INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in "sectioning" the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.

University
Microfilms
International

300 N. ZEEB ROAD, ANN ARBOR, MI 48106
18 BEDFORD ROW, LONDON WC1R 4EJ, ENGLAND

8116599

HOLT, JACK RUSSELL, II

STUDIES IN THE DINOFLAGELLATE GENERA, PERIDINIUM AND PERIDINIOPSIS

The University of Oklahoma PH.D. 1981

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STUDIES IN THE DINOFLAGELLATE GENERA, *PERIDINIUM* AND *PERIDINIOPSIS*

A DISSERTATION

SUBMITTED TO THE GRADUATE COLLEGE

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

By

JACK R. HOLT

Norman, Oklahoma

1981

STUDIES IN THE DINOFLAGELLATE GENERA, *PERIDINIUM* AND *PERIDINIOPSIS*

By

Lois A. Lyster

John A. Fistedis

John J. Skvarla

Ronald L. Perkins

ACKNOWLEDGMENTS

I express my deepest appreciation to Dr. Lois Pfiester and other members of my advisory committee (Dr. John Skvarla, Dr. John Fletcher, and Dr. Don Perkins) for advice and comments on my dissertation and my research. A special debt of gratitude is due to Dr. Lois Pfiester and Dr. J. Russell Carefoot, without whose pioneering work in the field of freshwater dinoflagellate biology, my research could not have been accomplished. Others outside of my committee who freely offered advice and aid were Dr. Norman Boke, Mr. Dan Butler, Dr. Barbara Hayhome, Dr. Bruce Kimmel, and Dr. Karen Steidinger.

I also thank my typists, Jerry Green, Barbara Holt, Sidonie Holmes, and Lisa Weir, who gave freely of their time and effort in preparation of this manuscript. I extend my sincere thanks to my family and friends for their help and understanding in this difficult time of my life.

ABSTRACT

Five *Peridinium* (*P. willei* Huit.-Kaas, *P. volzii* Lemm., *P. cinctum* (O.F.M.) Ehrenberg, *P. limbatum* (Stokes) *Lemm.*, and *P. inconspicuum* Lemm.) and one *Peridiniopsis* (*P. polonicum* (Wolosz.) Bourrelly) species were tested for auxotrophy, photoheterotrophy, nitrogen source utilization, pH optima, and chromosome numbers. *Peridiniopsis polonicum* requires vitamin B_{12}, and *Peridinium limbatum* requires thiamin for growth. Unlike marine *Peridinium* species, *P. willei*, *P. volzii*, *P. cinctum*, and *P. inconspicuum* do not display auxotrophy. *Peridinium volzii* is strongly inhibited by concentrations of biotin above 1 μgL^{-1}.

Photoheterotrophy occurs in *Peridinium willei*, *P. limbatum*, *P. inconspicuum*, and *Peridiniopsis polonicum*. All species tested exhibited photoheterotrophy except *P. volzii*. Growth of all species was strongly depressed by addition of lactate and propionate; while additions of glucose, glycerol, malonate and sucrose generally enhanced growth. Those species with apical pores (*Peridinium limbatum*, *P. inconspicuum*, and *Peridiniopsis polonicum*) generally demonstrated more pronounced growth enhancement on organic substrates than did species without apical pores. No species demonstrated dark growth on any organic substrate.

Growth of *Peridinium willei*, *P. volzii*, *P. cinctum*, *P. limbatum*, and *Peridiniopsis polonicum* is pH specific while *Peridinium*

inconspicuum shows no pH preference in the range of 5.5-8.5. Growth remained constant for most species tested on ammonium and nitrate from concentrations of 2.94×10^{-4} to 2.94 mM. Urea and nitrite were universally toxic at 2.94 mM but generally provided growth at lower concentrations. Indeed, urea often provided highest growth rates and was utilized by all six species. *Peridiniopsis polonicum* could not utilize ammonium or nitrite for growth.

A technique to stain, squash, and enumerate chromosomes of armored dinoflagellates is presented using a cellulase incubation and propionocarmine stain. Chromosome numbers for six freshwater armored dinoflagellates (*Peridinium cinctum, P. inconspicuum, P. limbatum, P. volzii, P. willei,* and *Peridiniopsis polonicum)* range from 41 *(P. inconspicuum)* to 210 *(P. cinctum)*. Evidence is presented to indicate dinoflagellate aneuploidy in culture.

TABLE OF CONTENTS

LIST OF TABLES

PAPER I

PAPER II

PAPER IV

Page

APPENDICES

LIST OF FIGURES

Page

STUDIES IN THE DINOFLAGELLATE GENERA, *PERIDINIUM* AND *PERIDINIOPSIS*

INTRODUCTION

Prager (1963) admonished workers to "amass information of dinoflagellate...physiology, nutrition and life histories." Though some attention has been brought to these areas of dinoflagellate biology, our knowledge is still scattered and superficial. Indeed, most of the specific classification is based on tabulation of plates forming the outer covering. Yet problems with our current understanding of dinoflagellates were lamented by Paulsen in 1949 who stated that peridinians are too variable to be classified by tabulation alone. Though problems such as polyploidy, aneuploidy and/or other mutations occur in culture (Loper et al., 1980), these problems cannot be elucidated without the isolation and culturing of dinoflagellate species. Thus, one does not have to rely on field correlations which may or may not reflect cause and effect, but one can subject cells to strict controlled experimentation. This argument may seem trivial; yet, prior to this report, physiological experimentation had been performed on only one of the 44 freshwater *Peridinium* species (Carefoot 1968, Pfiester 1974). Much more information on freshwater dinoflagellate biology is available since five more species have been brought into culture (four *Peridinium*, one *Peridiniopsis*).

Following is a description of all species used in this study (Fig. 1).

Peridinium willei Huit.-Kaas UTEX 2028. *Peridinium willei*, a very common plankter in Oklahoma, was first isolated in 1974 from shallow, weedy bodies of water. Cells measure 40-60 µm long and 45-70 µm wide.

Peridinium willei exhibits maximum growth at pH 7.5. Highest autotrophic growth rate (NO_3^-) is 0.33 generations per day while highest heterotrophic growth (rhamnose) is 0.38 generations per day. This species requires no vitamins. Glucose, glycerol, malonate, pyruvate, rhamnose and sucrose enhance growth while citrate, lactate, malate, mannose, propionate, rhamnose, and succinate inhibit growth. Nitrate, nitrite, ammonium and urea all serve as nitrogen sources. This species has 115±8.1 chromosomes.

Peridinium volzii Lemm. UTEX 2176. *Peridinium volzii*, uncommon in Oklahoma, was isolated from rock pools in the Wichita Mountains Wildlife Refuge in 1976. Cells measure about 38-52 µm long and wide. This species is heterothallic (Pfiester and Skvarla 1979).

Peridinium volzii exhibits maximum growth at pH 6.5. Highest autotrophic growth rate (NO_3^-) is 0.18 generations per day while maximum heterotrophic growth (sucrose) is only 0.14 generations per day. This species is inhibited by biotin concentrations above $1 \mu g L^{-1}$. Only sucrose of the substrates tested enhance growth while acetate, alpha-ketoglutarate, citrate, fructose, galactose, lactate, malate, maltose, mannose, propionate, pyruvate, rhamnose, and succinate strongly inhibit growth. Nitrate, nitrite, ammonium and urea all serve as nitrogen

sources though growth on nitrite is significantly lower. This species has 98±4.4 chromosomes.

Peridinium cinctum (O.F.M.) Ehrenberg UTEX 1336. *Peridinium cinctum* was isolated from Nebraska by Carefoot (1968). Cells are 45-60 µm long and 35-55 µm wide.

Peridinium cinctum exhibits maximum growth in the range of pH 6.5-7.5. Highest autotrophic growth (NO_3^-) is 0.23 generations per day while maximum heterotrophic growth (malonate) is 0.28 generations per day. This species does not require vitamins for growth. Galactose, glucose, malate and malonate enhance growth while alpha-ketoglutarate, citrate, fructose, lactate, maltose, mannose, propionate, rhamnose, succinate and sucrose inhibited growth. Nitrate, nitrite, ammonium and urea all serve as nitrogen sources though growth on nitrite and ammonium is significantly depressed. This species has 210±10 chromosomes.

Peridinium limbatum (Stokes) Lemm. UTEX 2195. *Peridinium limbatum* was collected in bloom condition from a bog pond in Southeastern Oklahoma in 1977. Cells are about 83-84 µm long and 64-66 µm wide and have an apical pore.

Peridinium limbatum exhibits maximum growth at pH 5.5. Highest autotrophic growth rate (NO_3^-) is 0.21 generations per day while maximum heterotrophic growth (glycerol) is 0.32 generations per day. This species is auxotrophic requiring thiamine for growth. Acetate, fructose, glucose, glycerol, malonate, rhamnose and sucrose enhance growth while alpha-ketoglutarate, citrate, lactate, propionate and succinate inhibit growth. Nitrate, nitrite, ammonium, and urea all serve as nitrogen sources though growth on nitrite is significantly

lower. Only highest concentrations of ammonium support growth. This species has 70±3 chromosomes.

Peridinium inconspicuum Lemm. UTEX 2255. *Peridinium inconspicuum* was collected from an experimental sewage lagoon pond on the University of Oklahoma campus in 1979. Cells are 15-20 µm long and 12-25 µm wide and have an apical pore.

Peridinium inconspicuum exhibits maximum growth at the entire range of pH tested (5.5-8.5). Highest autotrophic growth rate (NO_3^-) is 0.31 generations per day while maximum heterotrophic growth (glycerol) is 0.41 generations per day. This species does not require vitamins for growth. Fructose, glucose, glycerol, malate, malonate, pyruvate and sucrose enhance growth while only lactate and propionate inhibit growth. Nitrate, nitrite, ammonium and urea all serve as nitrogen sources. It has 41±2 chromosomes.

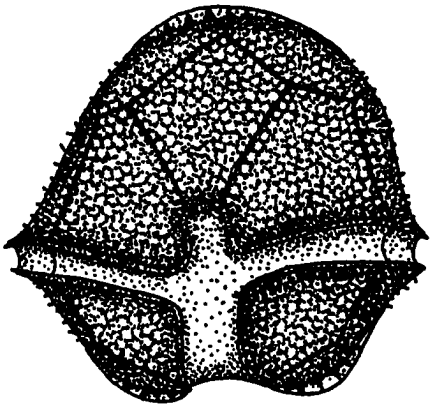
Peridiniopsis polonicum (Wolosz.) Bourrelly UTEX 2257.

Peridiniopsis polonicum was collected as a plankter from Lake Thunderbird Reservoir in Norman, Oklahoma in 1979. Cells are about 40 µm long and 35 µm wide and have an apical pore.

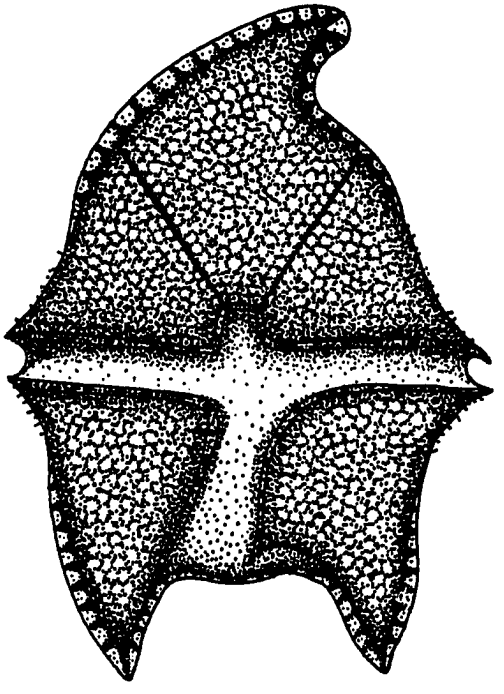
Peridiniopsis polonicum exhibits maximum growth at pH 7.5. Highest autotrophic rate (NO_3^-) is 0.21 generations per day while maximum heterotrophic growth rate (rhamnose) is 0.29 generations per day. This species is auxotrophic requiring vitamin B_{12} for growth. Fructose, galactose, glycerol, malonate, maltose, pyruvate, rhamnose and sucrose enhance growth while only lactate and propionate inhibit growth. Of the nitrogen sources, only nitrate and urea support growth while nitrite and ammonium support no growth. It has 56±3 chromosomes.

