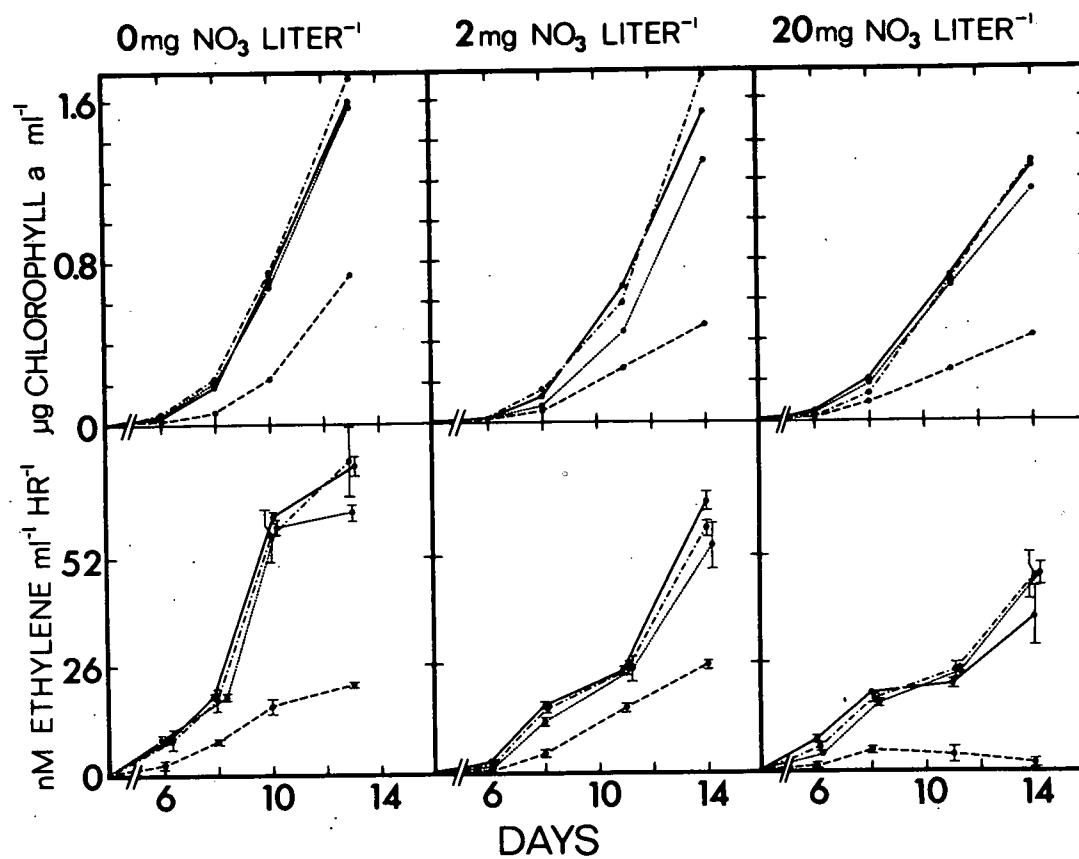
Figure 1. Concentration of chlorophyll a and rates of acetylene reduction of axenic <u>Anabaena</u> <u>cylindrica</u> over time at four concentrations of sodium and three concentrations of nitrate. Standard deviations are given for acetylene reduction unless one standard deviation is less than the diameter of the point. Sodium concentrations (mg liter^{-1}): 0 ·----·; 5 ·——·; 10 ·-·-·; 50 ······.

chlorophyll a, used as an estimate of increasing biomass,
closely resembled increases in rates of acetylene reduction
(Figure 1). The only exception to this was the treatment
in which cultures grown with 0 mg Na^+ liter^{-1} and an
initial concentration of 20 mg NO_3 liter^{-1} reached a maximum
acetylene reduction rate on day 8 and decreased steadily to
an almost negligible rate on day 14. However, concentra-
tions of chlorophyll a in the same treatment continued to
increase over the same period of time. This was the only
treatment in which the acetylene reduction rate decreased
with time.

