1978

Photoassimilation of organic compounds by two blue-green algae and natural phytoplankton communities.

Raymond Anthony. Allan

University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

Recommended Citation
https://scholar.uwindsor.ca/etd/3086

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.
NAME OF AUTHOR/NOM DE L'AUTEUR: Raymond Anthony Allan

TITLE OF THESIS/TITRE DE LA THÈSE: Photoassimilation of organic compounds by two blue-green algae and natural phytoplankton communities.

UNIVERSITY/UNIVERSITÉ: University of Windsor, Windsor, Ontario

DEGREE FOR WHICH THESIS WAS PRESENTED/GRÂDE POUR LEQUEL CETTE THÈSE FUT PRÉSENTÉE: M.Sc.

YEAR THIS DEGREE CONFERRED/ANNÉE D'OBTENTION DE CE GRÂDE: October 1978

NAME OF SUPERVISOR/NOM DU DIRECTEUR DE THÈSE: Dr. Donald W. Allen

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

DATED/DATÉ: June 7 1978

SIGNED/SIGNÉ: Raymond Anthony Allan

PERMANENT ADDRESS/RÉSIDENCE FIXÉE:
NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la these soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS RÉCU
PHOTOASSIMILATION OF ORGANIC COMPOUNDS BY TWO BLUE-GREEN ALGAE AND NATURAL PHYTOPLANKTON COMMUNITIES

by

Raymond Anthony Allan

A Thesis submitted to the Faculty of Graduate Studies through the Department of Biology in Partial Fulfillment of the requirements for the Degree of Master of Science at The University of Windsor

Windsor, Ontario, Canada

1978
to the birds........
ABSTRACT

PHOTOASSIMILATION OF ORGANIC COMPOUNDS BY TWO BLUE-GREEN ALGAE AND NATURAL PHYTOPLANKTON COMMUNITIES

by

Raymond Anthony Allan

Gloeocapsa alpicola and Synechococcus cedrorum took up arginine, glycine, aspartate and alanine at different rates in the light. Variation in the concentration of exogenous aspartate revealed saturation kinetics in G. alpicola and S. cedrorum with a $K_c$ of 1.05 and 1.3 nM respectively, at pH 8.0; corresponding $V_{max}$ values were 14.4 and 16.8 nM mg protein$^{-1}$ min$^{-1}$ respectively. Synechococcus cedrorum had a $K_c$ of 10 nM for arginine but G. alpicola did not show saturation kinetics. More than 79% of the $^{14}$C-labelled amino acids appeared in the ethanol-insoluble fraction. The ethanol-soluble fraction contained $^{14}$C-labelled amino acids, sugars and organic acids, at approximate ratios of 77.3 : 2.1 : 11.5 respectively. Flow of $^{14}$C-carbon from $^{14}$C-amino acids into metabolic pathways occurred with greater rapidity and diversity in G. alpicola.

Uptake of aspartate and arginine by G. alpicola and aspartate by S. cedrorum was lower in the dark or in the presence of DCMU, than in the light. However, uptake of arginine by S. cedrorum was similar for light, dark and DCMU-treated cells. $^{14}$C-CO$_2$ production in the light, from $^{14}$C-labelled exogenously supplied arginine, aspartate and
glycine was similar to dark $^{14}$C-CO$_2$ production in *S. cedrorum*. $^{14}$C-CO$_2$ from $^{14}$C-arginine was much reduced in the dark when metabolised by *G. alpicola*. Oxygen uptake in the dark was similar, for both blue-green algae, in the presence or absence of aspartate or arginine. The presence of glycine increased the oxygen uptake of *G. alpicola* but had no effect on oxygen consumption of *S. cedrorum*.

Uptake of organic compounds by naturally occurring phytoplankton communities in four arctic ponds revealed, in general, more organic carbon fixation occurring in the light than in the dark. Turnover times of substrates ranged from 37 to 238 h. The photosynthetic production values were intermediate in the Devon Island ponds relative to the Baffin Island ponds. The overall range being 3.55 to 14.4 mg C$_{ass}$ m$^{-3}$ h$^{-1}$. 
ACKNOWLEDGEMENTS

I wish to express my deepest thanks to Dr. Donald Wallen for his advice and criticism throughout this study. I would also like to thank Dr. D. T. N. Pillay and Dr. N. F. Taylor for providing helpful comments on the final draft. Dr. Paul Hebert kindly provided the opportunity for study in the Arctic. I acknowledge the assistance and support of many other people during the course of this study, especially Mona A. Mort, Fred Garnish and the members of the 5th Acadia Arctic Biology Expedition.
<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>xiii</td>
</tr>
</tbody>
</table>

CHAPTER I PHOTOASSIMILATION OF ORGANIC COMPOUNDS
BY GLOEOCAPSA ALPICOLA AND SYNECHOCOCCUS
CEDRORUM

INTRODUCTION ........................................... 3
MATERIALS AND METHODS ................................. 6
RESULTS ................................................... 12
DISCUSSION .............................................. 56

CHAPTER II UPTAKE OF ORGANIC COMPOUNDS BY
NATURAL PHYTOPLANKTON COMMUNITIES
IN FOUR ARCTIC PONDS

INTRODUCTION .......................................... 64
MATERIALS AND METHODS ............................... 65
RESULTS .................................................. 67
DISCUSSION ............................................. 78

LITERATURE CITED ...................................... 82
VITA AUCTORIS ........................................... 91
APPENDIX A ............................................. 88
APPENDIX B ............................................. 90
LIST OF TABLES

Table:                              Page
1. Growth constant (k) and mean generation time (T) in
   days, of *G. alpicola* grown in the presence of various
   concentrations of amino acids in the absence of N-NO₃,
   except where indicated........................................ 21
2. Uptake rates of ¹⁴C-amino acids by the blue-green algae,
   *S. cedrorum* and *G. alpicola*................................. 22
3. Distribution of ¹⁴C among the ethanol-insoluble and
   ethanol-soluble fractions in *G. alpicola* and *S.
   cedrorum............................................................. 24
4. Distribution of ¹⁴C in the amino acids, sugars and organic
   acids in the ethanol-soluble fraction after one hour
   incubation in ¹⁴C-labelled amino acids. (a) *G. alpicola*
   (b) *S. cedrorum................................................... 25
5. ¹⁴C among compounds of the ethanol-soluble fraction in
   *G. alpicola* incubated in ¹⁴C-labelled amino acid........ 27
6. ¹⁴C among sugars of the ethanol-soluble fraction in
   *G. alpicola* incubated in ¹⁴C-glycine and ¹⁴C-arginine
   .............................................................................. 31
7. ¹⁴C among compounds of the ethanol-soluble fraction
   in *S. cedrorum* after incubation in ¹⁴C-labelled amino
   acid........................................................................... 28
8. Release of ¹⁴C-CO₂ from accumulated ¹⁴C-labelled amino
   acids. (a) *G. alpicola* (b) *S. cedrorum................. 54
Table

9. Respiration rates, ul O$_2$ h$^{-1}$ mg protein$^{-1}$, of (a) G. alpicola and (b) S. cedrorum, in the dark, with or without an amino acid present. .......................... 55

10. Mean daily irradiance during the sample period........... 71

11. Primary production in the four arctic ponds, August 1977. ......................................................... 72

12. Velocity of $^{14}$C-glucose, $^{14}$C-aspartate and $^{14}$C-glycine uptake in arctic ponds. .......................... 76

13. Turnover times for glucose, aspartate and glycine by pond communities on Baffin and Devon Island, August 1977. ......................................................... 81
LIST OF FIGURES

Figure                                  Page

1. Growth of *Gloeocapsa alpicola* (cell number \( x 10^5 \text{ ml}^{-1} \)) throughout an 18 day period, in the absence of N-NO\(_3\). The media contained various concentrations of arginine. ................................................................. 14

2. Growth of *Gloeocapsa alpicola* (cell number \( x 10^5 \text{ ml}^{-1} \)) throughout a 12 day time period, in the absence of N-NO\(_3\). The media contained various concentrations of aspartate. ................................................................. 16

3. Growth of *Gloeocapsa alpicola* (cell number \( x 10^5 \text{ ml}^{-1} \)) throughout a 12 day time period, in the absence of N-NO\(_3\). The media contained various concentrations of glycine. ................................................................. 18

4. Growth of *Gloeocapsa alpicola* (cell number \( x 10^5 \text{ ml}^{-1} \)) throughout an 18 day time period, in the presence of N-NO\(_3\). The media contained various concentrations of aspartate. ................................................................. 20

5. Effect of light, DCMU and dark on arginine uptake (nM mg protein\(^{-1}\)) by *Synechococcus cedrorum*, over a 48 h time period. ................................................................. 33

6. Effect of light, DCMU and dark on arginine uptake (pM mg protein\(^{-1}\)) by *Gloeocapsa alpicola*, over a 48 h time period. ................................................................. 35

7. Effect of light, DCMU and dark on aspartate uptake (nM mg protein\(^{-1}\)) by *Synechococcus cedrorum*, over a 48 h time period. ................................................................. 37
8. Effect of light, DCMU and dark on aspartate uptake (nM mg protein$^{-1}$) by Gloeocapsa alpicola, over a 48 h time period................................. 39
9. Influx of aspartate at different external concentrations into S. cedrorum and G. alpicola......................... 41
10. Lineweaver-Burke plot for aspartate uptake by S. cedrorum and G. alpicola............................. 44
11. Lineweaver-Burke plot for arginine uptake by S. cedrorum........................................... 46
12. Influx of arginine at different external concentrations into G. alpicola................................. 48
13. Influx of glucose at different external concentrations into S. cedrorum................................. 50
14. Lineweaver-Burke plot for glucose uptake by S. cedrorum........................................... 52
15. Map of Canada showing location of arctic study sites on Baffin and Devon Islands....................... 69
16. Production, ug C l$^{-1}$ h$^{-1}$ and ug C ly$^{-1}$ h$^{-1}$ in pond 4, Devon Island............................ 74
ABBREVIATIONS.