This study is presented in the form of four separate papers. Papers I, II and III were prepared according to the instructions for contributors to the *Journal of Phycology*. Paper IV was prepared according to the instructions for contributors to the *American Journal of Botany*.

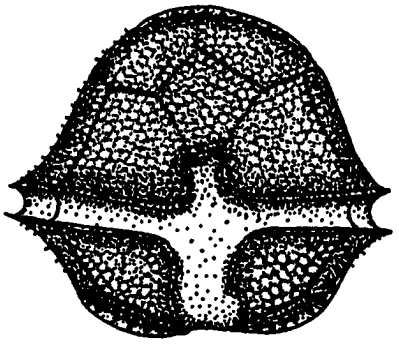
Figure 1. Vegetative cells of species studied (ventral view). Cells are drawn to scale.



a. Peridinium willei



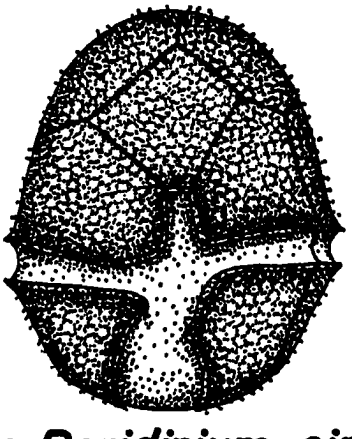
d. Peridinium limbatum



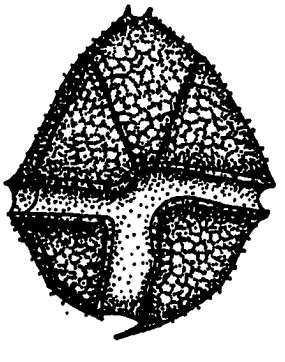
b. Peridinium volzii

e. Peridinium inconspicuum

20 µm



c. Peridinium cinctum



f. Peridiniopsis polonicum

Figure 1

Carefoot, J. R. 1968. Culture and heterotrophy of the freshwater dinoflagellate, *Peridinium cinctum* fa. *ovoplanum* Lindemann. *J. Phycol*. 4:129-31.

Loper, C. L., Steidinger, K. A. & Walker, L. M. 1980. A simple chromosome spread technique for unarmored dinoflagellates and implications of polyploidy in algal cultures. *Trans. Amer. Micros. Soc*. 99:343-6.

Paulsen, O. 1949. Observations on dinoflagellates. *Biol. Skrif*. 6:1-67.

Pfiester, L. A. Effects of nitrogen on asexual and sexual reproduction of *Peridinium cinctum* f. *ovoplanum* Lindemann. Ph.D. Dissertation. The Ohio State University. pp. 129.

Prager, J. C. 1963. Fusion of the family Glenodiniaceae into the Peridiniaceae, with notes on *Glenodinium foliacium* Stein. *J. Protozool*. 10(2):204-7.

PAPER I

A SURVEY OF AUXOTROPHY IN FIVE FRESHWATER DINOFLAGELLATES (PYRRHOPHYTA)

A SURVEY OF AUXOTROPHY IN FIVE FRESHWATER DINOFLAGELLATES (PYRRHOPHYTA)

ABSTRACT

Peridiniopsis polonicum (Wolosz.) Bourrelly requires vitamin B_{12}, and *Peridinium limbatum* (Stokes) Lemm. requires thiamin for growth. Unlike marine *Peridinium* species, *Peridinium willei* Huit.-Kaas, *P. volzii* Lemm., and *P. inconspicuum* Lemm. do not display auxotrophy. *Peridinium volzii* is strongly inhibited by concentrations of biotin above 1 µg L^{-1}.

INTRODUCTION

Auxotrophy in dinoflagellates has long been established (Provasoli and Carlucci 1974). Information on freshwater *Peridinium* auxotrophy, however, has been limited to *P. cinctum* (O.F.M.) Ehrenberg (Carefoot 1968) as other species have only recently been brought into culture. Although all marine and brackish water *Peridinium* thus far tested have a B_{12} requirement, (Provasoli and McLaughlin 1955, Iwasaki 1969, Droop 1958), *P. cinctum* has no vitamin requirement. The purpose of this survey is to test the freshwater dinoflagellates, *Peridiniopsis polonicum, Peridinium willei. P. volzii, P. inconspicuum* and *P. limbatum,* for biotin, thiamin and B_{12} requirements.

MATERIALS AND METHODS

Five axenic *Peridinium* species (*P. limbatum* [UTEX 2195], *P. inconspicuum* [UTEX 2255], *P. cinctum* [UTEX 1336], *P. volzii* [UTEX 2176], and *P. willei* [UTEX 2028]), and one *Peridiniopsis* species (*P. polonicum* UTEX 2257) were obtained by the method of Droop (1967) and were maintained in Modified Carefoot's Medium (Wynne and Berman 1980) with vitamins added. Log phase cells were washed in vitamin-free medium and innoculated into test solutions containing: no vitamins, thiamin, biotin, B_{12}, and all possible combinations of vitamins. Biotin and B_{12} were present in concentrations of 1 µg L^{-1} thiamine was present at 1 mg L^{-1}.

Culture vessels were 25 x 150 mm screw cap tubes containing 10 ml Modified Carefoot's Medium + vitamin treatments. Tubes were maintained in slants in a Percival model #PT80 growth chamber at 25° ± 1°C with 1000 ft-candles illumination on a 12-12h LD photoregime. Experiments were conducted for 25 days and all tests done in quadruplicate.

Cells were fixed with 0.5 ml isopropanol. Tubes were subsampled and counted using a Sedgewick-Rafter counting cell at 100X magnification with an ocular grid. All cells in three strips were counted yielding a correction factor of 3.865 (135.5 for *P. inconspicuum*).

RESULTS

Of the species tested, growth was affected in only *Peridinium limbatum, P. volzii* and *P. polonicum* by addition of vitamins (Table 1). *Peridinium limbatum* exhibited growth only in cultures containing thiamin while *P. polonicum* grew only when presented with B_{12}. There was no additional growth enhancement by the presence of vitamins other than those required by the respective organism.

Peridinium volzii, however, demonstrated a significantly depressed growth rate in the presence of biotin (Table 1). Indeed, a growth medium containing no vitamins gave the highest growth rate. In the concentration tested (1.0 µg L^{-1}), biotin did not completely inhibit growth.

Peridinium volzii was tested with varying concentrations of biotin (0, 0.1, 1.0, and 10.0 µg L^{-1}). When exposed to no biotin, *P. volzii* doubled every 3.73 days (Fig. 1). At 0.1 µg L^{-1} biotin, division rate was not significantly depressed. At 1.0 and 10 µg L^{-1} biotin, division rate was significantly depressed to 5.04 and 12.74 days, respectively.

DISCUSSION

Provasoli and Carlucci (1974) review of algal auxotrophy indicates 17 dinoflagellates require vitamin B_{12} alone, and 7 more require B_{12} in conjunction with thiamin and/or biotin. More recently, Bruno and McLaughlin (1977) demonstrated a vitamin B_{12} requirement by *Ceratium hirundinella*. Thus, it is not surprising that one of the 5 species tested, *Peridiniopsis polonicum*, exhibited a B_{12} requirement. Indeed, in light of the review by Provasoli and Carlucci (1974), it is surprising that none of the other species tested had a B_{12} requirement.

To my knowledge, *P. limbatum* is the only freshwater *Peridinium* species to demonstrate auxotrophy. The other auxotrophic *Peridinium* species, however, require B_{12} rather than thiamin. Indeed, *P. limbatum* is atypical among the dinoflagellates tested thus far in requiring thiamin alone. Of the 25 auxotrophic dinoflagellate species listed by Provasoli and Carlucci (1974), 6 require thiamin but in combination with B_{12} and/or biotin.

As in *P. cinctum* (Carefoot 1968), *P. inconspicuum* and *P. willei* show no significant difference in growth rate when grown with or without vitamins. Thus, these *Peridinium* species are unusual since virtually all other dinoflagellates tested thus far exhibited auxotrophy (Provasoli and Carlucci 1974, Bruno and McLaughlin 1977).

Peridinium volzii demonstrated a pronounced decline in growth rate when exposed to 1 µg L^{-1} or higher concentration of biotin. Such biotin sensitivity is not seen in other *Peridinium* species. Indeed, *P. cinctum* grows well on increased concentrations of biotin; perhaps, even to use it as a nitrogen source (Carefoot 1968).

This strain of *P. volzii* was isolated from ephemeral rock pools in the Wichita Mountains Wildlife Refuge of southwestern Oklahoma. Increased sensitivity to such a compound may serve as an environmental cue that the pool is drying out. As the pool dries, biotin may become more concentrated and stimulate cell encystment.

Certainly, more freshwater dinoflagellates should be surveyed before generalizations are made concerning their auxotrophy. Within the genus, *Peridinium,* marine and brackish species all exhibited a B_{12} requirement (Provasoli and McLaughlin 1955, Iwasaki 1969, and Droop 1958), while only *P. limbatum* of the 5 freshwater species tested had a vitamin requirement.

It may also be significant that those auxotrophic freshwater species which exhibited auxotrophy in this survey, both have apical pores. Only *P. inconspicuum* of the pore-bearing species surveyed did not exhibit a vitamin requirement. There is evidence that phagotrophy may be an important mode of nutrition in many members of the Dinophyceae (Spero and Moree 1979, Irish 1979). Perhaps, ingestion through the apical pore serves as a dietary supplement providing the necessary B_{12} or thiamin.

LITERATURE CITED

Bruno, S. F. & McLaughlin, J. J. A. 1977. The nutrition of the freshwater dinoflagellate *Ceratium hirundinella*. *J. Protozool.* 24: 548-53.

Carefoot, J. R. 1968. Culture and heterotrophy of the freshwater dinoflagellate, *Peridinium cinctum* fa. *ovoplanum* Lindeman. *J. Phycol.* 4: 129-31.

Droop, M. R. 1958. Requirement for thiamin among some marine and supralittoral protista. *J. Mar. Biol. Assoc.*, U. K. 37: 323-29.

_____. 1967. A procedure for routine purification of algal cultures with antibiotics. *Brit. Phycol. Bull.* 3: 295-7.

Irish, A. E. 1979. *Gymnodinium helviticum* Penard F. *achroum* Skuja a case of phagotrophy. *Br. Phycol. J.* 14: 11-5.

Iwasaki, H. 1969. Studies on the red tide dinoflagellates. III. On *Peridinium hangoei* Schiller appeared in Gokasho Bay, Shima Peninsula. *Bull. Plankton Soc. Jpn.* 16: 132-39.

Provasoli, L. & McLaughlin, J. J. A. 1955. Auxotrophy in some marine and brackish dinoflagellates. *J. Protozool.* 2(Suppl.): 10.

_____& Carlucci, A. F. 1974. Vitamins and growth regulators. *In* Stewart, W. D. P., [Ed.], *Algal Physiology and Biochemistry*, Blackwell Scientific Publications, Oxford, pp. 741-87.

Spero, H. & Moree, M. 1979. Phagotrophic feeding and its importance to the lifecycle of the holozoic dinoflagellate, *Gymnodinium fungiforme* Anissimova. *J. Phycol.* 15(Suppl.): 13.

Wynne, D. & Berman, T. 1980. Hot water extractable phosphorus--an indicator of nutritional status of *Peridinium cinctum* (Dinophyceae) from Lake Kinneret (Israel)? *J. Phycol.* 16: 40-6.

Table 1. Growth responses of tested dinoflagellates to thiamin (T), Biotin (Bn), and B_{12} (B) relative to growth in controls with no added vitamins.

Species	T	Bn	B	TBn	TB	BnB	TBnB
Peridinium willei	0	0	0	0	0	0	0
Peridinium volzii	0	-	0	-	0	-	-
Peridinium inconspicuum	0	0	0	0	0	0	0
Peridinium limbatum	+	0	0	+	+	0	+
Peridiniopsis polonicum	0	0	+	0	+	+	+

Figure 1. Growth response of *Peridinium volzii* to varying concentrations of biotin. Bars represent ± 2 standard errors.