Rates of ^{14}C assimilation reflected a pattern
similar to that of acetylene reduction; that is, carbon
uptake in cultures without sodium was consistently one-
third that of cultures grown at the three other concentra-
tions of sodium (Figure 2). No marked differences were
found among the higher sodium concentrations. In contrast
to rates of ^{14}C assimilation, rates of excretion of organic
carbon in cultures with no added sodium were approximately
one-half those of cultures with added sodium (Figure 2).
The relationship between rates of excreted organic carbon
and rates of ^{14}C assimilated can be seen more clearly when
excreted organic carbon is expressed as per cent carbon
assimilated (Figure 3). In all experiments a greater
percentage of carbon assimilated (as inorganic ^{14}C) was
excreted as organic carbon in cultures with no added sodium.
Although the actual percentages were small, this is a
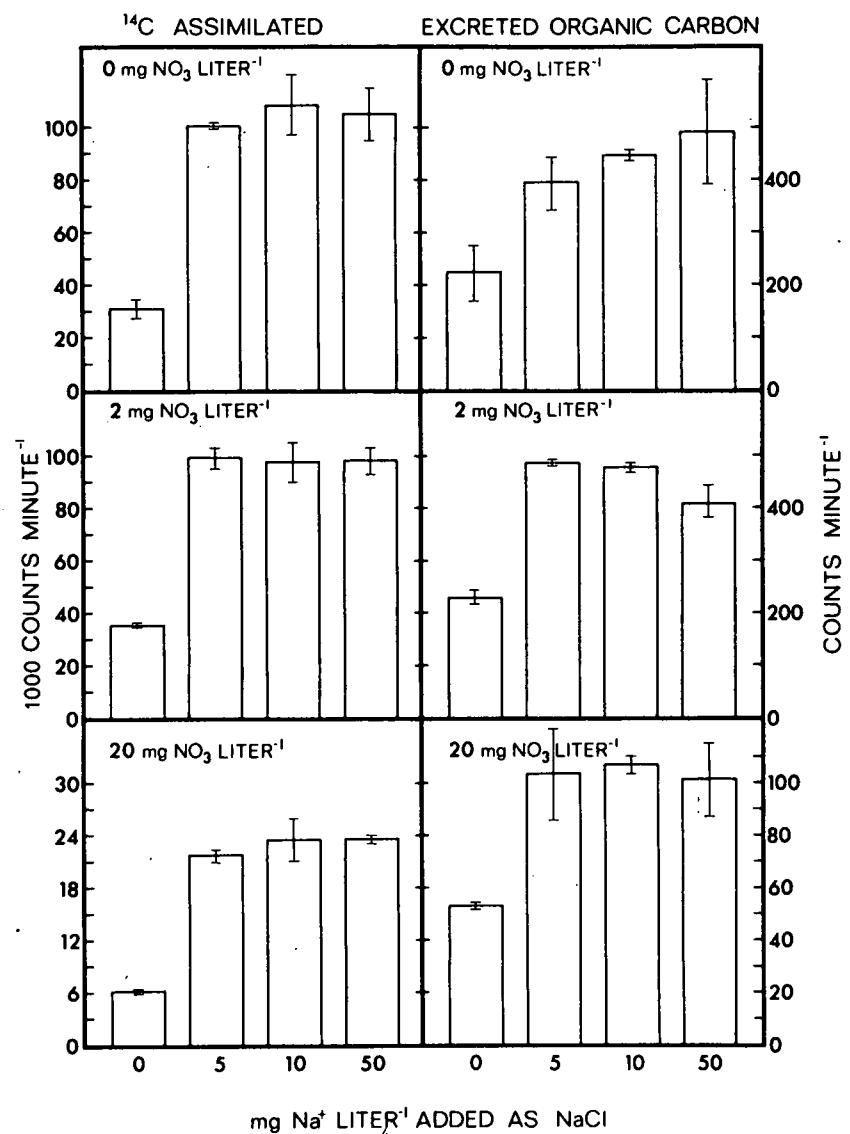
Figure 2. Rates of ^{14}C assimilation and excretion of organic carbon (± S.D.) of axenic _Anabaena_ _cylindrica_.
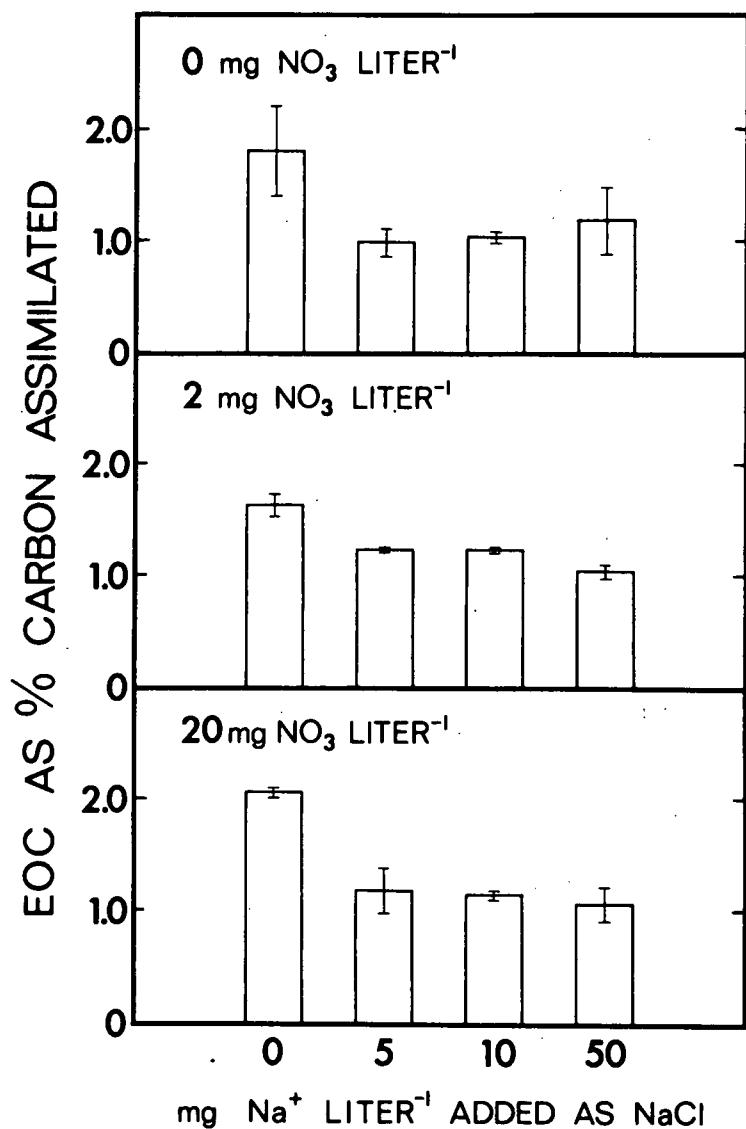
Figure 3. Excreted organic carbon as percentage
carbon assimilated (+ S.D.) of axenic
Anabaena cylindrica on day 14.

probable indication of a less efficient system in terms of carbon utilization in the sodium deficient cultures.

The quantity of particulate organic carbon present in cultures with added sodium was consistently twice that present in cultures without sodium (Figure 4). Since rates of carbon assimilation in cultures with added sodium were at least three times that of sodium deficient cultures, there appears to be a loss of carbon after assimilation in cultures with added sodium not entirely explicable in terms of excreted organic carbon. Possible explanations include: (1) Higher rates of CO_2 evolution occurred in sodium sufficient cultures. (2) Rates of ^{14}C assimilation in sodium sufficient cultures were less than three times that of sodium deficient cultures prior to day 14. Rates of ^{14}C assimilation were measured only on the last day of the experiment; the particulate organic carbon represented an accumulation over a 14-day period. Both explanations are entirely speculative since no data are available on CO_2 evolution or rates of ^{14}C assimilation prior to the 14th day of the experiments.