DCMU – 3- (3,4-dichlorophenyl-1) 1,1-dimethylurea
CCCP – carbonyl cyanide m-chlorophenyl hydrazone
ATP – adenosine triphosphate
NADP – nicotinamide adenine dinucleotide phosphate
NAD – nicotinamide adenine dinucleotide
CHAPTER I

PHOTOASSIMILATION OF ORGANIC COMPOUNDS BY

GLOHOCAPSA ALPICOLA AND SYNECHOCoccus CEPDRORUM
The ability of blue-green algae to dominate phytoplankton communities in organically rich environments has prompted investigation into their metabolism of exogenous carbon compounds. Thus, studies of their ability to utilize compounds in the light and dark is of ecological, physiological and systematic importance. Growth in the dark on an organic compound is defined as heterotrophy. This requires the use of an exogenous compound as a source of ATP, reducing power and carbon by the organism. Heterotrophy, as such, has been found to be a rather limited phenomenon among the blue-green algae. The filamentous organisms dominate in their heterotrophic capacity (Hoare et al., 1971; Kiyohara et al., 1960; Raboy et al., 1976; Van Baalen et al., 1971; White and Shilo, 1975). However, growth rates in the dark are extremely slow (White and Shilo, 1975). Ability to grow in the dark also infers the utilization of exogenous organic carbon in the light.

Kenyon et al. (1972) classified utilization of organic compounds into three divisions, the first being heterotrophy, as described above. Facultative photoheterotrophy is the ability to grow in the light on an organic substrate in the presence of DCMU. DCMU is an inhibitor of photosystem II (Bishop, 1958), and thus prevents the production of reducing power, but ATP synthesis still continues via cyclic photophosphorylation. Facultative photoheterotrophic organisms
have the capacity to use an organic compound for reducing power and carbon source. On the other hand, obligate photoheterotrophic organisms will not grow on an organic substrate in the dark or in the presence of DCMU if illuminated. They may photoassimilate and metabolize the organic compound but it is only used as a carbon source. One such genera, Anabaena, has been shown to photoassimilate and metabolize sugars (Wildon and Rees, 1965; Pearce and Carr, 1969; Rubin et al, 1977). Obligate autotrophs may also utilize a number of other organic compounds (Pearce and Carr, 1967; Hoare et al, 1967; Smith et al, 1967).

Research on the heterotrophic capacity of blue-green procaryotes has focused on carbohydrate compounds. Stanier et al (1971) examined forty strains belonging to the Order Chroococcales for growth on glucose in the dark. Rippka (1972) screened 38 unicellular strains for facultative photoheterotrophic ability. Seven strains were able to grow on glucose, in the light, in media containing $10^{-5}$M DCMU. One of these strains, Aphanocapsa 6714, has been further studied in its kinetics and metabolism of glucose (Pelroy et al, 1972, 1976; Pelroy and Bassham, 1973 a and b).

The reason why some blue-green algae behave in an obligate photoautotrophic manner, while others are facultatively photoheterotrophic or heterotrophic, has only partially been answered. Heterotrophy is also been found to be limited in most cases to growth on carbohydrate compounds. Smith et
al (1967) working with *Anacystis nidulans* (*Synechococcus* 6301) and *Glóeocapsa alpícola* (*Aphanocapsa* 6308) came to the conclusion that their obligate autotrophic mode of growth was due to their inability to couple ATP generation with breakdown of the organic substrate. This was due to the absence of NADH oxidase. Both organisms also possessed a blocked TCA cycle. This block is also found in *Aphanocapsa* 6714, a heterotrophic organism (Ihlenfeldt and Gibson, 1977). It appears that the only oxidative pathway which is producing reducing power in the blue-green algae is the pentose phosphate pathway (Pelroy et al, 1972). Biggins (1969) proposes that endogenous oxidation is linked solely NADP-linked dehydrogenases and therefore ATP is synthesised via NADP reoxidation through a respiratory chain. This would restrict the types of organic compounds that can be used for energy production to those which will readily enter the pentose phosphate pathway.

The first enzyme in the oxidative hexose phosphate pathway, glucose-6-phosphate dehydrogenase, is specifically and allosterically inhibited by the only sugar, ribulose-1,5-diphosphate, which is unique to the reductive pentose phosphate cycle (Calvin cycle). This reduces the contribution to the metabolism of the cell by glucose under illuminated conditions (Pelroy et al, 1972). They also suggest that the inability of *Synechococcus* 6301 and *Aphanocapsa* 6308 to grow on glucose under dark or light conditions, in the presence of the photosynthetic inhibitor, is mainly due to their lack of an effective glucose permease.
Because of the apparent lack of evidence for other respiratory mechanisms for the breakdown of organic compounds, the assimilation and metabolism of amino acids by blue-green algae has been neglected. Smith et al. (1967) examined the ability of *A. nidulans* and *G. alpicola* to incorporate leucine and aspartate into cell material. Ohki and Katoh (1975) found casamino acids enhanced organotrophic growth. The purple sulphur bacterium *Chromatium* has been shown to assimilate amino acids and metabolize them to a small degree (Wagner, 1973).

The objectives of this study are to investigate the ability of two unicellular obligate autotrophic blue-green algae to photoassimilate amino acids under natural growth conditions. There have been a limited investigation into the metabolism of exogenously supplied substrates by unicellular blue-green algae (Pelroy et al., 1972, 1976; Pelroy and Bassham, 1973 a and b; Smith et al., 1967). Amino acids could potentially be used as a nitrogen as well as a carbon source. Therefore, *Gloeocapsa alpicola*, a nitrogen fixer, and *Synechococcus cedrorum*, a non-nitrogen fixer were selected. Uptake kinetics studies and metabolic inhibitors were used to determine uptake mechanisms for amino acids. The ability of the organisms to use exogenously supplied amino acids as an energy and carbon source were examined by evaluation of assimilation and subsequent metabolism of a variety of amino acids, the affect of amino acids on oxygen uptake, and carbon dioxide release. When appropriate, dark uptake rates were compared to light uptake rates to examine the general hypothesis that these blue-green algae are obligate photoautotrophes.
MATERIALS AND METHODS

Axenic cultures of two blue-green algae, Gloeocapsa alpicola (Frenkel) and Synechococcus cedrorum (Gassner) were used in these experiments. The cultures were obtained from culture collections of Dr. H. McCurdy and Dr. R.C. Starr respectively. Both organisms are obligate photoautotrophic in nature. Gloeocapsa alpicola, which is about 4-12 μm in diameter, is a coccoid unicellular photosynthetic procaryote, with each individual enclosed by a lamellate, mucilaginous, thick sheath. Found in aggregations of 2 or 3 cells, Synechococcus cedrorum, 6 μm in diameter and 10 μm long, is rod shaped, lacks a sheath and forms chains of 2 to 4 cells.

Stock cultures were grown on Hughes medium as described by Allen (1968) and modified by Coleman (personal communication) (Appendix A). All chemicals used were Fisher reagent grade. This medium was used because of its dilute nature allowing for maximum blue-green algae growth and a reduced probability of bacterial contamination. The medium was sterilized by autoclaving for 15 minutes at 120 C and 15 psi. Stock cultures, maintained in 125 ml flasks containing 50 ml of the medium and shaken continuously at 100 rpm, were exposed to a light:dark regime of 14 : 10 in a Percival Environmental Chamber. The cells were grown at a temperature of 20 ± 1 °C and an irradiance of 7,000 ergs cm⁻². Routine checks of both stock and experimental cultures were made for evidence of conta-
mination; when present, these cultures were discarded.

Cultures were grown with continuous shaking at a temperature of 20 ± 1 °C in cotton stoppered 500 ml Erlenmeyer flasks containing 200 ml of sterile modified Hughes' medium. In one set of flasks the medium contained no nitrogen. The cultures were supplemented with 0.01, 0.05, 0.1, 0.5, or 1.0 x 10^-4 M glycine, aspartate and arginine, which were added aseptically to the growth flasks after the medium was autoclaved. Stock solutions of the amino acids were filter sterilized. The controls were not supplemented with amino acids. All cultures were adjusted to a pH of 8.0.

The inocula for the growth experiments were taken from stock cultures in the exponential phase of growth. The volume of each inoculum was adjusted to provide an initial concentration of 3 x 10^5 cells ml^-1. The cultures were incubated under the same conditions as the stock cultures. All experiments were run in duplicate. Routine checks for bacterial contamination of both stock and experimental cultures were made periodically by inoculation into nutrient broth.

Growth rates were determined by measuring the change in cell number using a model ZBI Coulter Counter. Cells were in 0.5% NaCl with a 50 μ aperture. The relative growth constant (k) and mean generation time (T) were calculated using the following formula:

\[ k = 2.3 \log_{10} \left( \frac{N - N_0}{N_0} \right) \]
\[ T = \log \frac{e^2}{k} = 0.693 \]

where \( N \) = cell number at time \( t \).
\[ N_0 = \text{cell number at time } t = 0. \]
\( t = \text{time in days.} \)

To measure photosynthetic rates, the radiocarbon technique was used (Strickland and Parsons, 1968). Two uCi of \( \text{^{14}CO}_3 \) as carrier free \( \text{Na}^{14}\text{CO}_3 \) were added to each of several replicates of 50 ml of cell suspension taken during the log phase of growth. After 3 h of incubation the experiment was terminated, cell numbers were determined, and the cell suspensions were filtered through a Sartorius membrane filter (47 mm, pore diameter 0.45 u). The filters were placed in 10 ml of scintillator and radioactivity measured on a Beckman model 3170 P scintillation spectrometer. Counting efficiency was calculated using the external standard. The concentration of inorganic C was calculated by titration (Appendix B).

For uptake studies 500 ml cell suspensions were incubated with 25 uCi of uniformly \( \text{^{14}C} \)-labelled L-amino acids (specific activity about 170-324 uCi umol\(^{-1}\), Amersham Searle Co) for 1 h. Two one ml aliquots were filtered on to a 0.8 u millipore filter, washed and placed in 10 ml of the scintillator (Wallen and Geen, 1968) for radioassay. The remaining cells were killed by addition of 1 ml of 40% formalin, then separated from the medium by centrifugation at 10,000 x 20 min (5-8 C)
in a Sorvall RC2-B centrifuge. Centrifugation was repeated by resuspending the cells in nutrient media to remove external $^{14}$C-amino acids. The cells were killed and extracted in boiling 80% ethanol for 30 min. The ethanol insoluble fraction was then removed with a Sartorius membrane filter and washed several times with ethanol. The filters were then prepared for radio assay.