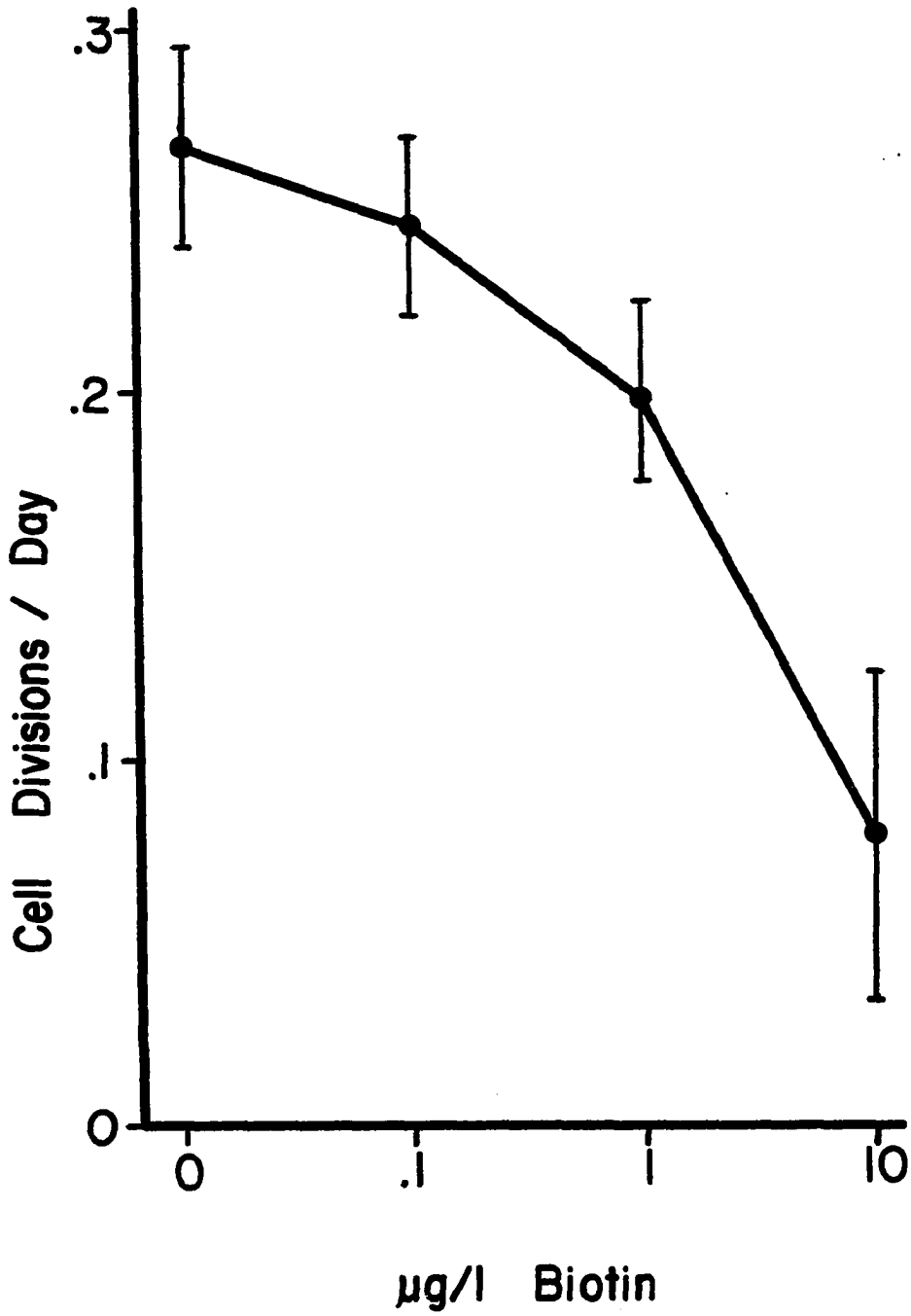


Figure 1

PAPER II

A SURVEY OF PHOTOHETEROTROPHY IN FIVE FRESHWATER DINOFLAGELLATES (PYRRHOPHYTA)

A SURVEY OF PHOTOHETEROTROPHY IN FIVE FRESHWATER DINOFLAGELLATES (PYRRHOPHYTA)

ABSTRACT

Photoheterotrophy occurs in *Peridinium willei* Huit.-Kaas, *Peridinium limbatum* (Stokes) Lemm., *Peridinium inconspicuum* Lemm., and *Peridiniopsis polonicum* (Wolosz.) Bourrelly. Only *Peridinium volzii* Lemm., of the species tested, did not exhibit photoheterotrophy. Growth of all species was strongly depressed by addition of lactate and propionate; while additions of glucose, glycerol, malonate and sucrose generally enhanced growth. Those species with apical pores (*Peridinium limbatum, Peridinium inconspicuum* and *Peridiniopsis polonicum)* generally demonstrated more pronounced growth enhancement on organic substrates than did species without apical pores. No species demonstrated dark growth on any organic substrate.

INTRODUCTION

Photoheterotrophy has been clearly demonstrated for a few dinoflagellates (Carefoot 1968, Loeblich 1966, Morrill and Loeblich 1978, Droop 1974). *Peridinium cinctum* Lindemann showed pH-dependent growth enhancement in seven different organic substrates (Carefoot 1968). With the culturing of four more freshwater *Peridinium (Peridinium willei, Peridinium volzii, Peridinium limbatum* and *Peridinium inconspicuum)* and one *Peridiniopsis (Peridiniopsis polonicum)* species, it is possible to make comparisons within freshwater Peridinales as to heterotrophy, photoheterotrophy or obligate phototrophy.

Peridinium limbatum, Peridinium inconspicuum and *Peridiniopsis polonicum* have an apical pore while the other *Peridinium* species tested do not. As suggested by the results of Spero and Moree (1979) and Irish (1980), the apical pore may be important in the nutrition of dinoflagellates in allowing the phagocytic uptake of particulate organics as well as whole cells. Perhaps these pore-bearing dinoflagellates are better able to take up and utilize dissolved organic compounds.

MATERIALS AND METHODS

Axenic cultures of five *Peridinium* species (*P. limbatum* [UTEX 2195], *P. inconspicuum* [UTEX 2255], *P. cinctum* [UTEX 1336], *P. volzii* [UTEX 2176], and *P. willei* [UTEX 2028]), and one *Peridiniopsis* species (*P. polonicum* [UTEX 2257]) were obtained by the method of Droop (1967) and Carefoot's Medium (Wynne and Berman 1980) with vitamins added. Log phase cells were innoculated into test solutions of seventeen different organic substrates.

Organic substrates tested were acetate, alpha-ketoglutarate, citrate, fructose, galactose, glucose, glycerol, lactate, malate, malonate, maltose, mannose, propionate, pyruvate, rhamnose, succinate and sucrose. Carbon-free solutions were also run simultaneously as controls against which to measure relative growth response to organic compounds provided. All organic substrate experiments run at four pH levels (5.5, 6.5, 7.5, and 8.5). Test solutions prepared after the method of Carefoot (1968). Organic substrates provided at concentrations of 2.5 mM.

Culture vessels were 25 x 150 mm screw cap tubes containing 10 ml solution. Tubes were maintained in slants in a Percival model #PT 80 growth chamber at 25°±1°C with 1000 ft-c illumination on a 12-12h photoregime. Experiments were conducted for 25 days and all tests done in quadruplicate.

Cells were fixed with 0.5 ml isopropanol. Tubes were subsampled and counted using a Sedgewick-Rafter counting cell at 100X magnification with an ocular grid. All cells in three strips were counted yielding a correction factor of 3.865 (135.5 for *P. inconspicuum*).

RESULTS

All six dinoflagellates studied were inhibited by lactate and propionate, low molecular weight organic acids. Malonate, another organic acid, enhanced growth in all species except *P. volzii* (Table 1). Indeed, only malonate, glycerol, glucose, and sucrose allowed growth in *P. volzii*.

Peridinium willei exhibited enhanced growth in glucose, glycerol, malonate, pyruvate, rhamnose and sucrose (Table 2). Growth inhibition was caused by addition of citrate, lactate, malate, mannose, propionate, rhamnose, and succinate. Rhamnose supported maximum heterotrophic growth with 0.38 generations per day above an autotrophic growth rate of 0.33 generations per day. Most pronounced growth enhancement occurred at pH 7.5.

Peridinium cinctum utilized galactose, glycerol, malate and malonate (Table 3). Growth was retarded by alpha-ketoglutarate, citrate, fructose, lactate, maltose, mannose, propionate, rhamnose, succinate, and sucrose. Greatest growth enhancement was seen with malonate which allowed a growth rate of 0.28 generations per day while strict autotrophic growth allowed only 0.23 generations per day. Maximum organic substrate utilization occurred at pH 6.5-7.5.

Peridinium limbatum exhibited enhanced growth in acetate, fructose, glucose, glycerol, malonate, rhamnose, and sucrose (Table 4). Growth was inhibited by alpha-ketoglutarate, citrate, lactate, propionate and succinate. Glycerol supported maximum growth at 0.32 generations per day over a maximum autotrophic growth rate of 0.21 generations per day. Maximum substrate utilization occurred at pH 5.5.

Peridinium inconspicuum utilized fructose, glucose, glycerol, malate, malonate, pyruvate and sucrose and was inhibited only by lactate and propionate (Table 5). Maximum growth enhancement was observed throughout the entire pH interval tested (pH 5.5-8.5). Glycerol supported maximum heterotrophic growth (0.41 generations per day) over a maximum autrotrophic growth rate of 0.31 generations per day.

Peridiniopsis polonicum demonstrated enhanced heterotrophic growth with fructose, galactose, glycerol, malate, malonate, pyruvate, rhamnose and sucrose (Table 6). Growth inhibition exhibited only by lactate and propionate. All enhanced growth occurred at pH 7.5. Maximum heterotrophic growth is seen with rhamnose (0.29 generations per day) over maximum autotrophic growth of 0.21 generations per day.

All species kept in total darkness exhibited no growth in all organic substrates.

DISCUSSION

Data confirm that dinoflagellate species tested are obligate phototrophs or photoheterotrophs. When these cells are kept in total darkness with an organic substrate, encystment must occur rapidly in that cell counts are comparable to initial innoculation concentration. Further, no motile cells are observed at the end of the 25 day incubation period. My experimental design does not, however, rule out secondary effects in which light is required in a developmental role.

Growth enhancement by various carbon sources appears to be strongly pH dependent as described by Carefoot (1968) for *P. cinctum*. This pH dependence merely seems to be a reflection of the species' pH optima (Holt and Pfiester in review a). One would expect growth and assimilation to be most efficient at pH conditions which are optimal for a species. *Peridiniopsis polonicum,* for example, grows well autotrophically only at pH 7.5 (Table 6); it also shows photoheterotrophic growth only at that pH.

Results of this research show that *Peridinium cinctum,* though in culture for over twelve years, responded very similarly to organic substrates as it did when first brought into culture (Carefoot 1968). That the effects of culture are minimal is essential if results are to have any meaning for the understanding of dinoflagellates in nature. Also, since *P. cinctum* has been in culture much longer than any other dinoflagellate tested, one may infer that changes in response to organic substrates has also been minimal, though chromosome studies indicate aneuploidy may have occurred (Holt and Pfiester in review b).

Peridinium volzii responded to most organic substrates by

decreased growth. Indeed, only glucose, glycerol, malonate and sucrose supported growth comparable to no carbon control growth rates. That these substrates allowed growth is interesting since they too supported increased growth in the other species tested (glucose supported increased growth in only *P. willei, P. limbatum* and *P. inconspicuum*). *Peridinium volzii* exhibits growth inhibition when exposed to biotin concentrations of 10 $\mu g\ L^{-1}$ (Holt and Pfiester in press). Perhaps the similar response of *P. volzii* to other organics is related to the same mechanism.

A comparison of growth responses between pore-bearing and non-pore-bearing dinoflagellates reveals possible increased ability to utilize organic substrates by species with an apical pore. Not only did the pore-bearing species utilize more substrates, but growth enhancement was clearly greater for the poroperidinium group.