Response of Anabaena cylindrica to Sodium and Nitrate

In order to investigate responses to nitrate and sodium, experiments were designed to compare two concentrations of sodium (0 and 5 mg Na^+ liter^{-1}) at three concentrations of nitrate (0, 2, and 20 mg NO_3 liter^{-1}). The two concentrations of sodium were found to produce

PARTICULATE ORGANIC CARBON
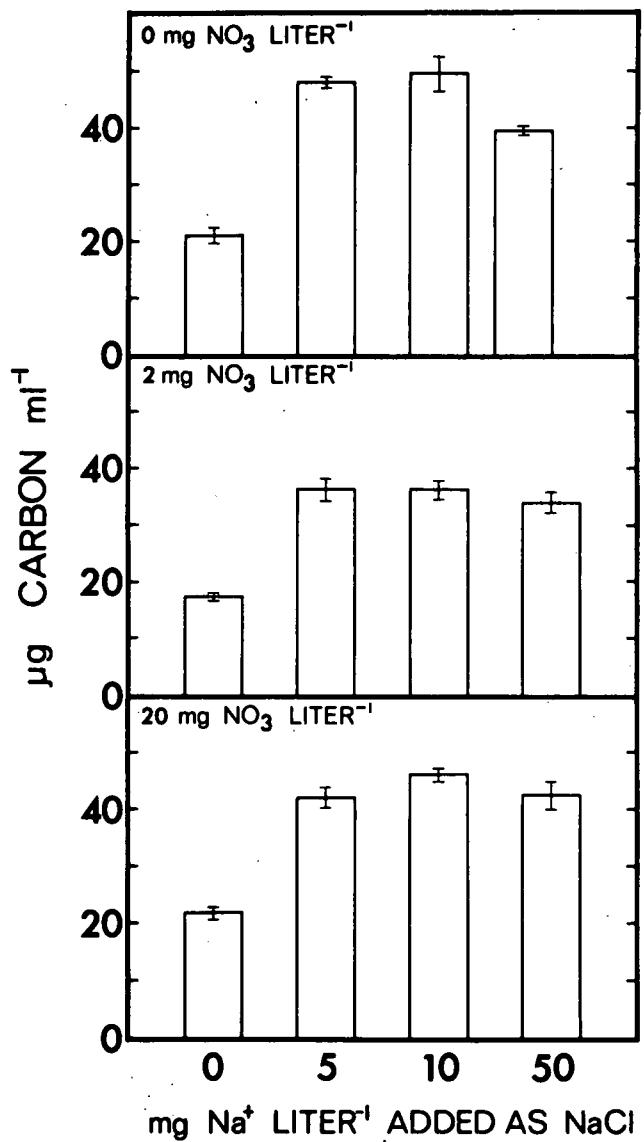
Figure 4. Concentration of particulate organic carbon (+ S.D.) of axenic <u>Anabaena cylindrica</u> on day 14.

highly significant results ($P \leq 0.01$) in all areas for which measurements are available (Table 1). Differing concentrations of nitrate produced significant results in four areas: acetylene reduction ($P \leq 0.01$), heterocyst numbers ($P \leq 0.01$), particulate organic nitrogen ($P \leq 0.05$), and excreted organic carbon as percent ^{14}C assimilated ($P \leq 0.01$). Three of the four areas are associated closely with facets of nitrogen metabolism. The addition of 2 mg NO_3 liter^{-1} appeared to make little difference in rates of acetylene reduction at either concentration of sodium compared to rates at 0 mg NO_3 liter^{-1} (Figure 5). However, the addition of 20 mg NO_3 liter^{-1} decreased the rates of acetylene reduction compared to 0 and 2 mg NO_3 liter^{-1} at both levels of sodium. Cultures grown at 5 mg Na^+ liter^{-1} continued to increase in rates of acetylene reduction through day 14, whereas cultures grown at 0 mg Na^+ liter^{-1} and 20 mg NO_3 liter^{-1} reached a maximum on day 11 and decreased to a negligible rate by day 14.

Heterocysts, generally accepted as the sites of nitrogen fixation under externally aerobic conditions (Wolk, 1973), decreased in numbers at higher concentrations of nitrate in both sodium sufficient and sodium deficient cultures. Cultures treated with the lowest sodium concentration (0 mg Na^+ liter^{-1}) and highest nitrate concentration (20 mg nitrate liter^{-1}) were the only ones having fewer heterocysts ml^{-1} than filaments ml^{-1} (Figure 6).

Table 1.--Results of two-way analysis of variance on data
from axenic cultures of <u>Anabaena</u> <u>cylindrica</u> in
response to sodium and nitrate, including levels
of significance: **, P≤0.01; *, P≤0.05; n.s.,
not significant.