The ethanol soluble fraction was taken down to a 2 ml volume "in vacuo" at room temperature. Two 50 ul aliquots were assayed for $^{14}$C activity. The amino acids, organic acids and sugars were separated on Rexyn 101 (H$^+$) and Rexyn 201 (OH$^-$) (Fisher Scientific Company) ion-exchange columns. Both columns were eluted with 50 ml of water to remove the sugar fraction. The Rexyn 101 column was eluted with 70 ml of 1N NH$_4$OH to remove the amino acids. The Rexyn 201 was eluted with 70 ml of 5N Formic acid to remove the organic acids. The three fractions were then evaporated down to 1 ml and 50 ul aliquots were counted for $^{14}$C activity.

The amino acids and sugars were chromatographed on Whatman 3 mm chromatography paper in two dimensions. The first solvent system consisted of phenol : ammonium hydroxide : water (300 : 75 : 1, v/v/v) and the second solvent system consisted of butanol : acetone : diethylamine : water (11 : 11 : 2 : 5, v/v/v). Chromatograms were exposed to Kodak X-ray film for 2 to 3 weeks to locate radioactive compounds. All radioactive spots were excised and radioactivity determined by placing the paper spots directly into a toluene based scintillator. Spots were identified
by the relative positions by spray reagents, fingerprinting
technique and co-chromatography. The amount of the compound
was determined by measuring the amount of radioactivity present
on the chromatograms.

Uptake rates of organic compound were calculated from the
equation:

\[ V = \frac{c(S_n + A)}{C ut} \]

where \( V = \text{nM taken up mg protein}^{-1} \text{h}^{-1} \); \( c = \text{radioactivity taken up by the cells; } S_n = \text{concentration of unlabelled} \)
substrate (zero); \( A = \text{concentration of added substrate}; \)
\( C = \text{cpm uCi}^{-1} \text{in the system; } u = \text{number of uCi added to} \)
sample; \( t = \text{incubation time (h) (Parsons and Strickland, 1962)}; \)
and expressed as \( \text{nM h}^{-1} \text{mg protein}^{-1} \). Protein was measured
with the phenol reagent of Folin and Ciocalteu (Lowry et al.,
1951). The cells were disrupted by boiling and solubilized
in a copper alkaline solution for 10 min. The optical density
of the column which developed on addition of the phenol reagent
was measured at 750 nm. The method was calibrated with bovine
serum albumin.

In one series of experiments uptake over 48 h was
measured by cells grown in either the light, the light with
\( 10^{-5} \text{M DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylene) (Dupont} \)
Company), or in the dark. One ml aliquots were removed
periodically and an uptake measured as described above.

For the uptake kinetics experiments the methods described
by Wright and Hobbie (1966) and Hobbie and Crawford (1969)
were used. Duplicate 5 ml samples were incubated with 0.4 uCi of uniformly $^{14}$C-labelled amino acid (specific activity 170-324 uCi umol$^{-1}$) for 1 h under the conditions described previously. The concentration of amino acid ranged from 1.0 to $10 \times 10^{-3}$ mM.

The release of $^{14}$C$^{-}$CO$_2$ during incubation was also measured. Each bottle was closed with a serum stopper and after 1 h, 0.2 ml NCS (Amersham/Searle Corporation) was injected onto a filter paper disc in a cup suspended from the serum stopper. Then, 0.5 ml 2 N H$_2$SO$_4$ was injected into the sample to drive off any $^{14}$C$^{-}$CO$_2$ and the samples were shaken gently for 1 h. The filter paper disc containing the absorbed $^{14}$C$^{-}$CO$_2$ was removed from the cup and $^{14}$C activity in it counted by liquid scintillation. The cells were filtered onto Sartorius membrane filters (0.45 u diameter) and radioactivity counted as measured above.

Respiration rates in the presence and absence of aspartate, glycine and arginine were compared using a YSI Model 53 biological oxygen monitor (Yellow Spring Instrument Co.). Amino acids were added to make a concentration of $2.5 \times 10^{-6}$ M in the culture. Temperature was maintained at 25°C in a Haake water bath. All experiments were conducted in at least triplicate, in the dark. Oxygen utilization was related to protein content of the cells. Readings of oxygen consumption were taken over a six hour period. The respiration rates were calculated for each treatment.
RESULTS

Variations in growth constant (k) and mean generation
time (T) of G. alpicola were examined in the presence of three
amino acids. The results are presented in Fig. 1-4 and Table 1.
The mean generation time decreased when arginine was added
to the medium containing no inorganic nitrogen (Fig. 1).
Aspartate at low concentrations had a similar affect on growth.
When the medium was supplemented with N-NO₃, (0.367 g l⁻¹)
aspartate had no affect on growth rates, except at a concentration
of 10⁻⁴ M or higher where it was inhibitory to growth.

Photosynthetic production under the low light conditions
used in these experiments was considerably higher in S. cedrorum
than in G. alpicola. Production rates were 0.301 and 0.528 mg
C mg protein⁻¹ h⁻¹, for G. alpicola and S. cedrorum respectively.

S. cedrorum and G. alpicola incorporated a variety of
amino acids at different rates. The uptake rates (nM h⁻¹ mg
protein⁻¹) are summarized in Table 2. Both organisms took up
and metabolized, to different degrees, all the amino acids
tested. Aspartate and arginine uptake rates by S. cedrorum
were slightly higher than by G. alpicola. Whereas, uptake
of glycine and alanine by G. alpicola was considerably higher
than by S. cedrorum. Glycine uptake, for example, was 8.5
nM h⁻¹ mg protein⁻¹ by G. alpicola and 2.2 nM h⁻¹ mg protein⁻¹
by S. cedrorum. Although aspartate and glutamate are very
similar in structure, the uptake rates for glutamate by G.
alpicola were much lower than for aspartate, 0.670 and
Fig. 1 Growth of *Gloeocapsa alpicola* (cell number x 10^5 ml⁻¹) throughout an 18 day time period, in the absence of N-NO₃. The media contained various concentrations of arginine, (○) zero; (△) 0.01 x 10⁻⁴ M; (▲) 0.05 x 10⁻⁴ M; (○) 0.1 x 10⁻⁴ M; (■) 0.5 x 10⁻⁴ M; (□) 1.0 x 10⁻⁴ M.
Fig. 1.
Fig. 2. Growth of *Gloeocapsa alpicola* (cell number x $10^5$ ml$^{-1}$) throughout a 12 day time period, in the absence of N-NO$_3$. The media contained various concentrations of aspartate, (○) zero; (△) 0.01 x 10$^{-4}$ M; (▲) 0.05 x 10$^{-4}$ M; (◎) 0.1 x 10$^{-4}$ M; (●) 0.5 x 10$^{-4}$ M; (□) 1.0 x 10$^{-4}$ M.
Fig. 2.
Fig. 3. Growth of *Gloecapsa alpicola* (cell number $\times 10^5$ ml$^{-1}$) throughout a 12 day time period, in the absence of N-NO$_3$. The media contained various concentrations of glycine, (○) zero; (△) 0.01 $\times 10^{-4}$ M; (▲) 0.05 $\times 10^{-4}$ M; (○) 0.1 $\times 10^{-4}$ M; (●) 0.5 $\times 10^{-4}$ M; (□) 1.0 $\times 10^{-4}$ M.
Fig. 4. Growth of *Gloeocapsa alpicola* (cell number x 10^5 ml⁻¹) throughout an 18 day time period, in the presence of N-NO₃. The media contained various concentrations of aspartate, (○) zero; (△) 0.01 x 10⁻⁴ M; (▲) 0.05 x 10⁻⁴ M; (○) 0.1 x 10⁻⁴ M; (●) 0.5 x 10⁻⁴ M; (□) 1.0 x 10⁻⁴ M.
Fig. 4.
Table 1. Growth constant (k) and mean generation time (T) in days, of *G. alpicola* grown in the presence of various concentrations of amino acids in the absence of N-NO₃, except where indicated.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Concentration x 10⁻⁴ M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.448</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.502</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.554</td>
</tr>
<tr>
<td>Aspartate*</td>
<td>0.845</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Mean generation time (T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>1.55</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.38</td>
</tr>
<tr>
<td>Glycine</td>
<td>1.25</td>
</tr>
<tr>
<td>Aspartate*</td>
<td>0.820</td>
</tr>
</tbody>
</table>

* Medium contained N-NO₃ source.
Table 2. Uptake rates of $^{14}$C-amino acids by the blue-green algae, *S. cedrorum* and *G. alpicola*. Experiments were run for 1 h, at 20°C and at a light intensity of 7,000 ergs cm$^{-2}$.

<table>
<thead>
<tr>
<th></th>
<th>Rate of uptake nM h$^{-1}$ mg protein$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aspartate</td>
</tr>
<tr>
<td><em>G. alpicola</em></td>
<td>2.528</td>
</tr>
<tr>
<td><em>S. cedrorum</em></td>
<td>3.298</td>
</tr>
</tbody>
</table>
2.528 nM h\(^{-1}\) mg protein\(^{-1}\) respectively. The uptake of proline by \textit{G. alpicola} (4.6 nM h\(^{-1}\) mg protein\(^{-1}\)) was higher than for the other amino acids, with the exception of glycine.

The distribution of \(^{14}\text{C}\) in the various fractions in \textit{G. alpicola} and \textit{S. cedrorum} after incubation in \(^{14}\text{C}\)-labelled amino acids is given in Table 3. Radioactivity in the ethanol-insoluble fraction was considerably higher than in the ethanol-soluble fraction, \(72\) to \(95\) % of the \(^{14}\text{C}\) assimilated. Some species differences were observed. When \(^{14}\text{C}\)-arginine or \(^{14}\text{C}\)-glycine was provided as a substrate the ethanol-insoluble fraction contained \(94.9\) and \(93.4\) % of the total \(^{14}\text{C}\) assimilated by \textit{S. cedrorum}. Conversely, the radioactivity incorporated into the ethanol-insoluble fraction when \textit{S. cedrorum} was provided \(^{14}\text{C}\)-aspartate, as substrate, was the lowest value obtained, \(79.2\) %. The values for this fraction in \textit{G. alpicola} were intermediate to this range.