A notable feature of these results is that acetate is only utilized by *P. limbatum*. Except *P. volzii*, which exhibited depressed growth with twelve other organic substrates, no other species responded to acetate. Though distinctions are often made between acetate and sugar algae (Droop 1974) acetate utilization (or oxytrophy) cannot be used to classify dinoflagellates. Indeed, all species, except *P. volzii*, utilized a mixture of organic acids and sugars.

LITERATURE CITED

Carefoot, J. R. 1968. Culture and heterotrophy of the freshwater dinoflagellate, *Peridinium cinctum* fa. *ovoplanum* Lindemann. *J. Phycol.* 4:129-31.

Droop, M. R. 1974. Heterotrophy of carbon. In: Stewart, W. D. P., ed. *Algal Physiology and Biochemistry,* Blackwell Scientific Publications, Oxford. pp. 530-59.

_____. 1967. A procedure for routine purification of algal cultures with antibiotics. Brit. Phycol. Bull. 3:295-7.

Holt, J. R. & Pfiester, L. A. in review a. A survey of nitrogen source utilization and pH optima in five freshwater dinoflagellates (Pyrrhophyta). *J. Phycol.*

Holt, J. R. & Pfiester, L. A. in review b. A technique for counting chromosomes of armored dinoflagellates, and chromosome numbers of six freshwater dinoflagellate species. *American Journal of Botany.*

_____. in press. A survey of auxotrophy in five freshwater dinoflagellates. *J. Phycol.*

Irish, A. E. 1979. *Gymnodinium helviticum* Penard f. *achroum* Skuja a case of phagotrophy. *Br. Phycol. J.* 14:11-5.

Loeblich, A. R. III. 1966. Aspects of the physiology and biochemistry of the Pyrrhophyta. *Phykos* 5:216-55.

Morrill, L. C. & Loeblich, A. R. III. 1978. Photoheterotrophy in the Pyrrhophyta. *J. Phycol.* 14(Suppl.):39.

Spero, H. & Moree, M. 1979. Phagotrophic feeding and its importance to the life-cycle of the holozoic dinoflagellate, *Gymnodinium*

fungiforme Anissimova. *J. Phycol.* 15(Suppl.):13.

Wynne, D. & Berman, T. 1980. Hot water extractable phosphorus - an indicator of nutritional status of *Peridinium cinctum* (Dinophyceae) from Lake Kinneret (Israel)? *J. Phycol.* 16:40-6.

Legend for Tables 1-6.

Growth response to 17 organic substrates at pH 5.5, 6.5, 7.5, and 8.5. Growth expressed relative to control mean generation times (cell divisions per day).

- \- growth inhibition at .05 level
- \-- growth inhibition at .01 level
- \--- growth inhibition at .001 level
- \+ growth enhancement at .05 level
- \++ growth enhancement at .01 level
- \+++ growth enhancement at .001 level
- 0 no significant difference between experimental and control growth rates.

Table 1. *Peridinium volzii* growth response to 17 organic substrates at pH 5.5, 6.5, 7.5, 8.5.

ORGANIC SUBSTRATE	pH			
	5.5	6.5	7.5	8.5
Acetate	---	---	---	---
a-Ketoglutarate	---	---	---	---
Citrate	---	---	---	---
Fructose	---	---	---	---
Galactose	---	---	---	---
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	---	---	---	---
Malate	--	-	---	---
Malonate	0	0	0	0
Maltose	---	---	---	---
Mannose	---	---	---	---
Propionate	---	---	---	---
Pyruvate	---	---	--	---
Rhamnose	--	--	---	---
Succinate	-	---	---	--
Sucrose	0	0	0	+
CONTROL (mean generation time)	.17	.18	.13	.11

Growth expressed relative to control mean generation times (cell divisions per day).

Table 2. *Peridinium willei* growth response to 17 organic substrates at pH 5.5, 6.5, 7.5, and 8.5.

ORGANIC SUBSTRATE	pH			
	5.5	6.5	7.5	8.5
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	---	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	+	++	0
Glycerol	0	+	+++	0
Lactate	0	---	---	---
Malate	--	0	0	0
Malonate	0	++	+++	0
Maltose	0	0	0	0
Mannose	---	---	---	---
Propionate	---	---	---	---
Pyruvate	0	++	+++	+
Rhamnose	-	++	+++	0
Succinate	0	---	---	---
Sucrose	++	0	0	0
CONTROL (mean generation time)	.13	.31	.33	.25

Growth expressed relative to control mean generation times (cell divisions per day).

Table 3. *Peridinium cinctum* growth response to 17 organic substrates at pH 5.5, 6.5, 7.5, and 8.5.

ORGANIC SUBSTRATE	pH			
	5.5	6.5	7.5	8.5
Acetate	0	0	0	0
a-Ketoglutarate	---	0	---	---
Citrate	0	---	---	---
Fructose	--	---	---	---
Galactose	0	+	0	0
Glucose	0	0	0	0
Glycerol	++	+++	++	0
Lactate	--	---	---	---
Malate	0	+	+	0
Malonate	0	0	+++	+
Maltose	0	---	---	---
Mannose	0	0	-	-
Propionate	---	---	---	---
Pyruvate	0	0	0	0
Rhamnose	0	0	---	---
Succinate	---	0	0	0
Sucrose	---	0	0	0
CONTROL (mean generation time)	.10	.22	.23	.14

Growth expressed relative to control mean generation times (cell divisions per day).

Table 4. *Peridinium limbatum* growth response to 17 organic substrates at pH 5.5, 6.5, 7.5, and 8.5.

ORGANIC SUBSTRATE	pH			
	5.5	6.5	7.5	8.5
Acetate	++	0	+	0
a-Ketoglutarate	--	--	---	---
Citrate	-	0	0	---
Fructose	++	++	0	0
Galactose	0	0	0	0
Glucose	+++	++	++	0
Glycerol	+++	++	++	+
Lactate	---	---	---	---
Malate	0	0	0	0
Malonate	++	+	++	+
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	---	---	---	---
Pyruvate	0	0	0	0
Rhamnose	+++	+++	++	0
Succinate	---	---	---	---
Sucrose	+++	+	0	0
CONTROL (mean generation time)	.20	.18	.15	.07

Growth expressed relative to control mean generation times (cell divisions per day).

Table 5. *Peridinium inconspicuum* growth response to 17 organic substrates at pH 5.5, 6.5, 7.5, and 8.5.

ORGANIC SUBSTRATE	pH			
	5.5	6.5	7.5	8.5
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	+++	+++	+++	++
Galactose	0	0	0	0
Glucose	++	+++	+++	+++
Glycerol	+++	+++	+++	+++
Lactate	---	---	---	---
Malate	+	+++	+++	++
Malonate	+++	+++	++	+++
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	---	---	---	---
Pyruvate	+++	+++	+++	++
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	++	+++	+++	++
CONTROL (mean generation time)	.31	.31	.30	.31

Growth expressed relative to control mean generation times (cell divisions per day).

Table 6. *Peridiniopsis polonicum* growth response to 17 organic substrates at pH 5.5, 6.5, 7.5, and 8.5.

ORGANIC SUBSTRATE	pH			
	5.5	6.5	7.5	8.5
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	+	0
Galactose	0	0	+	0
Glucose	0	0	0	0
Glycerol	0	0	++	0
Lactate	---	--	---	0
Malate	0	0	++	0
Malonate	0	0	++	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	---	---	---	0
Pyruvate	0	0	++	0
Rhamnose	0	0	++	0
Succinate	0	0	0	0
Sucrose	0	0	+	0
CONTROL (mean generation time)	.08	109	.21	.00

Growth expressed relative to control mean generation times (cell divisions per day).

PAPER III

A SURVEY OF NITROGEN SOURCE UTILIZATION AND pH OPTIMA IN FIVE FRESHWATER DINOFLAGELLATES (PYRRHOPHYTA)

A SURVEY OF NITROGEN SOURCE UTILIZATION AND pH OPTIMA IN FIVE FRESHWATER DINOFLAGELLATES (PYRRHOPHYTA)

ABSTRACT

Growth of *Peridinium willei* Huit.-Kaas, *P. volzii* Lemm., *P. cinctum* (O.F.M.) Ehrenberg, *P. Limbatum* (Stokes) Lemm., and *Peridiniopsis polonicum* (Wolosz.) Bourrelly is very pH specific while *Peridinium inconspicuum* Lemm. shows no pH preference in the range of 5.5-8.5. Growth remained constant for most species tested for ammonium and nitrate from concentrations of 2.94×10^{-4} to 2.94 mM. Urea and nitrite were universally toxic at 2.94 mM but generally provided growth at lower concentrations. Indeed, urea often provided highest growth rates and was utilized by all six species. *Peridiniopsis polonicum* could not utilize ammonium or nitrite for growth.

INTRODUCTION

Pfiester (1974) demonstrated the importance of nitrogen to the life cycle of *Peridinium cinctum*. She further showed that the utilization of various nitrogen substrates (nitrate, ammonium, and urea) was dependent upon concentration as well as pH. Defined growth media for *P. cinctum* require relatively high $NaNO_3$ concentrations (Carefoot 1968, Lindström and Rodhe 1978) as does a defined medium for *Ceratium hirundinella* (O.F.M.) Dujardin (Bruno and McLaughlin 1980).

Continued growth of *P. cinctum* in Lake Kinneret despite high intracellular C:P ratios (greater than 300:1) indicates fluctuations of phosphorus may not be important in governing dinoflagellate blooms (Serruya and Berman 1975). *Peridinium* responds quickly, however, to nitrogen-free environments (Pfiester 1975, 1976, 1977, Pfiester and Skarvla 1979) and are readily induced to undergo sexual reproduction.

The purpose of this research is to examine the growth rate of six armored dinoflagellate species (*P. willei, P. volzii, P. cinctum, P. limbatum, P. inconspicuum* and *Peridiniopsis polonicum*) with respect to pH (5.5, 6.5, 7.5 and 8.5), nitrogen source (nitrate, nitrite, ammonium and urea), and concentration (2.94×10^{-4}, 2.94×10^{-3}, 2.94×10^{-2}, 2.94×10^{-1}, and 2.94 mM).

MATERIALS AND METHODS

Axenic cultures of five *Peridinium* species (*P. limbatum* [UTEX 2195], *P. inconspicuum* [UTEX 2255], *P. cinctum* [UTEX 1336], *P. volzii* [UTEX 2167], and *P. willei* [UTEX 2028]) and one *Peridiniopsis* species (*P. polonicum* [UTEX 2257]) were obtained by the method of Droop (1967) and were maintained in Modified Carefoot's Medium (Wynne and Berman 1980) with vitamins (1 μg L^{-1} biotin and B_{12} and 1 mg L^{-1} thiamin). Log phase cells were washed in nitrogen-free medium and innoculated into test solutions.

Culture vessels were 25 x 150 mm screw cap tubes containing 10 ml Modified Carefoot's Medium with nitrogen and pH treatments. HCl and NaOH were used to adjust pH to 5.5, 6.5, 7.5, and 8.5 for both pH and nitrogen treatments. Nitrate, nitrite, ammonium, and urea were used at 2.94 mM (an equivalent molar concentration to nitrate in Modified Carefoot's Medium). All nitrogen sources were tested at 0, 2.94×10^{-4}, 2.94×10^{-3}, 2.94×10^{-2}, 2.94×10^{-1}, and 2.94 mM concentrations. Constant solute concentration was maintained by appropriate additions of NaCl.

Tubes were placed in slants in a Percival Model #PT 80 growth chamber at 25° ± 1°C with 1000 ft-c run for 25 days. All experiments were conducted in quadruplicate.

Cells were fixed with 0.5 ml isopropanol. Tubes were subsampled and counted using a Sedgewick-Rafter counting cell at 100X magnification with an ocular grid. All cells in three strips were counted yielding a correction factor of 3.87 (135.48 for *P. inconspicuum*).