	Sodium	Nitrate	S X N
Acetylene reduction	**	**	n.s.
Heterocyst numbers	**	**	*
Particulate organic nitrogen	**	*	n.s.
^{14}C assimilation	**	n.s.	n.s.
Excreted organic carbon	**	n.s.	n.s.
Particulate organic carbon	**	n.s.	n.s.
% Excreted organic carbon	**	**	*
Chlorophyll a	**	n.s.	n.s.
Filament numbers	**	n.s.	n.s.
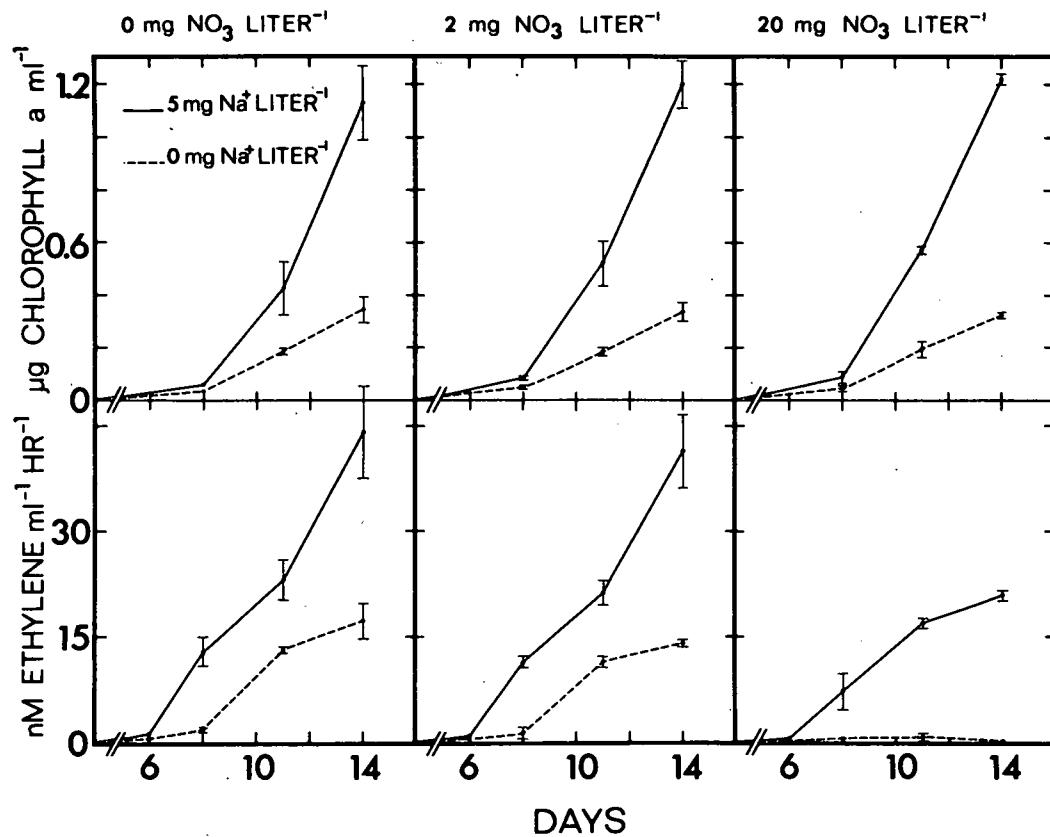
Figure 5. Concentration of chlorophyll a and rates of acetylene reduction of
           Anabaena cylindrica over time at two concentrations of sodium and
           three concentrations of nitrate. Standard deviations are included
           unless one standard deviation was less than the diameter of the point.
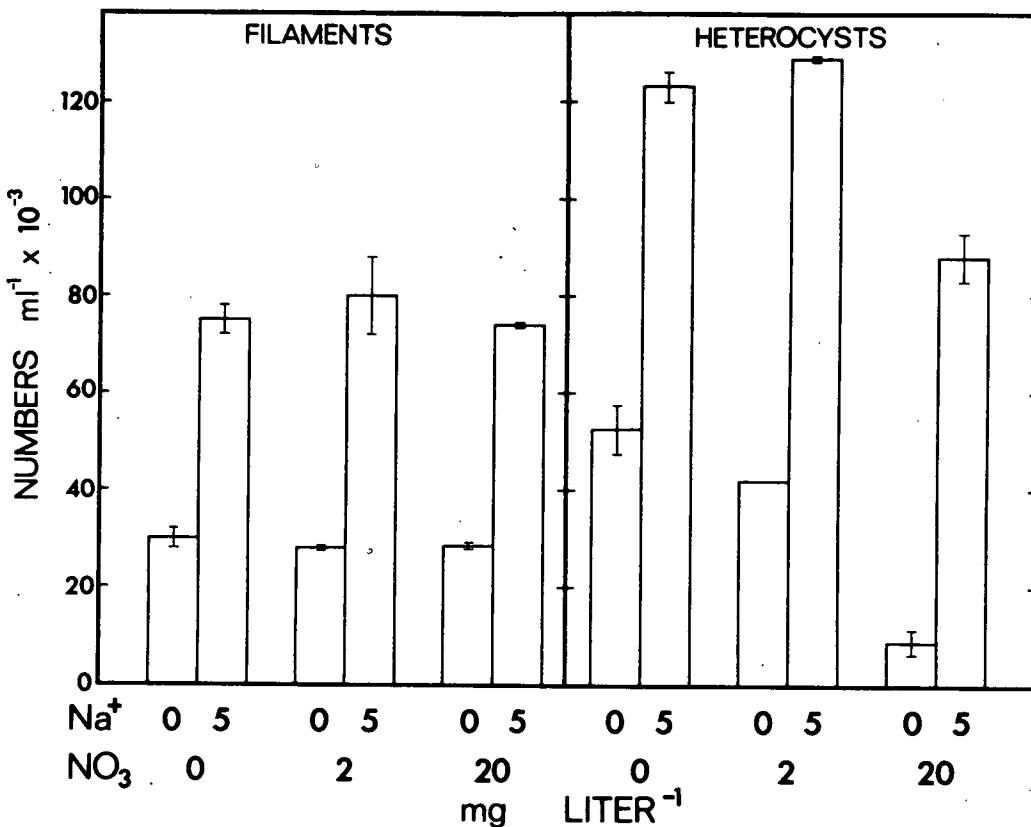
Figure 6. Density of filaments and heterocysts (ml^{-1}) on day 14 of axenic _Anabaena cylindrica_ in response to variations in sodium and nitrate. Standard deviations are included unless one standard deviation was less than the width of the line.

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Particulate organic nitrogen content of cultures grown with 5 mg Na^+ liter^{-1} did not vary with increasing nitrate concentration (Figure 7), indicating that cultures grown at the higher nitrate concentrations, where acetylene reduction decreased, were efficiently supplementing elemental nitrogen fixation with reduction of nitrate. However, sodium deficient cultures decreased in particulate organic nitrogen with increasing nitrate concentration, indicating a lag in nitrogen assimilation at higher concentrations of nitrate.