The distribution of \(^{14}\text{C}\) within the ethanol-soluble fraction is shown in Table 4. For both species of blue-green algae, more than \(75\) % of the \(^{14}\text{C}\) assimilated was in the amino acid fraction. This fraction in \textit{S. cedrorum} was considerably more than \(10\) % higher than in \textit{G. alpicola}. Most of the remaining \(^{14}\text{C}\) activity was found in the organic acid fraction. The relative amount of \(^{14}\text{C}\) in the sugar and organic acid fractions, were greater when \(^{14}\text{C}\)-arginine and \(^{14}\text{C}\)-aspartate were provided as substrate.

Tables 5 and 7 give the distribution of \(^{14}\text{C}\) among compounds of the ethanol-soluble fraction amino acids of \textit{G. alpicola} and \textit{S. cedrorum} respectively. Both blue-green
Table 3. Distribution of $^{14}$C among the ethanol-insoluble and ethanol-soluble fractions in *G. alpicola* and *S. cedrorum*, after utilization of $^{14}$C-amino acids for 1 h, at 20°C and at a light intensity of 7,000 ergs cm$^{-2}$.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Total fixation of $^{14}$C (uCi) $10^7$ cells$^{-1}$</th>
<th>Radioactivity as % of total $^{14}$C fixed ethanol-insoluble fraction</th>
<th>ethanol-soluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. alpicola</em></td>
<td>aspartate</td>
<td>0.024</td>
<td>88.9</td>
<td>10.1</td>
</tr>
<tr>
<td></td>
<td>arginine</td>
<td>0.026</td>
<td>85.7</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>glycine</td>
<td>0.061</td>
<td>83.6</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>alanine</td>
<td>0.027</td>
<td>90.2</td>
<td>9.8</td>
</tr>
<tr>
<td><em>S. cedrorum</em></td>
<td>aspartate</td>
<td>0.012</td>
<td>79.2</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>arginine</td>
<td>0.003</td>
<td>94.9</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>glycine</td>
<td>0.004</td>
<td>93.4</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>alanine</td>
<td>0.002</td>
<td>88.8</td>
<td>11.2</td>
</tr>
</tbody>
</table>
Table 4. Distribution of $^{14}\text{C}$ in the amino acids, sugars and organic acids in the ethanol-soluble fraction after one hour incubation in $^{14}\text{C}$-labelled amino acids. (a) G. alpicola (b) S. cedrorum

<table>
<thead>
<tr>
<th>Exogenous substrate</th>
<th>Fraction % of total $^{14}\text{C}$ fixed in the ethanol-soluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aspartate</td>
</tr>
<tr>
<td>Amino acid</td>
<td>76.4</td>
</tr>
<tr>
<td>Sugar</td>
<td>1.8</td>
</tr>
<tr>
<td>Organic acid</td>
<td>21.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Fraction % of total $^{14}\text{C}$ fixed in the ethanol-soluble fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amino acid</td>
</tr>
<tr>
<td></td>
<td>Sugar</td>
</tr>
<tr>
<td></td>
<td>Organic acid</td>
</tr>
</tbody>
</table>
Table 5. $^{14}$C among amino acids of the ethanol-soluble fraction in G. alpicola after incubation in $^{14}$C-labelled amino acids for 1 h, at 20°C and at a light intensity of 7,000 ergs cm$^{-2}$.
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>14C-amino acid % recovery</th>
<th>glycine</th>
<th>alanine</th>
<th>glutamate</th>
<th>arginine</th>
<th>aspartate</th>
<th>proline</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspartate and glutamate</td>
<td>10.0</td>
<td>10.0</td>
<td>92.4</td>
<td>5.9</td>
<td>87.3</td>
<td>15.6</td>
<td></td>
</tr>
<tr>
<td>glutamine</td>
<td>11.6</td>
<td>--</td>
<td>4.0</td>
<td>51.8</td>
<td>2.1</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>proline</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.9</td>
<td>3.8</td>
<td>75.5</td>
<td></td>
</tr>
<tr>
<td>arginine</td>
<td>0.5</td>
<td>--</td>
<td>--</td>
<td>24.8</td>
<td>0.5</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>ornithine</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>14.1</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>asparagin</td>
<td>0.4</td>
<td>--</td>
<td>--</td>
<td>0.3</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>tyrosine</td>
<td>2.1</td>
<td>--</td>
<td>--</td>
<td>1.0</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>glycine</td>
<td>60.3</td>
<td>--</td>
<td>--</td>
<td>1.0</td>
<td>0.3</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>alanine</td>
<td>2.2</td>
<td>82.0</td>
<td>3.5</td>
<td>--</td>
<td>0.1</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>serine</td>
<td>4.6</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.6</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>leucine</td>
<td>0.8</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>threonine</td>
<td>1.0</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1.3</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>methionine</td>
<td>1.2</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>valine</td>
<td>0.3</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.2</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>hydroxyproline</td>
<td>4.1</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.5</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>lysine</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.3</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>isoleucine</td>
<td>--</td>
<td>--</td>
<td>2.0</td>
<td>--</td>
<td>0.6</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>unknown 1</td>
<td>1.8</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>0.4</td>
<td>--</td>
<td></td>
</tr>
</tbody>
</table>
Table 7. $^{14}$C among compounds of the ethanol-soluble fraction in *S. cedrorum* after incubation in $^{14}$C-labelled amino acids.

<table>
<thead>
<tr>
<th>$^{14}$C-amino acid</th>
<th>aspartate</th>
<th>arginine</th>
<th>glycine</th>
<th>alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$C-amino acid</td>
<td>88.5</td>
<td>4.8</td>
<td>2.6</td>
<td>2.9</td>
</tr>
<tr>
<td>aspartate and glutamate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glutamine</td>
<td>4.2</td>
<td></td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>arginine</td>
<td>--</td>
<td>95.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>glycine</td>
<td>--</td>
<td></td>
<td>82.7</td>
<td>--</td>
</tr>
<tr>
<td>alanine</td>
<td>5.9</td>
<td></td>
<td>--</td>
<td>96.3</td>
</tr>
<tr>
<td>threonine</td>
<td>0.8</td>
<td></td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>methionine</td>
<td>--</td>
<td></td>
<td>--</td>
<td>0.9</td>
</tr>
<tr>
<td>valine</td>
<td>--</td>
<td></td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>lysine</td>
<td>0.4</td>
<td></td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>isoleucine</td>
<td>0.3</td>
<td></td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>unknown 1</td>
<td>--</td>
<td></td>
<td>2.2</td>
<td>--</td>
</tr>
</tbody>
</table>
algae metabolized arginine more readily than aspartate. In addition to aspartate, glutamine, proline and threonine were detected in \textit{G. alpicola}.

Only 24.8\% of $^{14}$C-arginine was left after one hour of utilisation by \textit{G. alpicola}. A large percent of $^{14}$C was resident in glutamine (51.8\%) and to a lesser extent in ornithine and aspartate/glutamate. In contrast \textit{S. cedrorum} had most of the $^{14}$C activity found in arginine and the only other amino acids labelled were aspartate/glutamate. Both organisms exhibited poor metabolism of alanine, more than 82\% of the radioactivity remained as $^{14}$C-alanine after 1 h. Aspartate contained most of the metabolised $^{14}$C.

Similar to previous trends the redistribution of $^{14}$C from glycine by \textit{G. alpicola} was far greater than that of \textit{S. cedrorum}. The major fraction of the label, in both blue-green algae was located in aspartate and glutamine, however, in \textit{G. alpicola} a variety of other amino acids were labelled, hydroxyproline (4.1\%), serine (4.6\%), tyrosine (2.05\%) and alanine (2.2\%) are the more important ones.

Unlike aspartate, glutamate appears to be metabolized to a much lesser degree. Glutamate retained 92.4\% of the label. Proline as metabolized by \textit{G. alpicola} followed similar lines to arginine metabolism. The majority of the redistributed label being in aspartate/glutamate and glutamine. However, ornithine was not labelled when proline was the substrate.

The ethanol-soluble sugars in uptake experiments were
analysed by chromatography. In only two experiments the percent $^{14}C$ activity in the sugars of the ethanol-soluble fraction was high enough to be detected by radiographic techniques. This occurred when *G. alpicola* was provided either $^{14}C$-glycine or $^{14}C$-arginine as substrates. The data is presented in Table 6. Three detectable sugars were derived from $^{14}C$-glycine but 72.3% of the label was in an unidentified spot. The other sugars were ribose and glucose. Five sugars were detected when the cells were grown in $^{14}C$-arginine, galactose (76.3%) and to a lesser extent, glucose (10.2%), arabinose (8.9%), with very small amounts of fructose and ribose.

The influx of $^{14}C$-arginine or $^{14}C$-aspartate into *G. alpicola* grown in the light-dark regime, with or without DCMU, or in the dark is given in Figs. 5-8. Uptake by the light grown cells over the first 24 h was considerably higher for both amino acids than the influx into either DCMU-treated or dark grown cells, with the exception of $^{14}C$-arginine influx into *S. cedrorum* (Fig. 5). The influx of $^{14}C$-aspartate into *S. cedrorum* was lower after 48 h than aspartate uptake by DCMU-treated or dark grown cells (Fig. 7). There was no significant difference in the uptake by cells grown in DCMU or in the dark for either amino acid.