RESULTS

Most species tested demonstrated optimal growth at a narrow range of pH (Figs. 1-6). *Peridinium willei, P. limbatum* and *Peridiniopsis polonicum* had the most narrow pH optimum ranges with maximum growth at pH 7.5, 5.5 and 7.5, respectively. *Peridinium volzii* and *P. cinctum* both had broader pH requirements with maximum growth at pH 5.5-6.5 and 6.5-7.5, respectively. Only *P. inconspicuum* of the species tested maintained optimal growth throughout the pH interval examined.

As concentrations of nitrogen sources ranged from 2.94×10^{-4} mM to 2.94 mM, growth rate generally remained constant for ammonium and nitrate growth cells (Figs. 7-12). *Peridinium willei, P. volzii* and *P. inconspicuum* had similar growth rates in both ammonium and nitrate media (Figs. 7, 8, 11).

At the highest concentration (2.94 mM), nitrite proved to be toxic to all species tested except *P. inconspicuum* where growth was depressed (Fig. 11). *Peridiniopsis polonicum* could not utilize nitrite at any concentration tested (Fig. 12). In all cases (except *P. polonicum*), growth rate rose significantly when the nitrite concentration dropped from 2.94 mM to 2.94×10^{-1} mM.

Similarly, growth response to urea was only measurable in *P. cinctum* and *P. inconspicuum* at the highest concentration. In every case growth rates increased sharply as cells were grown in more dilute urea media. The highest recorded nitrogen source growth rate was for *P. inconspicuum* at 2.94×10^{-1} mM urea.

DISCUSSION

In freshwater, nitrogen is commonly available to algae as either nitrate or ammonium ions. Though almost all algae, with few exceptions (Cain 1965), can use nitrate they utilize ammonium preferentially when both ammonium and nitrate are present (Morris 1974, Syrett 1962). Despite ammonium preference, growth rates are usually very close when using either ammonium or nitrate as the sole nitrogen substrate (Morris 1974). With the exception of *Peridiniopsis polonicum* and *Peridinium limbatum,* nitrate and ammonium growth responses were very similar (Figs. 7-12).

As Pfiester (1974) first demonstrated for *P. cinctum,* urea is a very good substrate for *Peridinium* and *Peridiniopsis* growth. Perhaps these dinoflagellates are utilizing urea both as a nitrogen and as a carbon source as Carefoot (1968) speculated *P. cinctum* utilized biotin. Though nitrite proved to be an adequate nitrogen source for all species except *Peridiniopsis polonicum,* depressed growth rates were seen in *Peridinium volzii, P. cinctum* and *P. limbatum* indicating possible toxicity at all concentrations tested.

Chromosome data indicate that dinoflagellates undergo polyploidy or aneuploidy in culture (Holt and Pfiester in review, Loper et al. 1980). Though *P. cinctum* had been in culture for 7-8 years between the time of my experiments and those of Pfiester (1974), nitrogen utilization results are quite similar in both cases. Indeed, pH growth results are identical to those of Carefoot (1968). After having been in culture for years, growth responses have not changed. Thus, *P. cinctum* serves as a control for all other dinoflagellate species tested.

Results of the pH growth experiments reflect characteristics of the environment from which the dinoflagellates were isolated. *Peridinium inconspicuum,* a generalist in terms of nitrogen utilization, was isolated from a sewage lagoon. Fluctuations in pH of a highly productive, eutrophic environment are to be expected and, therefore, organisms isolated from such an environment should show a broad pH growth response.

Similarly, *Peridiniopsis polonicum,* a euplankter isolated from Lake Thunderbird reservoir, Cleveland County, Oklahoma, has a very narrow optimal pH range. Perhaps this reflects rather constant pH conditions in a well buffered open water environment. *Peridinium limbatum,* collected from a bog pond in Southeastern Oklahoma, grew best at the most acid pH tested. Again, acid conditions are to be expected in a bog environment.

Growth response is constant through a 10,000 fold difference in nitrate concentration. Even down to 2.94×10^{-4} mM nitrate, growth rate appears to be independent of nitrogen concentration. These results appear to be at odds with those of Carefoot (1968) who claimed maximum *P. cinctum* growth occurs at 2.94 mM nitrate. Other dinoflagellate growth media also contain relatively high nitrate or ammonium concentrations (Lindström and Rodhe 1978, Loeblich 1975).

In a number of reports Pfiester (1974, 1975, 1977, Pfiester and Skvarla 1979) demonstrate that nitrogen deprivation induces sexual reproduction in *Peridinium.* The nitrogen threshold below which *Peridinium* is induced to undergo sexual reproduction must be below 2.94×10^{-4} mM. Indeed, nitrogen free controls showed no growth for all species tested.

Although cells were washed in nitrogen-free medium prior to each experiment, they may have stored excess nitrogen while growing in the standard Carefoot's Medium. If nitrogen is stored, extracellular nitrogen should have little effect on growth rate. This model, however, implies that nitrogen-free medium should support growth until intracellular nitrogen is used up, but induction of *Peridinium* sexuality prevents the elucidation of this question.

LITERATURE CITED

Bruno, S. F. & McLaughlin, J. J. A. 1977. The nutrition of the freshwater dinoflagellate *Ceratium hirundinella*. *J. Protozool.* 24:548-53.

Cain, J. 1965. Nitrogen utilization in 38 freshwater chlamydomonad algae. *Can. J. Bot.* 43:1367-78.

Carefoot, J. R. 1968. Culture and heterotrophy of the freshwater dinoflagellate, *Peridinium cinctum* fa. *ovoplanum* Lindemann. *J. Phycol.* 4:129-31.

Droop, M. R. 1967. A procedure for routine purification of algal cultures with antibiotics. *Brit. Phycol. Bull.* 3:295-7.

Holt, J. R. & Pfiester, L. A. in review. A technique for counting chromosomes of armored dinoflagellates, and chromosome numbers of six freshwater dinoflagellate species. *American Journal of Botany*.

Lindström, K. & Rodhe, W. 1978. Selenium as a micronutrient for the dinoflagellate *Peridinium cinctum* fa. *westii*. *Mitt. Internat. Verein. Limnol.* 21:168-73.

Loeblich, A. R. III. 1975. A seawater medium for dinoflagellates and the nutrition of *Cachonina niei*. *J. Phycol.* 11:80-6.

Loper, C. L., Steidinger, K. A. & Walker, L. M. 1980. A simple chromosome spread technique for unarmored dinoflagellates and implications of polyploidy in algal cultures. *Trans. Amer. Micros. Soc.* 99:343-6.

Morris, I. 1974. Nitrogen assimilation and protein synthesis. In: Stewart, W. D. P., ed. *Algal Physiology and Biochemistry,*

Blackwell Scientific Publications, Oxford, pp. 583-609.

Pfiester, L. A. 1974. Effects of nitrogen on asexual and sexual reproduction of *Peridinium cinctum* f. *ovoplanum* Lindemann. Ph.D. Dissertation. The Ohio State University. pp. 129.

_____. 1975. Sexual reproduction of *Peridinium cinctum* f. *ovoplanum* (Dinophyceae). *J. Phycol.* 11:259-65.

_____. 1977. Sexual reproduction of *Peridinium gatunense* (Dinophyceae). *J. Phycol.* 13:92-5.

_____& Skvarla, J. J. 1979. Heterothallism and thecal development in the sexual life history of *Peridinium volzii* (Dinophyceae). Phycologia 18:13-18.

Serruya, C. & Berman, T. 1975. Phosphorus, nitrogen and the growth of algae in Lake Kinneret. *J. Phycol.* 11:155-62.

Syrett, P. J. 1962. Nitrogen assimilation. In: Lewin, R. A., ed. *Physiology and Biochemistry of Algae.* Academic Press, New York & London. pp. 171-88.

Wynne, D. & Berman, T. 1980. Hot water extractable phosphorus--an indicator of nutritional status of *Peridinium cinctum* (Dinophyceae) from Lake Kinneret (Israel)? *J. Phycol.* 16:40-6.

LEGENDS FOR FIGURES

Figure 1. *Peridinium willei* growth rate versus pH. Bars represent ±2 standard errors.

Figure 2. *Peridinium volzii* growth rate versus pH. Bars represent ±2 standard errors.

Figure 3. *Peridinium cinctum* growth rate versus pH. Bars represent ±2 standard errors.

Figure 4. *Peridinium limbatum* growth rate versus pH. Bars represent ±2 standard errors.

Figure 5. *Peridinium inconspicuum* growth rate versus pH. Bars represent ±2 standard errors.

Figure 6. *Peridiniopsis polonicum* growth rate versus pH. Bars represent ±2 standard errors.

Figure 7. *Peridinium willei* growth rate versus nitrogen source concentration at pH 6.5. Bars represent ±2 standard errors.

Figure 8. *Peridinium volzii* growth rate versus nitrogen source concentration at pH 6.5. Bars represent ±2 standard errors.

Figure 9. *Peridinium cinctum* growth rate versus nitrogen source concentration at pH 6.5. Bars represent ±2 standard errors.

Figure 10. *Peridinium limbatum* growth rate versus nitrogen source concentration at pH 5.5. Bars represent ±2 standard errors.

Figure 11. *Peridinium inconspicuum* growth rate versus nitrogen source concentration at pH 6.5. Bars represent ±2 standard errors.

Figure 12. *Peridiniopsis polonicum* growth rate versus nitrogen source concentration at pH 7.5. Bars represent ±2 standard errors.