Particulate organic carbon, rates of ^{14}C assimilation, and rates of excretion of organic carbon (Figures 7 and 8) did not vary with increases in nitrate concentration. As in the other experiments, particulate organic carbon in sodium sufficient cultures (5 mg Na^+ liter^{-1}) was approximately twice that in sodium deficient cultures, whereas rates of ^{14}C assimilation were approximately three times greater. The percentage of ^{14}C assimilated and then excreted as organic carbon increased with increasing nitrate concentration in sodium deficient cultures (Figure 9).

In summary, when no sodium was added to cultures of Anabaena cylindrica, rates of acetylene reduction, ^{14}C assimilation, and excretion of organic carbon were significantly reduced as well as concentrations of chlorophyll a and particulate organic carbon compared with cultures grown with 5, 10, and 50 mg Na^+ liter^{-1}. No marked differences
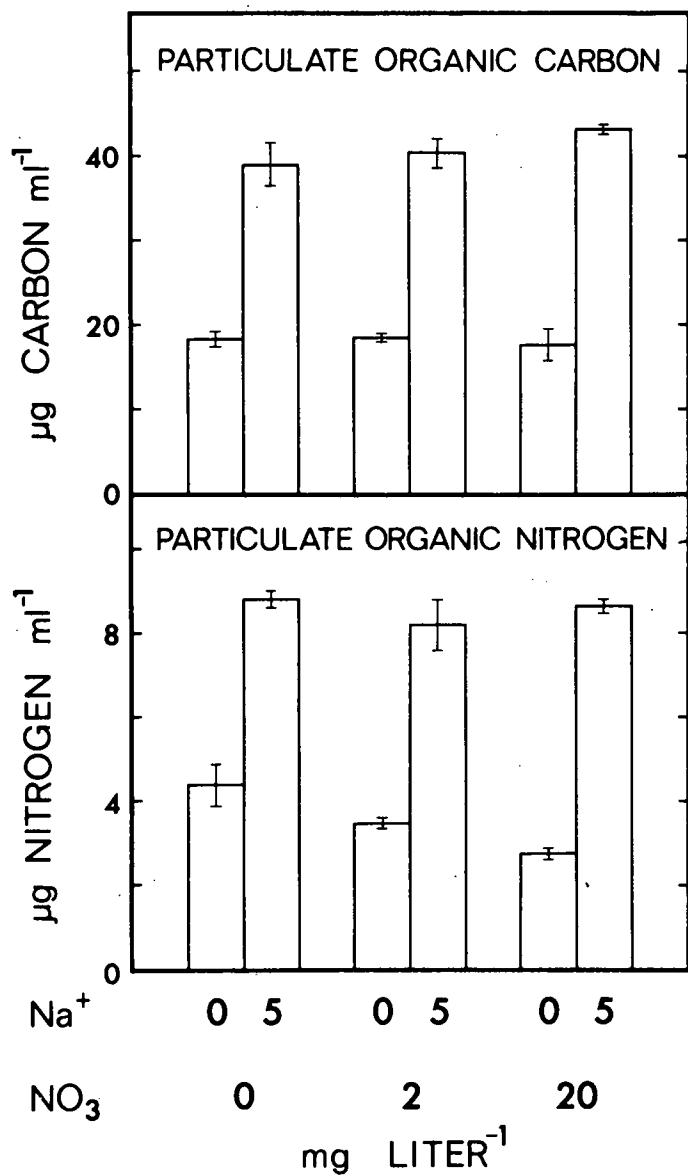
Figure 7. Concentrations (+ S.D.) of particulate organic carbon and particulate organic nitrogen on day 14 of <u>Anabaena cylindrica</u> in response to variations in sodium and nitrate.
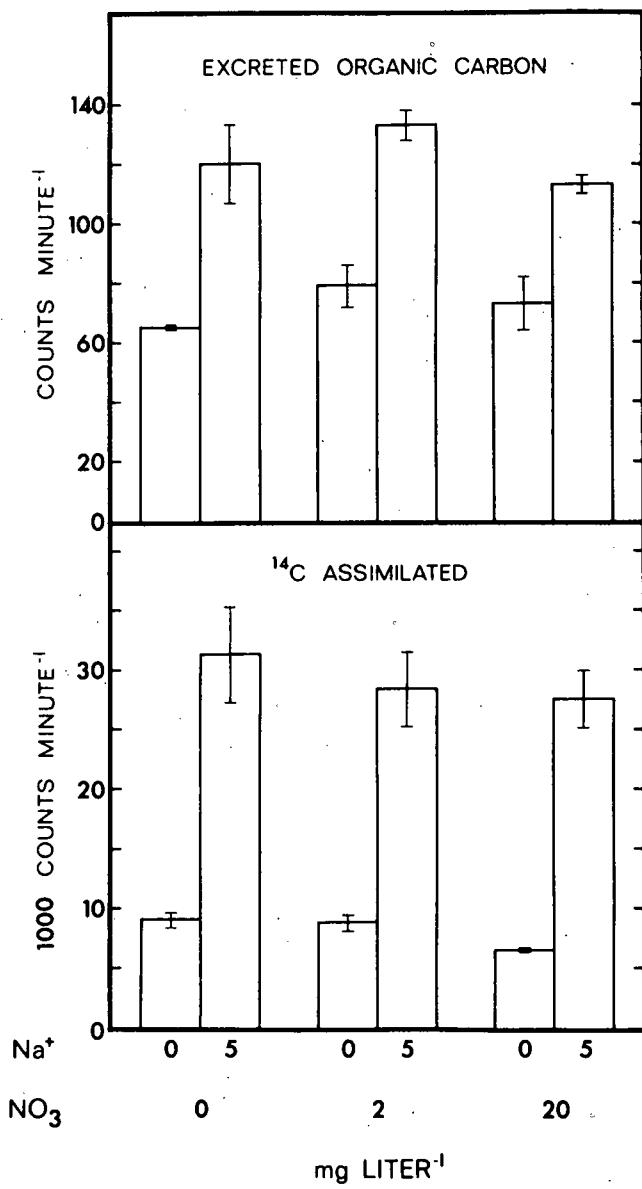
Figure 8. Rates of ^{14}C assimilation and excretion of
organic carbon ($\pm$ S.D.) on day 14 of
<u>Anabaena</u> <u>cylindrica</u> in response to varying
sodium and nitrate.