The effect of external amino acid concentrations on initial uptake rates in the two algae is shown for $^{14}C$-aspartate in Fig. 9. The Lineweaver-Burk plot (a plot of the inverse of the uptake against the inverse of the substrate concentration) results in straight lines from which the
Table 6. $^{14}$C among sugars of the ethanol-soluble fraction in *G. alpicola* incubated in $^{14}$C-glycine and $^{14}$C-arginine.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>$^{14}$C-amino acid</th>
<th>Glycine</th>
<th>Arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ribose</td>
<td>% recovery</td>
<td>12.7</td>
<td>1.1</td>
</tr>
<tr>
<td>glucose</td>
<td></td>
<td>15.0</td>
<td>10.2</td>
</tr>
<tr>
<td>fructose</td>
<td>--</td>
<td>--</td>
<td>3.4</td>
</tr>
<tr>
<td>arabinose</td>
<td>--</td>
<td>--</td>
<td>8.9</td>
</tr>
<tr>
<td>galactose</td>
<td>--</td>
<td>--</td>
<td>76.3</td>
</tr>
<tr>
<td>unknown 1</td>
<td></td>
<td>72.3</td>
<td>--</td>
</tr>
</tbody>
</table>
Fig. 5. Effect of light, DCMU and dark on arginine uptake (nM mg protein$^{-1}$) by *Synechococcus ectorum*, over a 48 h time period. (○—○) light; (●—●) 10$^{-5}$ M DCMU, in the light; (●—●) dark.
Fig. 6. Effect of light, DCMU and dark on arginine uptake (pm mg protein⁻¹) by Gloecapsa alpicola, over a 48 h time period. (O—O) light; (•--•) 10⁻⁵ M DCMU, in the light; (•—•) dark.
Fig. 7. Effect of light, DCMU and dark on aspartate uptake (nM mg protein\(^{-1}\)) by *Synechococcus cedrorum*, over a 48 h time period. (○—○) light; (■—■) 10\(^{-5}\) M DCMU, in the light; (●—●) dark.
Fig. 8. Effect of light, DCMU and dark on aspartate uptake (nM mg protein$^{-1}$) by Gloeocapsa alpicola, over a 48 h time period. (○—○) light; (●—●) $10^{-5}$ M DCMU, in the light; (●—○) dark.
Fig. 9. Influx of aspartate at different external concentrations into *Synechococcus cedrorum* (○), and *Gloecapsa alpicola* (●). pH 8.0; Temperature of 20°C.
following parameters for uptake kinetics were determined. The transport constant ($K_t$) for *S. cedrorum* is 1.3 nM and the $V_{max}$ 16.8 nM min$^{-1}$ mg protein$^{-1}$. The $K_t$ and $V_{max}$ for *Gloecapsa* are 1.1 nM and 14.4 nM min$^{-1}$ mg protein$^{-1}$ (Fig 10). For both algae, at high concentrations a second uptake mechanism may be involved. Thus there may exist two uptake mechanisms with differing affinities for aspartate (Fig. 10). The transport system for the amino acid in both blue-green procaryotes has a high substrate affinity and exhibits saturation (Michaelis-Menten) kinetics. The affect of $^{14}$C-arginine influx into *S. cedrorum* is similar, (Fig. 11). The $K_t$ and $V_{max}$ are 10 nM and 64.0 nM min$^{-1}$ mg protein$^{-1}$ respectively. The substrate affinity for arginine is lower than that for aspartate. Uptake of arginine by *G. alpicola* did not show rate limitation with increasing concentration. Instead the influx appeared to increase regularly over a considerable range of substrate concentrations (Fig. 12) suggesting that arginine influx is by diffusion. *Synechococcus cedrorum* exhibited saturation kinetics when $^{14}$C-glucose, commonly used in heterotrophic studies, was provided as a substrate (Figs. 13-14): $K_t = 1.1$ nM and $V_{max} = 8.3$ nM min$^{-1}$ mg protein$^{-1}$. The high substrate affinity of this system is similar to that for aspartate. Glucose was not taken up by *G. alpicola*, nor was there any evidence that uptake could be induced.

The release of $^{14}$C-$CO_2$ from assimilated $^{14}$C-amino acids
Fig. 10. Lineweaver-Burk plot for aspartate uptake by *Synechococcus cedrorum* (○), \( r = .998, p < .001 \) and *Gloeocapsa alpicola* (●), \( r = .998, p < .001 \).

\( V_{\text{max}} \) = maximum rate of influx, \( K_t \) = transport constant.

\( 1/V \) = inverse of the influx (nM min\(^{-1}\) mg protein\(^{-1}\)).

\( 1/S \) = inverse of the substrate concentration (μM).
$K_t = 1.3 \text{ nM}$

$V_{max} = 16.8 \text{ nM min}^{-1} \text{ mg prot}^{-1}$

$K_t = 1.05 \text{ nM}$

$V_{max} = 14.4 \text{ nM min}^{-1} \text{ mg prot}^{-1}$
Fig. 11. Lineweaver-Burk plot for arginine uptake by *Synechococcus cedrorum* \((r = .997, p .001)\).

\[ V_{\text{max}} \] = maximum rate of influx, \( K_c \) = transport constant.

\[ 1/V \] = inverse of the influx (nM min\(^{-1}\) mg protein\(^{-1}\)).

\[ 1/S \] = inverse of the substrate concentration (uM).
$\frac{1}{V} \propto \frac{1}{S}$

$K_t = 10 \text{nM}$

$V_{max} = 64 \text{nM min}^{-1} \text{mg prot}^{-1}$
Fig. 12. Influx of arginine at different external concentrations into Gloeocapsa alpicola. pH 8.0; Temperature of 20 C.
Fig. 12
Fig. 13. Influx of glucose at different external concentrations into *Synechococcus cedrorum*. pH 8.0; Temperature of 20 C.
Fig. 13
Fig 14. Lineweaver-Burk plot for glucose uptake by *Synechococcus cedrorum* (*r* = .999, *p* < .001).

$V_{\text{max}}$ = maximum rate of influx, $K_t$ = transport constant.

$1/V$ = inverse of the influx (nM min$^{-1}$ mg protein$^{-1}$)

$1/S$ = inverse of the substrate concentration (uM).
$K_t = 1.1 \text{ nM}$

$V_{\text{max}} = 8.3 \text{ nM min}^{-1} \text{ mg prot.}^{-1}$

Fig. 14
is given in Table 8. In general, $^{14}\text{C-CO}_2$ production rates in the light were similar for the two algae with the exception of $^{14}\text{C-CO}_2$ released from arginine. From 78 to 94% of accumulated $^{14}\text{C-arginine}$ by G. alpicola reappeared as $^{14}\text{C-CO}_2$. The release of $^{14}\text{C-CO}_2$ from G. alpicola when grown with either $^{14}\text{C-aspartate}$ or $^{14}\text{C-arginine}$ decreased from an initial high rate after 0.5 to 1 h to a lower rate after 3 h. For example, 34% of accumulated $^{14}\text{C-aspartate}$ was released as $^{14}\text{C-CO}_2$ after 0.5 h compared to 20% after 3 h. In contrast, when S. cedrorum was incubated with either $^{14}\text{C-amino acid}$, $^{14}\text{C-CO}_2$ release increased with time up to 3 h. $^{14}\text{C-CO}_2$ released from accumulated $^{14}\text{C-glycine}$ remained constant for 3 h, 9% and 5% for G. alpicola and S. cedrorum respectively.

The release of $^{14}\text{C-CO}_2$ in the dark, by G. alpicola from $^{14}\text{C-arginine}$ accumulated was considerably lower than release in the light, 21% (Table 8B). Dark release of $^{14}\text{C-CO}_2$ from $^{14}\text{C-aspartate}$ and $^{14}\text{C-glycine}$ was similar to production in the light. $^{14}\text{C-CO}_2$ released by S. cedrorum from dark accumulation of $^{14}\text{C-glycine}$ was higher, 14%, and $^{14}\text{C-aspartate}$ lower, 7%, than production in the light. Dark production from $^{14}\text{C-arginine}$, 29%, was similar to light release.

Respiration rates in the dark for both procaryotes are given in Table 9. In the majority of cases the presence of an amino acid had no effect on the respiration rate over a period of 6 h. Glycine, however, did significantly (p < .05) increase the respiration rate of G. alpicola.
Table 8. Release of $^{14}$C-CO$_2$ from accumulated $^{14}$C-labelled amino acid. (a) *G. alpicola* (b) *S. cedrorum*

A. Light Incubation

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>aspartate</th>
<th>arginine</th>
<th>glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>34</td>
<td>89</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>94</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>87</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>78</td>
<td>9</td>
</tr>
<tr>
<td>mean</td>
<td>26.8</td>
<td>87.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>aspartate</th>
<th>arginine</th>
<th>glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>23</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>37</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>32</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>31</td>
<td>5</td>
</tr>
<tr>
<td>mean</td>
<td>30.0</td>
<td>27.5</td>
<td>5.0</td>
</tr>
</tbody>
</table>

B. Dark Incubation

<table>
<thead>
<tr>
<th></th>
<th><em>G. alpicola</em></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td><em>S. cedrorum</em></td>
<td>7</td>
<td>29</td>
</tr>
</tbody>
</table>
Table 9: Respiration rates, $\mu$ l $O_2 \cdot h^{-1} \cdot mg \cdot protein^{-1}$, of
(a) G. alpicola and (b) S. cedrorum, in the dark,
with or without an amino acid present.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Test</th>
<th>Control</th>
<th>Sign. t</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspartate</td>
<td>18.9</td>
<td>16.2</td>
<td>--</td>
</tr>
<tr>
<td>arginine</td>
<td>36.4</td>
<td>37.9</td>
<td>--</td>
</tr>
<tr>
<td>glycine</td>
<td>31.5</td>
<td>24.3</td>
<td>*</td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Test</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>aspartate</td>
<td>34.3</td>
<td>30.0</td>
</tr>
<tr>
<td>arginine</td>
<td>39.6</td>
<td>37.0</td>
</tr>
<tr>
<td>glycine</td>
<td>22.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

* p .05, blanks indicate non-significance.
DISCUSSION

The inhibitory effect of amino acids on growth, in the presence of inorganic nitrogen, observed in these experiments (Fig. 4) has also been detected in other organisms. The bacterium *Thiobacillus* was inhibited by amino acids (Lu et al, 1971) as were the diatoms examined by Hellébust (1970) and Hayward (1965).

It is of interest to note that restriction of nitrogen in the growth medium produces an increase in the growth rate of *G. alpicola* (Table 1). The growth of *Chloroglea fritschii*, also a nitrogen fixer, was enhanced on glycine and glutamine (Fay, 1965). The growth of *G. alpicola* was increased when arginine or aspartate were present in the growth medium but glycine had no effect. In contrast, in the presence of nitrogen aspartate did not effect growth rates of *G. alpicola* or *A. nidulans* (Smith et al, 1967). Some carbohydrates, in particular glucose, have been reported to stimulate the growth of blue-green algae (Fay, 1965; Hoare et al, 1971; Raboy et al, 1976; Van Baalen et al, 1971).