PERIDINIUM WILLEI

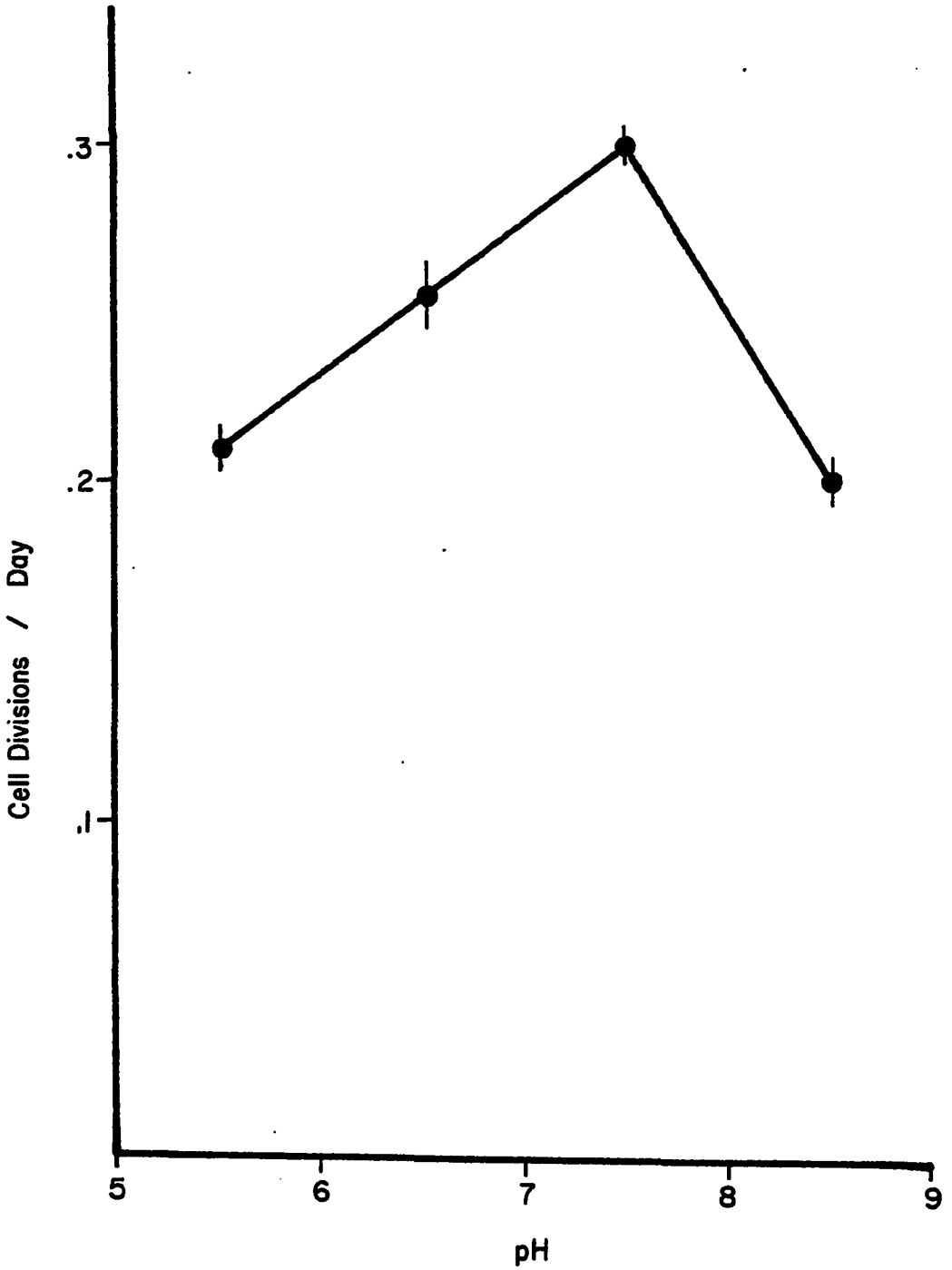


Figure 1

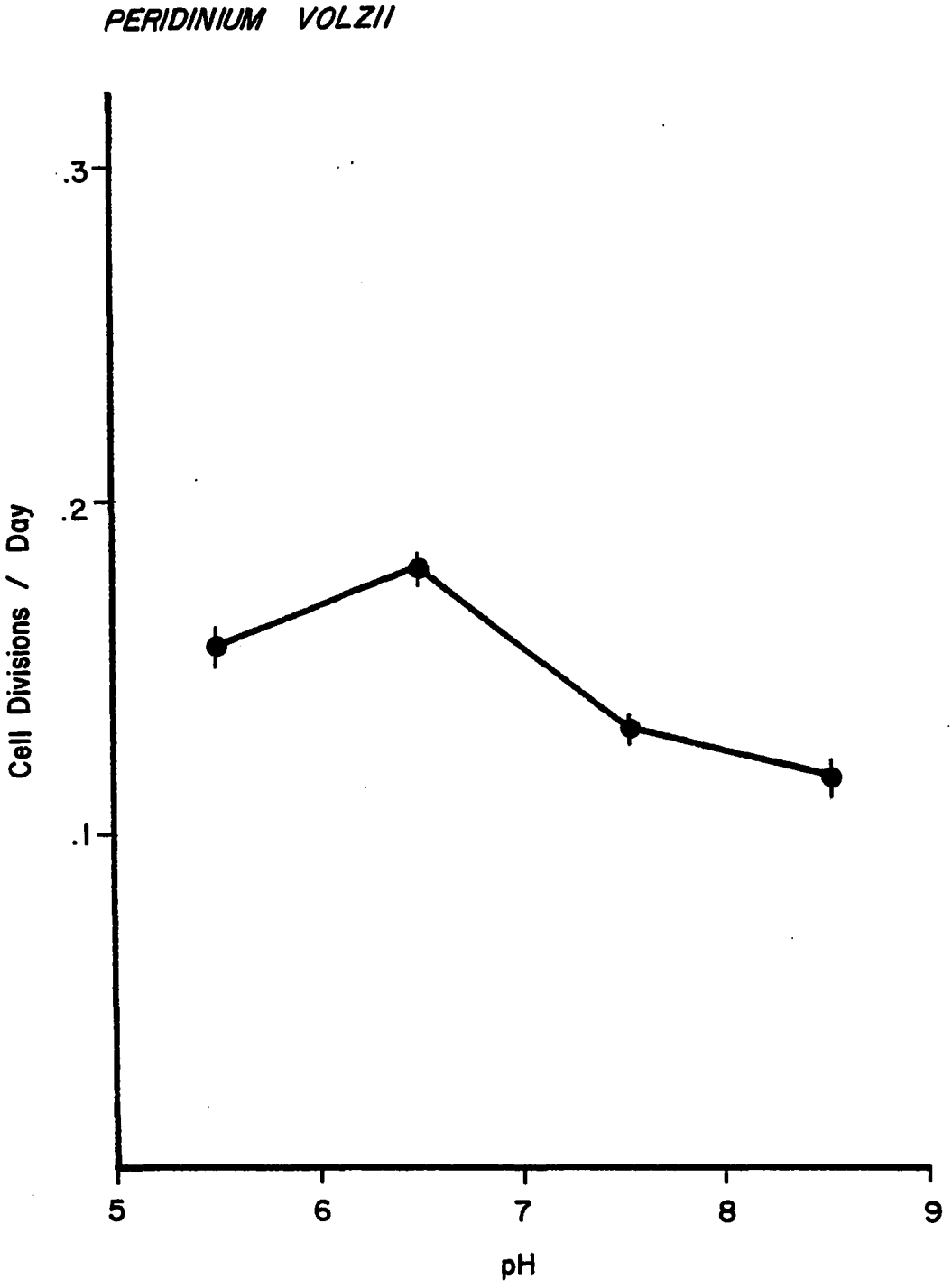


Figure 2

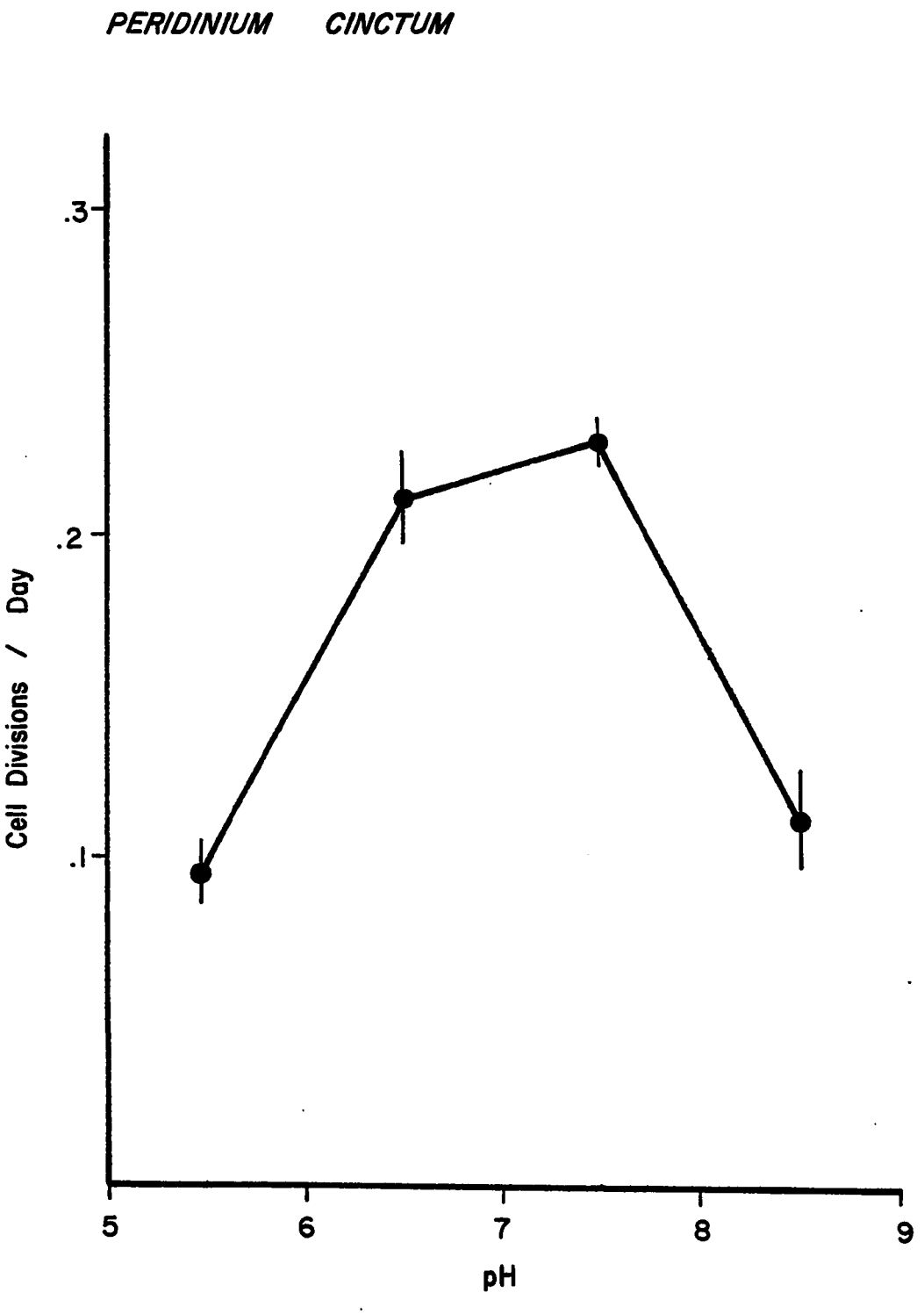


Figure 3

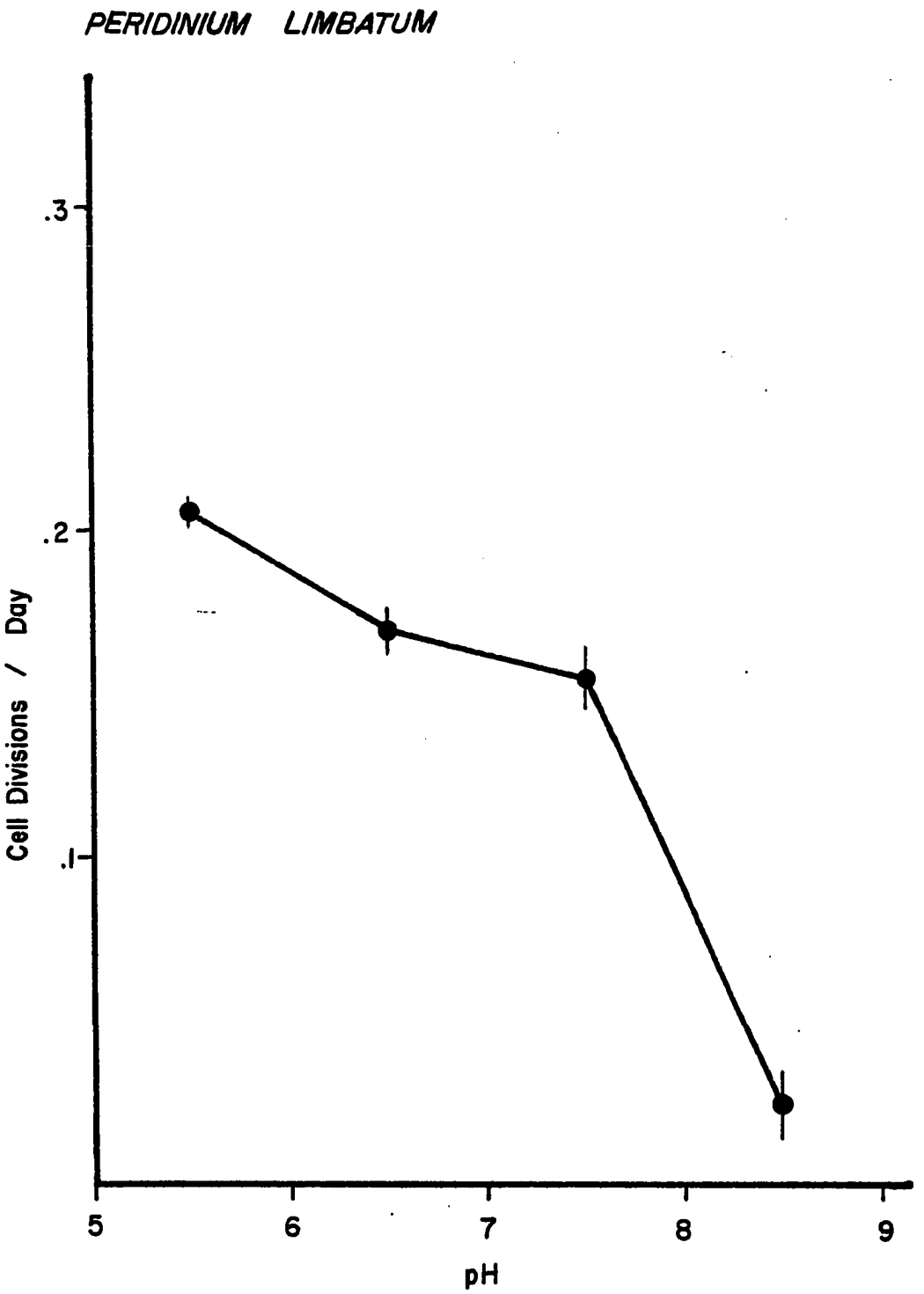


Figure 4

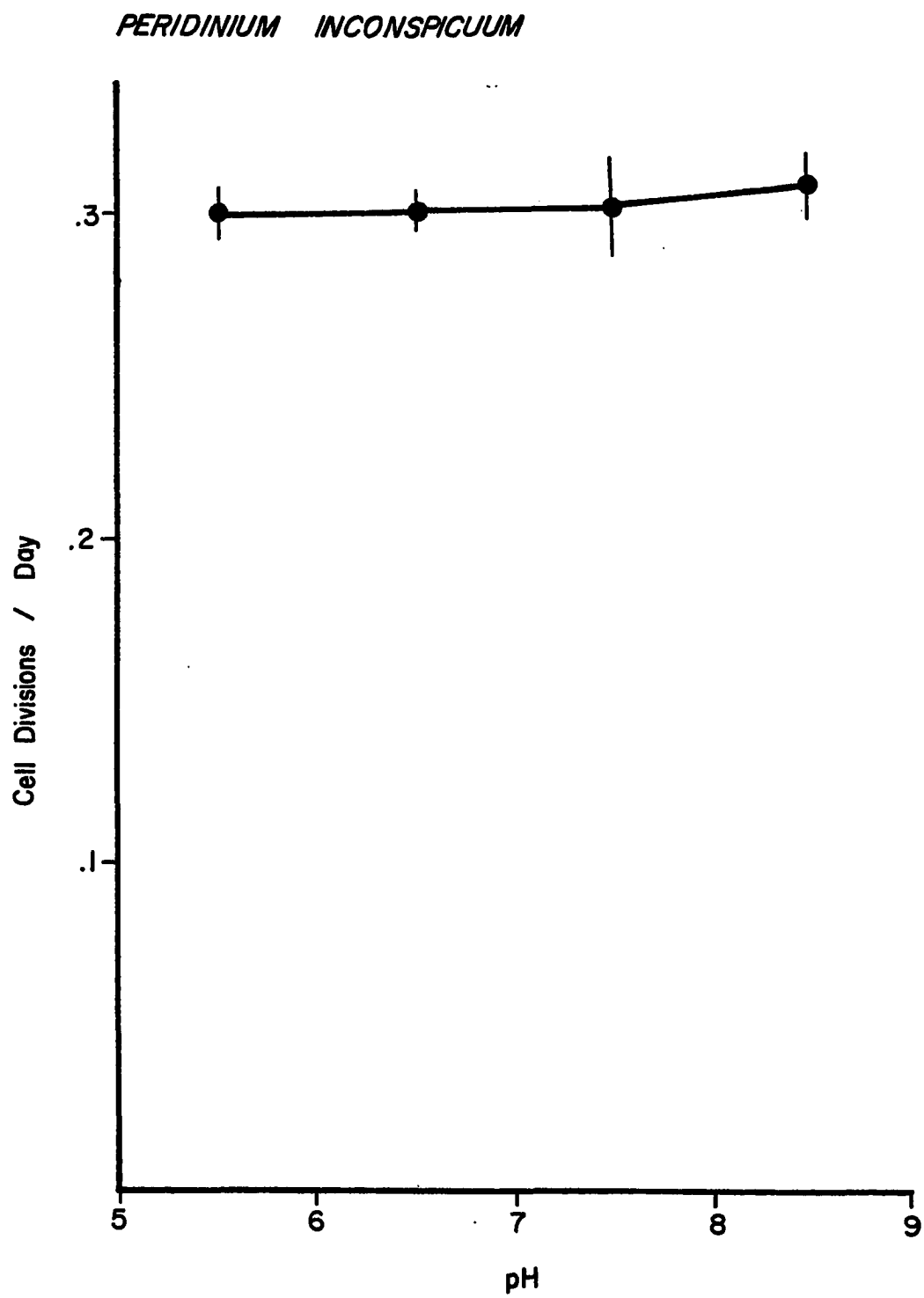