EOC AS %
CARBON ASSIMILATED
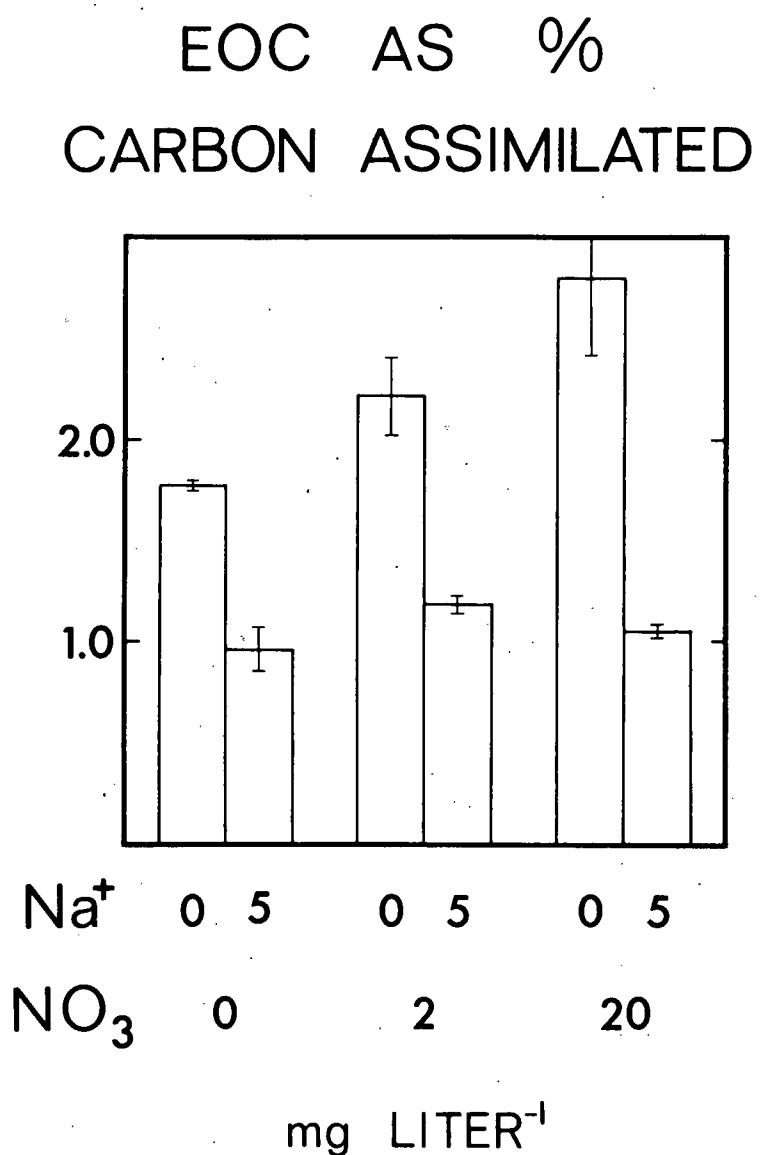
Figure 9. Excreted organic carbon as percentage carbon assimilated (+ S.D.) on day 14 of Anabaena cylindrica in response to varying sodium and nitrate.

were found in cultures supplied with 5, 10, and 50 mg Na^+ liter^{-1}. Increases in nitrate concentrations resulted in a decrease in acetylene reduction and heterocyst number in both sodium deficient and sodium sufficient cultures; however, a decrease in particulate organic nitrogen was observed only in sodium deficient cultures as nitrate concentrations were increased. An increase in the percentage organic carbon excreted was also apparent in sodium deficient cultures with increasing nitrate concentrations. The effects of high nitrate concentrations on sodium deficient cultures observed in this study are congruous with the results reported by Brownell and Nicholas (1967) in which toxic levels of nitrite accumulated when nitrate was present in sodium deficient cultures. That nitrite was accumulating in sodium deficient cultures in this study is a possibility; however, if so, it did not affect significantly aspects of carbon metabolism (rates of ^{14}C assimilation, excretion of organic carbon and particulate organic carbon) by day 14 of the incubation period. Highest nitrate concentrations used in this study (20 mg NO_3 liter^{-1}) were much lower than those used in the Brownell and Nicholas (1967) study (10 mM KNO_3) and are closer to those found in natural aquatic systems.

Response of Natural Populations of Blue-green Algae to Sodium Enrichment

In situ investigations were initiated in order to describe the response of natural populations of

heterocytous blue-green algae to additions of sodium. Typically, the cation composition of hard-water, marl lakes of southwestern Michigan is completely dominated by calcium and magnesium. The preponderance of divalent cations and the consequent low monovalent:divalent cation ratio has been proposed as one factor, among several, which functions in a cyclical manner to suppress productivity in marl lakes (Wetzel, 1969).

Enrichment studies were conducted in the summers of 1973 and 1974 in Wintergreen Lake, a eutrophic hard-water system which receives significant amounts of allochthonous and autochthonous organic input. The sodium concentrations rarely deviate from approximately 5 mg Na^+ $liter^{-1}$, a concentration differing little from that of other more oligotrophic hard-water lake systems in the area. Extensive work has been done on the lake describing basic limnological parameters as well as studies with particular emphasis on carbon and nitrogen metabolism (Duong, 1972; Manny, 1972; and Wetzel, et al., 1974). Characteristically, a dense heterocystous blue-green population develops in the summer; it was during a period of such development that the enrichment experiments were conducted.