This study shows that cells of *S. cedrorum* and *G. alpicola* take up amino acids at different rates, and apparently through transport systems. Transport systems for amino acids studied, with the exception of arginine in *G. alpicola* obey saturation kinetics (Fig. 9-12) indicating that the uptake process is carrier-mediated. These organisms possess transport systems with considerably higher substrate affinities (inverse of $K_t$) than many eucaryotic algae. For example, Lui and Hellebust
(1974 a and b), Wallen (1973), Hellebust (1970) and Stephens et al (1969) have reported on transport systems for amino acids ranging from $10^{-6}$ to $10^{-3}$ M. On the other hand transport systems with substrate affinities as high as observed in this study have been reported. Hobbie, Crawford and Webb (1968) found $K_t$'s for amino acid transport by bacterial populations ranging from $4 \times 10^{-9}$ to $1 \times 10^{-6}$ M. Vaccaro and Jannasch (1966) obtained a $K_t$ for glucose transport by bacterial populations of $2 \times 10^{-8}$ M at 6 C. Inlenfeldt and Gibson (1977) obtained high $K_t$ values for acetate uptake by strains of Synechococcus and Aphanocapsa.

The uptake of arginine by *G. alpicola* did not exhibit Michaelis-Menten kinetics, however the uptake increased linearly with increasing concentration up to the highest concentration tested (Fig. 12). It may be concluded that the influx of arginine into this alga is accomplished through diffusion. However, the significant reduction of arginine uptake in the dark relative to the light, and in the presence of the metabolic inhibitor DCMU (Fig. 6) indicates that an energy requiring mechanism of very low affinity may be involved.

Most of the recently incorporated radioactivity from the supplied organic substrates was recovered in the ethanol-insoluble fraction of the cells (Table 3). It is assumed that the radioactivity found in this fraction is actually contained in the proteins (Smith et al. 1967; Britten and McLue, 1962). Hence, this data suggest that the main contribution of exogenously supplied amino acids is to the cellular protein
in these procaryotes. Wagner et al. (1973) reported that almost all of the exogenously supplied leucine and valine appeared in the protein fraction of *Chromatium* sp. However, Hellebust (1970) and Hoare and Moore (1965) using amino acids and acetate respectively, found only a small proportion of the radioactivity in the protein fraction of the organisms studied.

The distribution of $^{14}$C into the sugar component of the ethanol-soluble fraction resulting from the incorporation of amino acids was very low (Table 4). Conversion of exogenously supplied amino acids into carbohydrates has been observed in diatoms (Hellebust, 1970) and the characean alga *Nitella flexilis* (Wallen, 1973). For gluconeogenesis to take place these amino acids may have entered and followed the reverse of the glycolytic pathway to produce triose phosphate and hence carbohydrates. Stanier and his co-workers (Smith et al., 1967) have reported that some blue-green algae lack an operative tricarboxylic acid cycle, and phosphoenolpyruvate synthetase.

Under these conditions, the photosynthetic generation of a cellular environment with high NADH$_2$/NAD for reversal of the pathway, as suggested for *Nitella*, could not be used.

The incorporation pattern of $^{14}$C into the amino acids provide further support for the observation of Smith et al., (1967) and Ihlenfeldt and Gibson (1977) that the TCA cycle is inoperative. If this is correct compounds derived from assimilation of $^{14}$C-glutamate should be restricted to the amino acids of the glutamate family. The data in Table 5 support this inference.
The distribution of radioactivity from the other amino acids was similarly restricted to related amino acids to a large extent or minimal in the case of alanine. When \( ^{14}\text{C-aspartate} \) was supplied as a substrate to \textit{G. alpicola} radioactivity was recovered from the aspartate, glutamate and serine families of amino acids. Similar results have been observed for \textit{Anacystis nidulans} (Geunter, 1974). The serine family of amino acids are synthesized from 3-phospho-glycerate, thus implying, for both organisms, that the enzyme phosphoenolpyruvate carboxykinase is active. A similar observation has been made by Smith et al (1967). The recovery of a high proportion of \( ^{14}\text{C-label} \) in glutamine from cells incubated in \( ^{14}\text{C-glycine} \) or \( ^{14}\text{C-arginine} \) supports the observation of Smith et al (1967) that the block in the TCA cycle is due to the absence of \( \alpha \)-ketoglutarate dehydrogenase. Hence glutamate cannot enter the TCA cycle and some is converted to glutamine.

The appearance of \( ^{14}\text{C} \) in the organic acid fraction of the two algae (Table 4) provides evidence that the exogenously supplied amino acids are being deaminated or transaminated and may thus be a potential nitrogen source for the cell. Wagner et al (1973) reported that \textit{Chromatium} sp. used glutamate, glutamine and aspartate as nitrogen sources. Hood (1971) found a 20 % increase in the enzymes arginase and arginine deaminase after inclusion of arginine in the growth medium of the blue-green alga \textit{Anacystis variabilis}. Deamination and/or transamination of glycine and alanine by \textit{S. cedrorum} did not occur to the same
extent as in \textit{G. alpicola}. In general, amino acids were more extensively utilized by \textit{G. alpicola} than by \textit{S. cedrorum}.

Amino acid uptake in cells incubated either in the dark or light in the presence of DCMU was much reduced relative to the light uptake (Figs. 5-7). Arginine influx by \textit{S. cedrorum} proved to be an exception to this (Fig. 8). This could indicate a low energy requiring transport system. Pelroy and Bassham (1973 b) proposed that the turnover rates of metabolites in dark incubated cells and in DCMU treated, light incubated cells were reduced to 80% of that in photosynthetically grown cells. Other workers have found large reductions in the uptake of organic compounds by blue-green algae when placed in the dark (Ihlenfeldt and Gibson, 1977; Hoare and Moore, 1965; Smith et al, 1967). Glucose and acetate uptake in the presence of $10^{-5}$M DCMU has been demonstrated to be less compared to light uptake (Ihlenfeldt and Gibson, 1977; Hoare et al, 1967). CCCP (carbonyl cyanide m-chlorophenyl hydrazone; Calbiochem, San Diego, California) an electron uncoupler, had a similar effect to DCMU in reducing uptake of acetate (Ihlenfeldt and Gibson, 1977). A preliminary experiment verified a similar reduction in uptake of amino acids by \textit{G. alpicola} in the presence of CCCP in the light.

$^{14}\text{C-CO}_2$ production from $^{14}\text{C}$-labelled amino acids was monitored in the light and dark (Table 8). Energy production from amino acids in the light would require a mechanism other than the TCA cycle or the oxidative hexose monophosphate pathway as both are shut off (Pelroy et al, 1972). However,
in the dark the oxidative hexose monophosphate pathway is operational. Smith et al. (1967) stated that, "the only conceivable way in which the obligate autotroph could generate ATP during the metabolism of organic compounds would be by substrate-level phosphorylation accompanying glycolysis". 

$^{14}\text{C-CO}_2$ production observed in these experiments may be due to decarboxylation of organic molecules rather than respiration. 

$^{14}\text{C-CO}_2$ production rates were greater than 80% of $^{14}\text{C}$-arginine assimilated from the medium by G. alpicola in the light. This decreased to 21% of the total arginine assimilated in the dark. The decrease in $^{14}\text{C-CO}_2$ production in the dark, may indicate some involvement or influence of the light reaction in this phenomenon. Linko et al. (1957) denoted that the urea cycle may in some way be involved in photosynthesis. However, recent studies have not indicated any involvement of the urea cycle intermediates in photosynthetic metabolism of G. alpicola (Pelroy and Bassham, 1972). Table 5 does indicate that the urea cycle intermediates are present during photoautotrophic growth of G. alpicola in the presence of $^{14}\text{C}$-arginine, but this does not infer any involvement in photosynthesis. The only other study of CO$_2$ release from blue-green algae is that of Oscillatoria jasorvensis by Stephens et al. (1967), who observed that 15% of recently assimilated glycine was released as CO$_2$. This value is slightly higher than the glycine values reported in this study (Table 8). With the exception of arginine dark release of $^{14}\text{C-CO}_2$ was generally similar to that of light. This does not correspond to the expected 80%
reduction in metabolism in the dark suggested by Pelroy and Bassham (1973 b). Carbohydrates are also known to be converted to $\text{CO}_2$ in the light. Pelroy et al (1972) observed that in *Aphanocapsa* 6714, a facultative heterotroph, 37% of the incorporated glucose was released as $\text{CO}_2$ in the light. *Aphanocapsa* 6308 however exhibited negligible $\text{CO}_2$ production because of its inability to take up exogenous glucose.

Respiration rates in the dark measured by oxygen electrodes were not enhanced by the presence of amino acid, with one exception (Table 9). Therefore, it would appear that $^{14}$C-$\text{CO}_2$ production in the dark does not contribute to ATP generation. This supports the suggestion that $^{14}$C-$\text{CO}_2$ release is due to decarboxylation of the amino acid. The possibility of energy production from substrate-level phosphorylation does exist but it is too small to be of significance to the cells catabolic metabolism.

*G. alpicola* and *S. cedrorum* possess high affinity transport systems for a number of amino acids. Photoassimilation does occur under the conditions of these experiments. However, the metabolism of amino acids appears to be of limited use as the amount going into energy production processes are extremely small. Respiration rates in the dark, are not affected by the presence of amino acids at concentrations of $2.5 \times 10^{-6}$M. These two blue-green algae would be capable of competition with other aquatic species for available amino acids. Uptake of amino acids by naturally occurring phytoplankton communities is discussed in Chapter II.
CHAPTER II

UPTAKE OF ORGANIC COMPOUNDS BY NATURAL PHYTOPLANKTON
COMMUNITIES IN FOUR ARCTIC PONDS
INTRODUCTION

In recent years there have been a number of studies on the phytoplankton communities of arctic ponds (Kalff, 1967; Alexander et al., 1972; Stross et al., 1972; Sheath et al., 1975). The majority of these investigations have been studied in alaskan waters. The limnology of arctic lakes, in Canada, has been extensively studied, Char and Mendotta lakes. (Morgan and Kalff, 1972; Welch, 1974; Schindler et al., 1974 a and b).