Figure 5

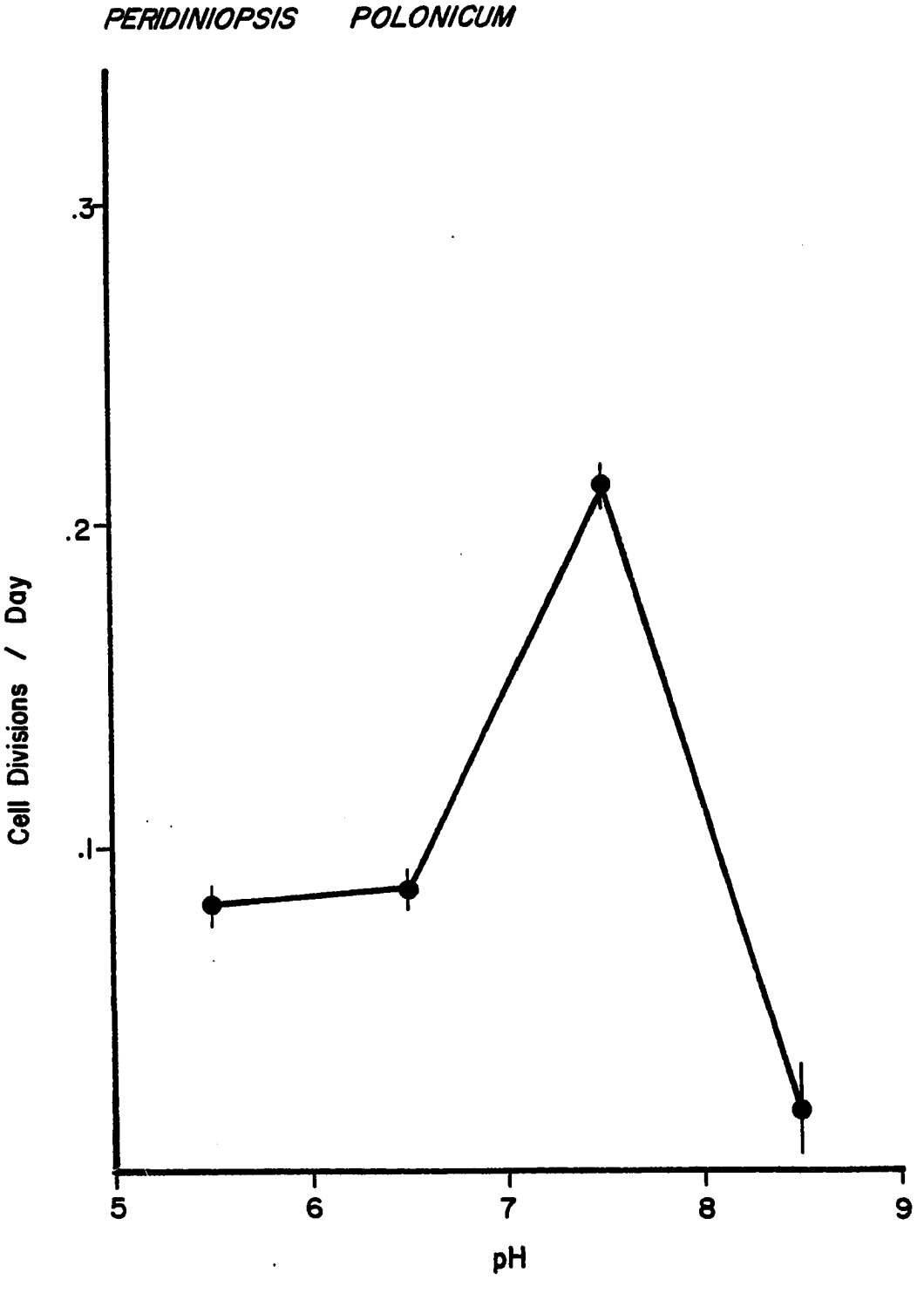


Figure 6

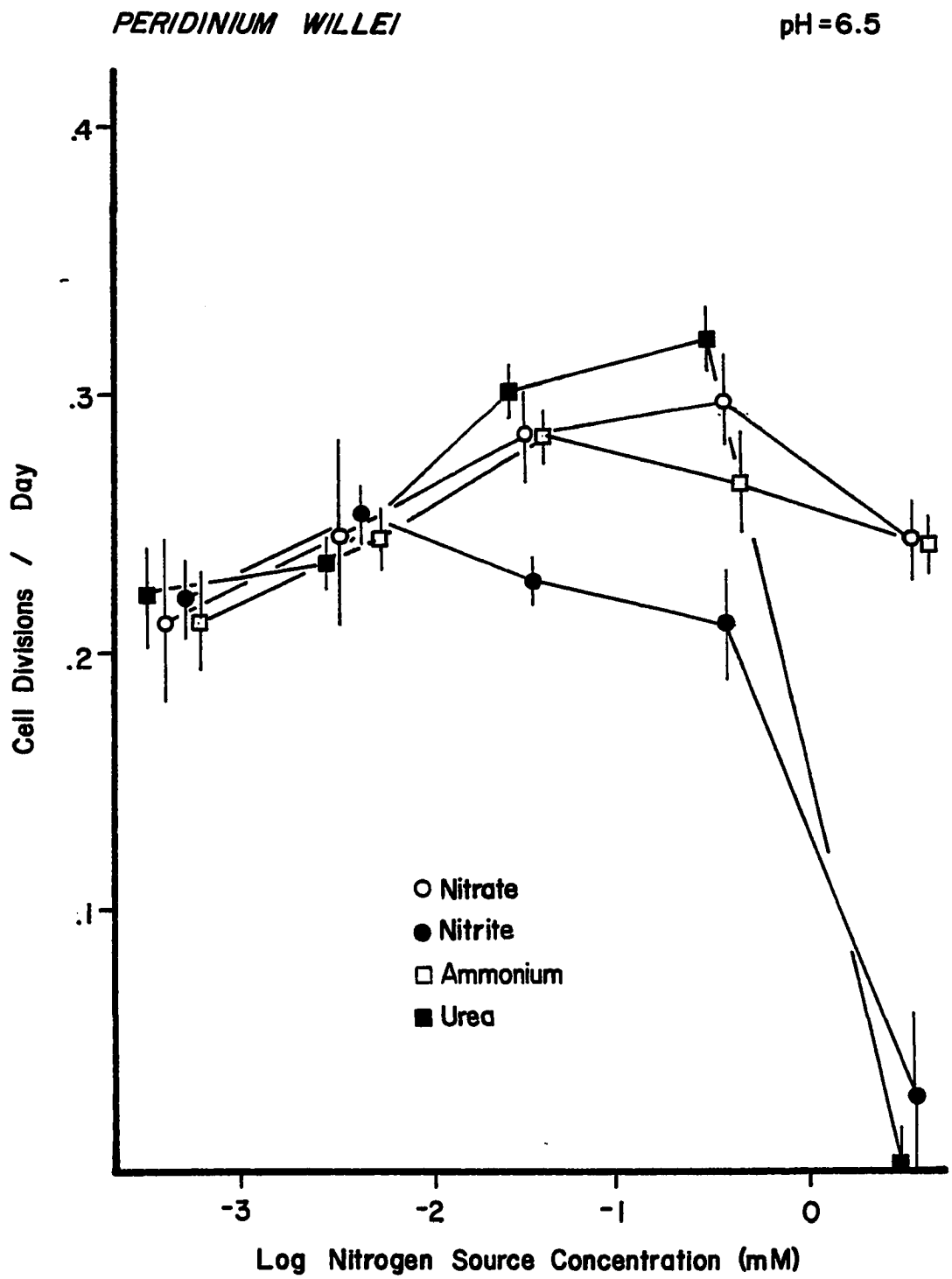

PERIDINIUM WILLEI
pH=6.5
Cell Divisions / Day
.4
.3
.2
.1
-3
-2
-1
0
Log Nitrogen Source Concentration (mM)
O Nitrate
● Nitrite
□ Ammonium
■ Urea