Figure 10 illustrates the effects of sodium added in concentrations of 50, 100, and 200 mg Na^+ $liter^{-1}$ above background lake water (4.98 mg Na^+ $liter^{-1}$) at a depth of one meter. The predominant alga in the phytoplankton was
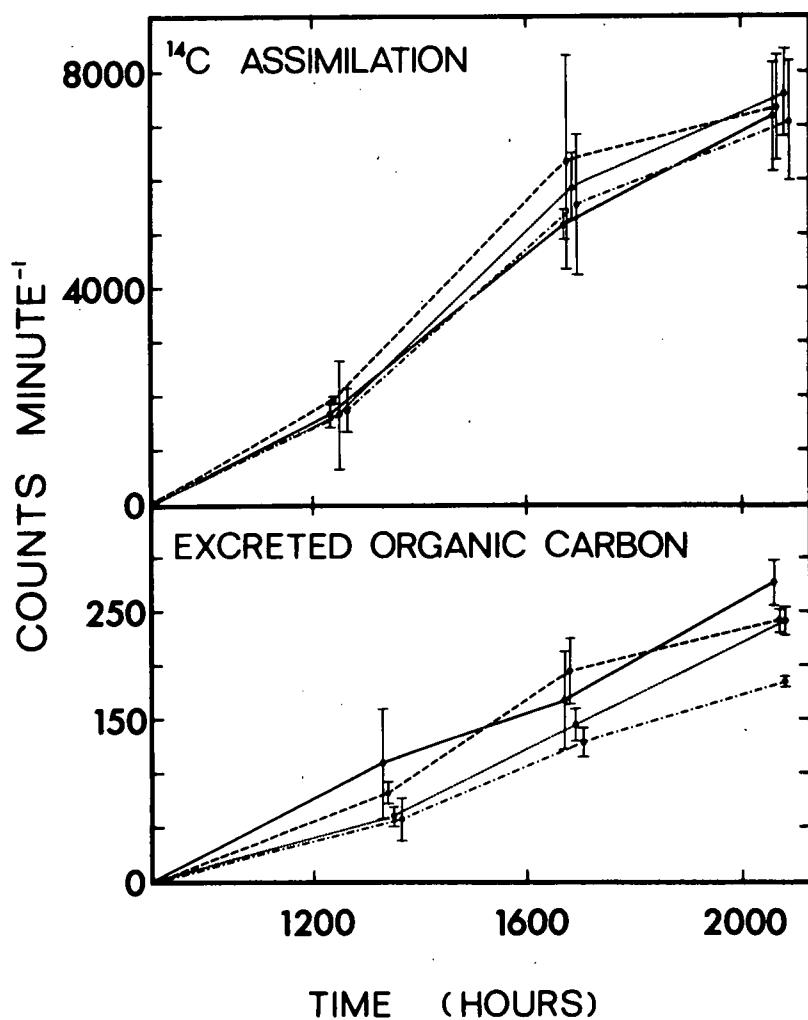
Figure 10. Photosynthetic carbon fixation and excreted organic carbon of phytoplankton populations in response to sodium enrichment, 16 July 73, in Wintergreen Lake over a 12-hour incubation period. Lakewater control . ___ .; 50 mg Na$^+$ liter^{-1} .---.; 100 mg Na$^+$ liter^{-1} .....; 200 mg Na$^+$ liter^{-1} .-.-.

Aphanizomenon sp. Although no marked enhancement of photo-synthetic carbon fixation was observed over untreated lakewater controls, neither was there a marked suppression over a 12-hour incubation period, which supplies further evidence for the halotolerant nature of blue-green algae (Pillai, 1954, 1955; Batterton & Van Baalan, 1971). In contrast to these results were those illustrated in Figure 11 in which a two- to three-fold stimulation in photosynthetic carbon fixation was caused by the addition of 5 mg Na^+ $liter^{-1}$ over background lakewater (4.99 mg Na^+ $liter^{-1}$) over a four hour incubation period. The addition of 50 mg Na^+ $liter^{-1}$ caused highly erratic results. The same concentrations of sodium did not increase the rates of acetylene reduction (Figure 11). Again, the dominant phytoplankter was Aphanizomenon (Table 2). These prelimi-nary bioassays suggest that, whereas the addition of large quantities of sodium have no effect on photosynthetic carbon fixation in this system, the addition of small quantities of sodium does enhance carbon fixation.