The arctic ponds are a different habitat from those found in glacially formed lakes. The depths are shallow, hence they tend to heat up more than lakes. In winter they freeze solid (Kalff, 1967; Hobbie, 1973). As a result there is no inoculum of active cells at ice-break as in deeper lakes. In ponds the spring population is composed of species able to resume active growth after being frozen or able to germinate rapidly from inactive spores (Sheath et al., 1975).

There remains a good deal to learn of production processes in Canadian arctic ponds. In August, 1977, I initiated a preliminary study of production processes in four arctic ponds, two on Baffin and two on Devon Islands, as part of the 5th Acadia Arctic Biology Expedition from August 1 to September 12, 1977. Photosynthetic production and heterotrophic utilization of glucose and amino acids were measured.
MATERIALS AND METHODS

The $^{14}$C method (Steemann and Neilsen, 1952; Strickland and Parsons, 1968) was used in the primary production measurement; the results therefore represent approximate net production. The water samples were collected just beneath the surface of the pond and filtered through a 164 μm mesh net to remove large zooplankton and detrital particles. Samples were poured into 150 ml pyrex bottles and incubated with 4.2 μCi of $^{14}$C as carrier free Na$_2$CO$_3$. Two light and one dark bottle were incubated at each station. The bottles were exposed, suspended from a tripod, at the sample depth (Kalff, 1967).

Incubation times on Baffin Island, ponds 1 and 2, were 6 h. Samples from Devon Island, ponds 3 and 4, were incubated for 12 h. In one series of experiments primary production of pond 4 was measured at 2 h intervals throughout the day. After incubation the samples were immediately filtered with a 47 μm Sartorius membrane filter (0.8 μm pores) using a vacuum equal to 25 cm of mercury. The vacuum was generated by a hand-held portable vacuum pump. Membranes were placed in scintillation vials containing 10 ml of the scintillator (Wallen and Geen, 1968). Vials were counted in a Beckman Scintillation Counter, model 3150P. Efficiency of counting was determined using the external standard technique. Counting times were preset to give a standard error of less than 1 %. Primary production was calculated as mg C$_{\text{ass}}$ m$^{-3}$ h$^{-1}$ and mg C$_{\text{ass}}$ ly$^{-1}$ h$^{-1}$. Total alkalinity was determined potentiometrically by titrating
against 0.02 N H₂SO₄ (Golterman and Clymo, 1969). Inorganic carbon was measured as mg l⁻¹ using the tabulation of Saunders et al (1962).

In all four ponds light and dark uptake of organic compounds by the bacteria and algae of the plankton was measured using the technique of Parsons and Strickland (1962). Uniformly labelled ¹⁴C-glucose, aspartate and glycine were tested in ponds 1 and 2. Glycine was not tested in ponds 3 and 4. The labelled compounds were made up under sterile conditions in 2 ml glass ampoules with activities of 1 or 2 µCi. Water samples were collected and filtered as indicated above. Duplicate light and dark bottles were incubated with the substrate being tested. The ¹⁴C-substrate was transferred from the ampoule, using 1 ml sterile disposable pipets, into the bottom of the sample bottles. After mixing bottles were placed horizontally in the pond at a depth of 20-30 cm. Incubation times were 6 h in ponds 1 and 2, 12 h in ponds 3 and 4. The water samples were then filtered and prepared for radioassay.

Incipient irradiation at the water surface was recorded using a Kahlsico Bimetallic Actinograph (kahl scientific instrument corporation). The pH of each pond was measured using a Hach portable water analysis instrument and the water temperature recorded with a thermometer.
RESULTS

Experiments were conducted on two ponds on Southern Baffin Island and two ponds on South West Devon Island (Fig. 15). A synopsis of these habitats is given below.

Southern Baffin

Pond 1: This is a small oval shaped pond, about 30 x 20 m, at an elevation of 10 m, at Pritzler Harbour (62 07'N, 67 16'W). The pond, situated in a small sedge meadow, has a mud bottom, 0.5 m deep. The average depth of the pond is 30-40 cm. It is important to note that this pond is frequented by geese and a large number of goose fecal pellets were observed around the edge. A small snowbank fed into the pond. The major fauna of this pond is Daphnia middendorfiana.

Pond 2: This is a small rectangular pond, length 10 m, width 5 m, at an elevation of 20 m. Its mean depth is between 15 and 20 cm. The bottom consisted of rocks covered with a light algal mat composed of Oscillatoria limosa (Roth) C. A. Agardh. It is situated in a very rocky area and completely surrounded by large boulders. No run off was visible from the surrounding terrain. Zooplankton were absent from this pond.

South West Devon

Pond 3: This large circular shaped pond, 100 m in diameter, is situated in a sedge meadow, surrounded by raised beach about 500 m from Gasgoyne Inlet (74 40'N, 91 20'W). Like other ponds in the area it is shallow throughout with
Fig. 15. Map of Canada showing location of arctic study sites on Baffin Island (ponds 1 and 2) and Devon Island (ponds 3 and 4).
maximum depth of 0.75 m. The bottom is composed of a mixture of stones and mud. The depth of the latter varied, getting deeper towards the centre of the pond. The edge of the pond is surrounded by moss and sedge. Two fairy shrimp genera are present, Branchinecta and Artemiopsis.

Pond 4: This is a boomerang shaped pond, length 200 m, width 25 m. It is 20 m from the sea at one point. The mean depth is 0.5 m and the bottom consists of a mixture of rock and mud. The catchment area of this pond is mainly raised beach. The same fairy shrimp genera are present as in pond 3.

During the ten sampling days the water temperature ranged from 8-12°C at Baffin Island and 4-6°C at Devon Island. Light conditions were variable but more constant at Devon Island (Table 10). The pH varied from 7.8-8.8 at Baffin Island and 8.3-9.0 at Devon Island. Production was considerably higher in pond 1 than in the other three ponds and lowest in pond 2, 14.4 and 2.15 mg C\text{ass} m^{-3} h^{-1} respectively (Table 11). Production values in ponds on Devon Island were similar and slightly higher than pond 2. These values were 3.55 and 3.21 mg C\text{ass} m^{-3} h^{-1} respectively for ponds 3 and 4. Production as a function of irradiance showed similar trends (Table 11).

In a preliminary study of daily oscillations, production measurements were conducted in pond 4, at 2 h intervals from 10.00 to 22.00 h, August 26, (Fig. 16). A production peak occurred at 13.00 h. The data was also plotted as a function of irradiance to assess efficiency of production. Production efficiencies increased logarithmically from 10.00 to 22.00 h.
Table 10. Mean daily irradiance during the sample period.

<table>
<thead>
<tr>
<th></th>
<th>Southern Baffin Island</th>
<th>Southern Devon Island</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>langleys hour⁻¹</td>
<td>Date</td>
</tr>
<tr>
<td>August 7</td>
<td>44.8</td>
<td>August 23</td>
</tr>
<tr>
<td>8</td>
<td>21.6</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>32.7</td>
<td>25</td>
</tr>
<tr>
<td>10</td>
<td>12.2</td>
<td>26</td>
</tr>
<tr>
<td>11</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>47.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 11. Primary production in the four arctic ponds, August 1977.

<table>
<thead>
<tr>
<th></th>
<th>Pond 1</th>
<th>Pond 2</th>
<th>Pond 3</th>
<th>Pond 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. mg C(_{\text{ass}}) m(^{-3}) h(^{-1})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14.4</td>
<td>0.67</td>
<td>4.10</td>
<td>2.26</td>
<td></td>
</tr>
<tr>
<td>16.7</td>
<td>1.29</td>
<td>3.00</td>
<td>4.16</td>
<td></td>
</tr>
<tr>
<td>12.2</td>
<td>4.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>14.4</td>
<td>2.15</td>
<td>3.55</td>
<td>3.21</td>
</tr>
</tbody>
</table>

|                  |        |        |        |        |
| B. mg C\(_{\text{ass}}\) ly\(^{-1}\) h\(^{-1}\) |        |        |        |        |
| 0.322           | 0.021  | 0.190  | 0.089  |        |
| 0.773           | 0.106  | 0.153  | 0.214  |        |
| 0.258           | 0.100  |        |        |        |
| mean            | 0.452  | 0.076  | 0.172  | 0.151  |
Fig. 16. Production, ug C $1^{-1}$ h$^{-1}$ ($\bigcirc$) and ug C ly$^{-1}$ h$^{-1}$ ($\bullet$) in pond 4, Devon Island. Measured at 2 h intervals, from 10.00 til 22.00 h on 26th August, 1977.
Fig. 16
The uptake rates of the substrates tested are given in Table 12. On Baffin Island light uptake of glucose was significantly higher in pond 1 than pond 2, 30.3 and 10.8 ng C l\(^{-1}\) h\(^{-1}\) respectively. Dark uptake of glucose was lower than light uptake in both ponds but higher in pond 1 than pond 2, 14.0 and 8.1 ng C l\(^{-1}\) h\(^{-1}\). The light uptake rates of glucose in pond 3 and 4 were similar to each other and lower than rates in the Baffin Island ponds, 8.6 and 5.1 ng C l\(^{-1}\) h\(^{-1}\) respectively. In contrast to ponds 1 and 2 dark uptake in pond 3, 15.1 ng C l\(^{-1}\) h\(^{-1}\), was almost double the light uptake rate. However, in pond 4 the dark uptake showed the same trend as in ponds 1 and 2, being lower than uptake in the light, 4.7 ng C l\(^{-1}\) h\(^{-1}\).

The light uptake rates of aspartate in ponds 1 and 2 were 20.8 and 18.3 ng C l\(^{-1}\) h\(^{-1}\) and in ponds 3 and 4, 3.3 and 5.1 ng C l\(^{-1}\) h\(^{-1}\). The uptake rates of aspartate in the dark in ponds 3 and 4 was higher than light uptake, 5.6 and 5.4 ng C l\(^{-1}\) h\(^{-1}\) respectively. Dark uptake in pond 1 was lower than in pond 2, 10.9 and 14.1 ng C l\(^{-1}\) h\(^{-1}\).

Glycine uptake was only measured in the ponds on Baffin Island. Both light and dark uptake in pond 2, 19.0 and 13.3 ng C l\(^{-1}\) h\(^{-1}\), respectively, were higher than the corresponding uptake in pond 1, 17.8 and 7.3 ng C l\(^{-1}\) h\(^{-1}\).

The data obtained when uptake was measured at decreasing irradiance was quite variable but gave no indication that light uptake of the substrates tested was influenced by intensity. Uptake was not measured at irradiance below 50%
# Table 2. Velocity of $^{14}$C-glucose, $^{14}$C-aspartate and $^{14}$C-glycine uptake in arctic ponds

## A. Ponds 1 and 2, Baffin Island.

<table>
<thead>
<tr>
<th></th>
<th>$^{14}$C-glucose (ng C l$^{-1}$ h$^{-1}$)</th>
<th>$^{14}$C-aspartate (ng C l$^{-1}$ h$^{-1}$)</th>
<th>$^{14}$C-glycine (ng C l$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond 1 light</td>
<td>30.3</td>
<td>20.8</td>
<td>14.8</td>
</tr>
<tr>
<td>Pond 1 dark</td>
<td>14.0</td>
<td>10.9</td>
<td>7.3</td>
</tr>
<tr>
<td>Pond 2 light</td>
<td>10.8</td>
<td>18.3</td>
<td>22.7</td>
</tr>
<tr>
<td>Pond 2 dark</td>
<td>8.1</td>
<td>14.1</td>
<td>13.3</td>
</tr>
</tbody>
</table>

## B. Ponds 3 and 4, Devon Island.

<table>
<thead>
<tr>
<th></th>
<th>$^{14}$C-glucose (ng C l$^{-1}$ h$^{-1}$)</th>
<th>$^{14}$C-aspartate (ng C l$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond 3 light</td>
<td>8.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Pond 3 dark</td>
<td>15.1</td>
<td>5.6</td>
</tr>
<tr>
<td>Pond 4 light</td>
<td>5.1</td>
<td>5.1</td>
</tr>
<tr>
<td>Pond 4 dark</td>
<td>4.7</td>
<td>5.4</td>
</tr>
</tbody>
</table>
of the incident irradiance since the appropriate neutral
density filters were lost in transit.
DISCUSSION

Phytoplankton production rates were not corrected for a potential loss of organic carbon during filtration. This possible source of error was suggested by Arthur and Rigler (1967). Recent work by Nalewajko and Lean (1972) and McMahon (1973) questioned the validity of such a correction. Rudd and Hamilton (1973) demonstrated that cell breakage due to filtration is not likely responsible for the "filtration error".

The range of production values were similar to the range observed in alaskan ponds (Kalff, 1967; Alexander et al., 1972). Alexander and Coulon (1973) reported a considerable seasonal oscillation with a maximum after ice melt and again in August. Annual production in these alaskan ponds was very low, however exceeded that of Char Lake, the least productive of all freshwater bodies (Kalff and Welsh, 1974).

Low production in pond 2, relative to the other ponds studied may be due to nutrient limitation. This pond is located in rocky terrain with no obvious allochthonous nutrient source. Phytoplankton biomass was 36% of the biomass in pond 1. Pond 1 is surrounded by a large sedge meadow across which melt water drains from a snowbank. Further, the large number of goose droppings surrounding the pond undoubtedly
contribute a significant nutrient load (Manny et al., 1974). The terrain on Devon Island is relatively uniform with sparse vegetation around the ponds. The results from pond 3 are curious. The biomass was the lowest of the four ponds studied, yet mean primary production was similar to those of ponds 2 and 4. Production in pond 4 was not light limited (Fig. 16). Light limitation was likely absent in the other ponds.

Stross (1975) and Doty (1959) reported that daily oscillations in the photosynthetic capacity in the arctic have small amplitudes. Stross (1975) observed a peak in an Alaskan pond occurring in the late afternoon. In contrast I observed a peak at 1300, when the sun was at the zenith.

The efficiency of light utilisation was low, 0.262 μg C ly⁻¹ h⁻¹, at 1300 and showed a rapid linear increase up to the last measurement at 2100 h, 0.869 μg C ly⁻¹ h⁻¹, (Fig. 16) when irradiation was 22% that of the daily maximum. One may speculate that this reflects an adaptive response to maximize production, within the physiological limits of the phytoplankton, during the ice-free period while exposed to virtually continuous light of fluctuating irradiances.

Kalff (1967) demonstrated that many organisms in arctic ponds at Barrow, Alaska were facultative heterotrophs capable of using glucose. A number of workers have demonstrated that certain algae are capable of growing on organic substrates (Wheeler et al., 1977, 1974; Lui and Hellebust, 1974; Hellebust, 1971). Further it has been demonstrated that some alga grow better on organic substrates in the light than the
dark (Hellebust and Lewin, 1972; Lewin and Hellebust, 1976; Kalff, 1967; Wallen, 1974; Wright, 1974). In these studies the algae were generally given concentrations of dissolved organic carbon above those found in natural waters and thus did not demonstrate a photoheterotrophic capacity under natural conditions.

In this study the concentrations of organic carbon compounds in the water were not determined. Hence, calculations of velocity of uptake of organic compounds (µg C l⁻¹ h⁻¹) is an underestimate of actual values but it does give an indication of the capacities of the algae. The experiments demonstrated that photoheterotrophy took place and that, in general, more organic carbon fixation occurred in the light than in the dark. Exceptions to this observation were the uptake of aspartate in ponds 3 and 4 and glucose in pond 3, which were higher than light uptake.

The heterotrophic capacity of the ponds, indicated by turnover time of the substrate due to the uptake mechanism, ranged from 37 to 238 h for glucose (Table 13). This is within the range reported by Wright and Hobbie (1966) for Lake Erkén. The range of turnover times for aspartate and glycine were similar. The short turnover times suggest that these substrates are important components of a biologically active fraction of the dissolved organic matter in the ponds. There appears to be a widespread heterotrophic capability of the algal community in the dark, with photosynthesis enhanced by the availability of organic carbon in the light.
Table 13: Turnover times for glucose, aspartate and glycine by pond communities on Baffin and Devon Islands, August 1977.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Turnover Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pond 1</td>
</tr>
<tr>
<td>glucose</td>
<td></td>
</tr>
<tr>
<td>light</td>
<td>36.9</td>
</tr>
<tr>
<td>dark</td>
<td>60.0</td>
</tr>
<tr>
<td>aspartate</td>
<td></td>
</tr>
<tr>
<td>light</td>
<td>27.4</td>
</tr>
<tr>
<td>dark</td>
<td>52.2</td>
</tr>
<tr>
<td>glycine</td>
<td></td>
</tr>
<tr>
<td>light</td>
<td>92.6</td>
</tr>
<tr>
<td>dark</td>
<td>60.3</td>
</tr>
</tbody>
</table>
LITERATURE CITED


APPENDIX A

Taken from Allen (1968) as modified by Coleman.

Solution no. \[ \text{ml} \text{ l}^{-1} \]
1 200
2 20
3 1
4 1

Make up to volume with glass distilled water.

Solution no. 1

(a)
NaNO\textsubscript{3} 19.84 g
MgSO\textsubscript{4} \cdot 7H\textsubscript{2}O 3.00 g
NaCO\textsubscript{3} 0.80 g

Make up to 6 l with water.

(b)
CaCl\textsubscript{2} \cdot H\textsubscript{2}O 1.44 g

Make up to 1.5 l with water.

Add a and b and make up to 8 l.

Solution no. 2
K\textsubscript{2}HPO\textsubscript{4} 1.95 g l\textsuperscript{-1}

Solution no. 3

Gaffron's Minor elements.

* See next page.

Solution no. 4
FeCl\textsubscript{3} \cdot 6H\textsubscript{2}O 2.4 g l\textsuperscript{-1}
EDTA 1.86 g l\textsuperscript{-1}
Solution no. 3
Gaffron's Minor elements

<table>
<thead>
<tr>
<th>Compound</th>
<th>g l⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₃BO₃</td>
<td>3.100</td>
</tr>
<tr>
<td>MnSO₄·4H₂O</td>
<td>2.230</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>0.287</td>
</tr>
<tr>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>0.088</td>
</tr>
<tr>
<td>Co(NO₃)₂·4H₂O</td>
<td>0.146</td>
</tr>
<tr>
<td>Na₂WO₄·2H₂O</td>
<td>0.033</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>0.125</td>
</tr>
<tr>
<td>KBr</td>
<td>0.119</td>
</tr>
<tr>
<td>KI</td>
<td>0.083</td>
</tr>
<tr>
<td>Cd(NO₃)₂·4H₂O</td>
<td>0.154</td>
</tr>
<tr>
<td>NiSO₄[NH₄]₂SO₄·6H₂O</td>
<td>0.198</td>
</tr>
<tr>
<td>VoSO₄·2H₂O</td>
<td>0.020</td>
</tr>
<tr>
<td>Al₂(SO₄)₃K₂SO₄·24H₂O</td>
<td>0.474</td>
</tr>
<tr>
<td>Cr(NO₃)₂·7H₂O</td>
<td>0.037</td>
</tr>
</tbody>
</table>
Measurement of dissolved inorganic carbon.

1. Take 100 ml of incubation medium and adjust pH to 8.3 with dilute NaOH.

2. Titrate to 4.5 using a pH meter (stirring bar etc.) with 0.02 N HCl (standardized). Use a 5 ml buret. Record volume of acid used.

3. Boil sample for ca. 5 minutes to drive off CO₂—cool on ice back to room temperature (pH meter is temperature sensitive) and titrate back to pH 8.3 using 0.02 N NaOH (standardized, CO₂ free etc.). Record volume of alkali used.

\[ \text{Meq. HCl} - \text{Meq. NaOH} = \text{Meq. HCO}_3^- \text{ in sample} \]

\[ 1 \text{ Meq. HCO}_3^- = 12 \text{ mg C}. \]
VITA AUCTORIS

Born: September 30, 1951, Greenock, Scotland.

1974 - B. Sc. (Honours) Applied Microbiology, University of Strathclyde, Glasgow, Scotland.


1975 - 1977 - Teaching Assistant, Department of Biology, University of Windsor, Windsor, Ontario.

Major Field - Microbiology.