The development and associated literature of bio-assay studies as a tool in understanding nutrient relation-ships in laboratory cultures and in natural populations as well as the advantages and disadvantages of such a technique have been discussed by Wetzel (1965b). Ideally, enrichment bioassays provide a rapid determination of nutrients which may be in short supply and, hence, limiting productivity among natural phytoplankton populations. Problems exist
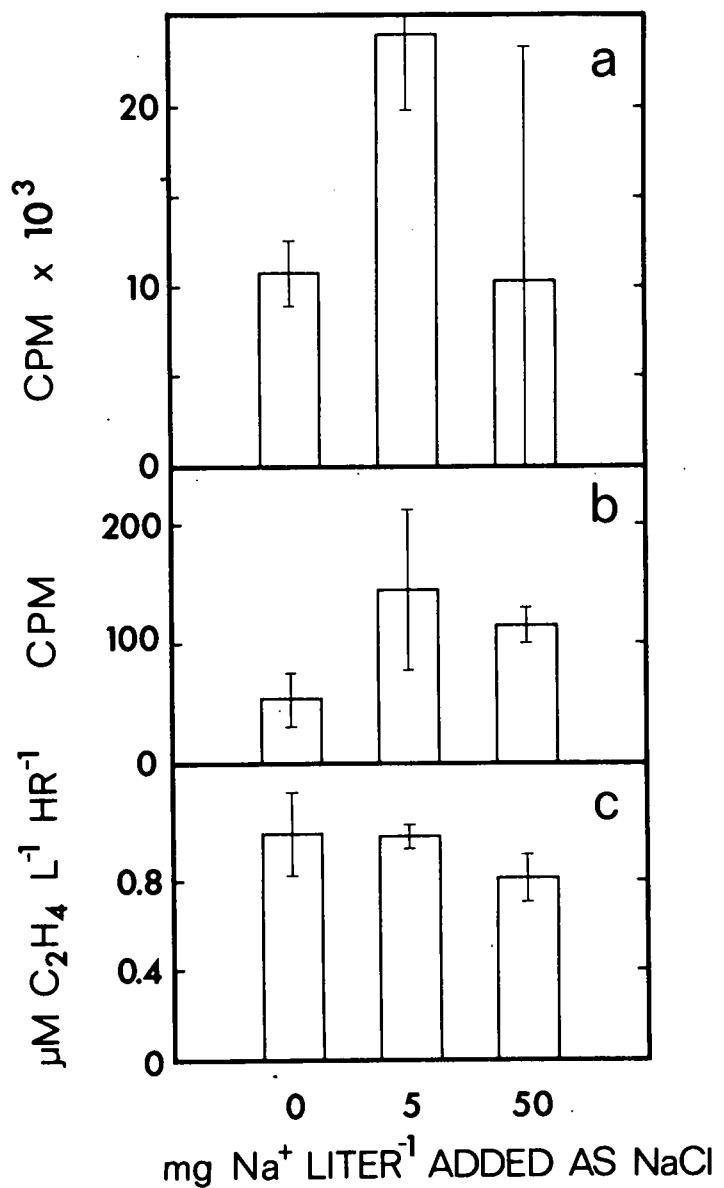
Figure 11. (a) Photosynthetic carbon fixation, (b) rates
of excretion of organic carbon, and
(c) acetylene reduction of phytoplankton
populations in response to sodium enrichment,
11 July 74, in Wintergreen Lake at 0.5
meters. 0 = lakewater control ($\pm$ S.D.).

Table 2.--Major groups of algae present on 11 July 74 at
0.5 M in Wintergreen Lake.

Genus	Number liter^{-1}
Aphanizomenon sp.	
filaments	16,524,300
heterocysts	2,988,200
Anabaena sp.	
filaments	89,200
heterocysts	22,300
Microcystis sp.	22,300
Ceratium sp.	423,700
Cryptomonas sp.	178,400
Trachelomonas sp.	66,900

in interpretation in that the response of a population may

vary not only among aquatic systems, but with time and at

differing concentrations of the added nutrient due to

changes in interacting factors within the system as well

as the interaction of such factors with the added nutrient.

The interpretation is compounded further when the physio-

logical function of the compound in unknown. The initial

bioassays performed in this study are certainly subject to

all of the above problems of interpretation. Although

sodium concentrations vary little within Wintergreen Lake

throughout the year, components which may alter the

response to sodium may vary. More specifically, the

response of the Aphanizomenon population may vary within

the period of the bloom. The population used in the

enrichments of 1974 was extraordinarily dense (16 million filaments liter^{-1}) and crashed dramatically a few days after the bioassays. That the population was stressed during the period of the experiment is probable; that this "stressed" condition is related to the positive response to added sodium is speculative.

Obviously, many questions remain with regard to the role of sodium in aquatic systems, particularly as it affects blue-green algal populations. Although direct extrapolation from axenic laboratory cultures is impossible, laboratory studies, by minimizing lack of control, make it possible, among other functions, to pinpoint areas of potential importance to natural populations. The response of sodium deficient cultures to nitrate in this study as well as the results reported by Brownell and Nicholas (1967) that sodium inhibits nitrate reductase suggest intriguing possibilities for the role of sodium in aquatic systems in terms of interactions between inorganic and elemental nitrogen assimilation.

SUMMARY AND CONCLUSIONS

The response of axenic cultures of <u>Anabaena</u>
<u>cylindrica</u> to four concentrations of sodium and three con-
centrations of nitrate were examined with special emphasis
on aspects of nitrogen and carbon metabolism. <u>In situ</u>
sodium enrichment bioassays were employed to ascertain the
response of natural populations of heterocystous blue-
green algae to additions of sodium. The acetylene reduction
technique was used as a comparative measure of the N_2-fixing
ability of blue-green algae, and the assimilation of
inorganic radioactive carbon provided a means of deter-
mining rates of photosynthetic carbon fixation. The
following conclusions can be drawn from this study:

1. Cultures with no NaCl added suffered from decreased
   rates of acetylene reduction, ^{14}C assimilation,
   excretion of organic carbon as well as lower con-
   centrations of chlorophyll a and particulate
   organic carbon as compared to cultures supplied
   with 5, 10, and 50 mg Na^+ $liter^{-1}$.

2. Sodium deficient cultures released a higher per-
   centage of previously fixed carbon as organic

carbon, possibly indicating a less efficient system in terms of carbon utilization.

3. No differences in any area measured were demonstrable among cultures grown with 5, 10, and 50 mg Na^+ $liter^{-1}$.

4. High nitrate concentrations (20 mg NO_3 $liter^{-1}$) resulted in decreased rates of acetylene reduction and heterocyst numbers in sodium sufficient and sodium deficient cultures; however, decreased particulate organic nitrogen content at high levels occurred only in sodium deficient cultures.

5. Higher percentages of excreted organic carbon occurred with increasing nitrate concentrations in sodium deficient cultures.

6. Preliminary sodium enrichment bioassays performed in Wintergreen Lake indicated increased photosynthetic carbon fixation with small additions of sodium (5 mg Na^+ $liter^{-1}$). Higher concentrations (50, 100, and 200 mg Na^+ $liter^{-1}$) elicited neither a stimulatory nor an inhibitory response in carbon fixation over untreated lakewater controls.

7. No increase in _in situ_ acetylene reduction rates occurred among natural populations with additions of sodium over the short incubation period utilized.

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