Figure 7

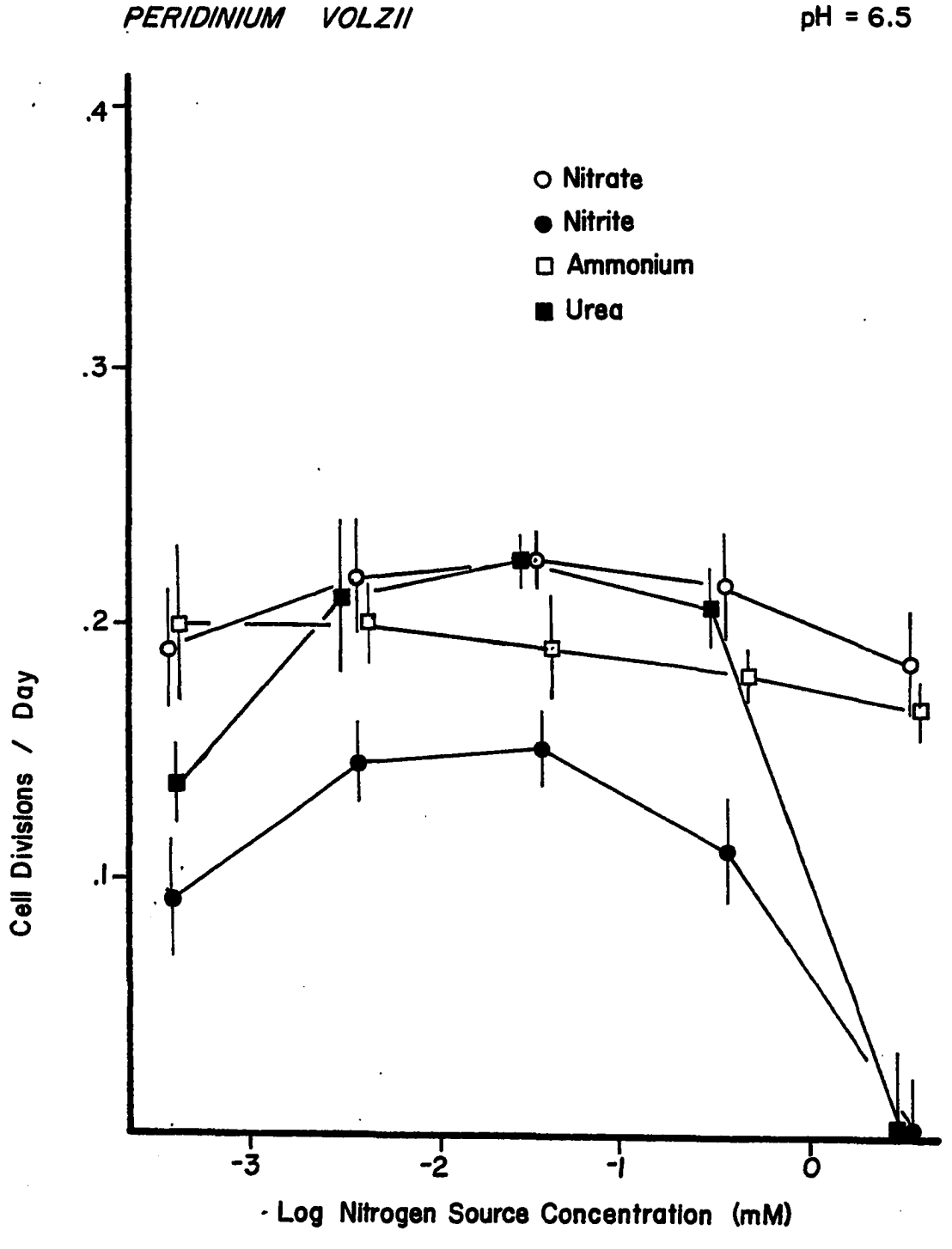

PERIDINIUM VOLZII
pH = 6.5
.4
.3
.2
.1
Cell Divisions / Day
Nitrate
Nitrite
Ammonium
Urea
-3
-2
-1
0
- Log Nitrogen Source Concentration (mM)

Figure 8

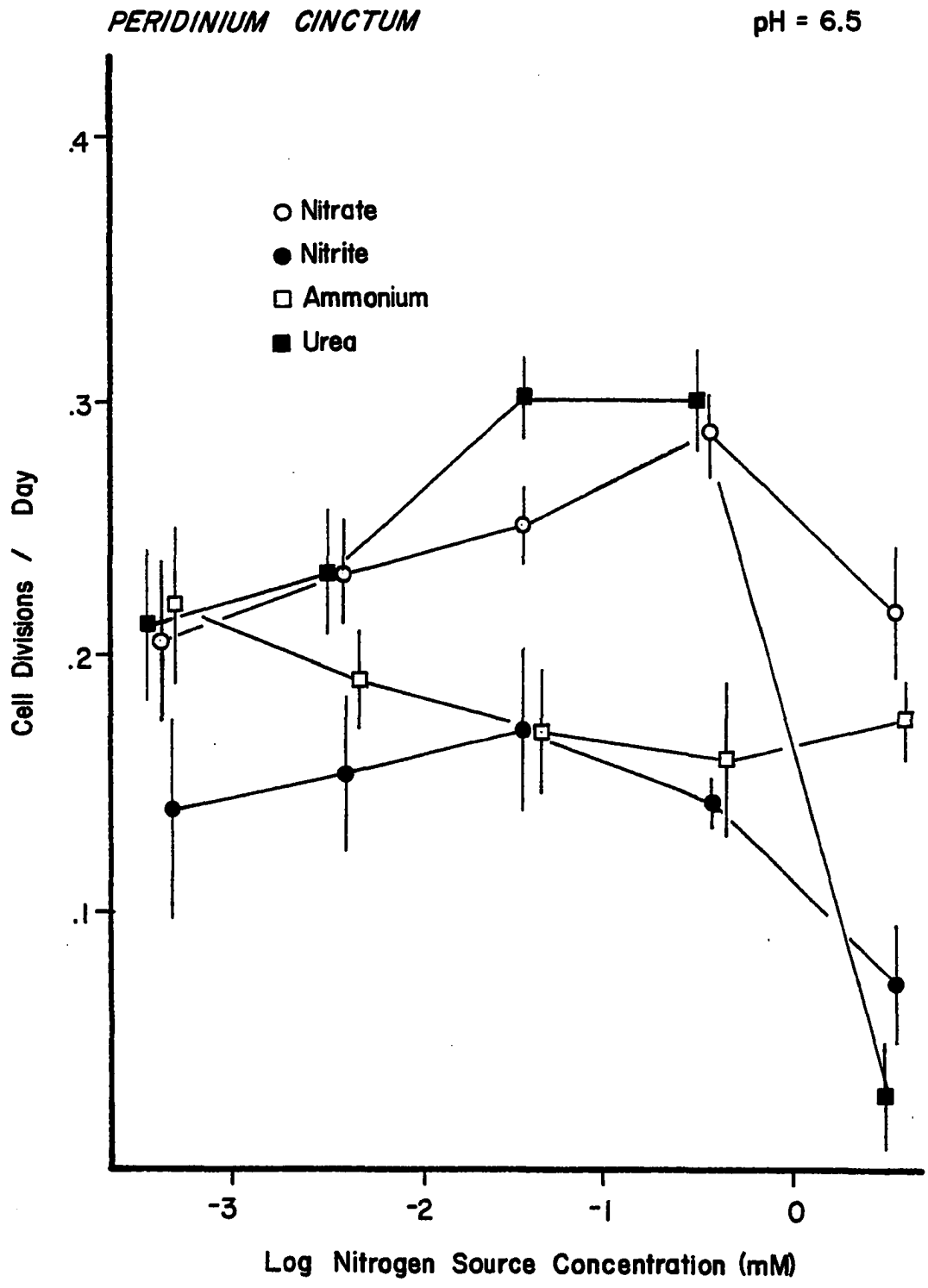

PERIDINIUM CINCTUM
pH = 6.5
Nitrate
Nitrite
Ammonium
Urea
Cell Divisions / Day
.4
.3
.2
.1
-3
-2
-1
0
Log Nitrogen Source Concentration (mM)

Figure 9

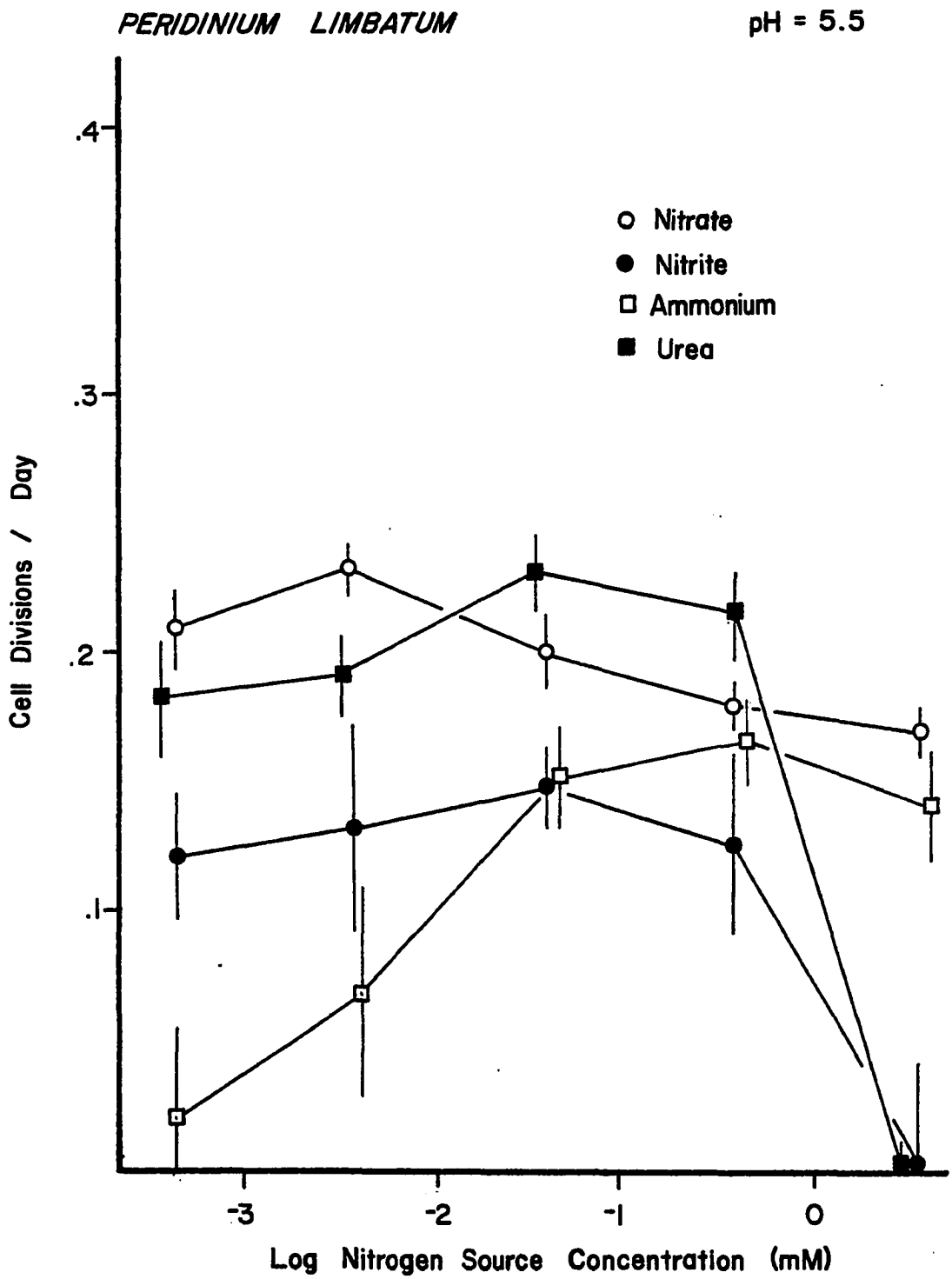

PERIDINIUM LIMBATUM
pH = 5.5
Nitrate
Nitrite
Ammonium
Urea
Cell Divisions / Day
.4
.3
.2
.1
-3
-2
-1
0
Log Nitrogen Source Concentration (mM)

Figure 10

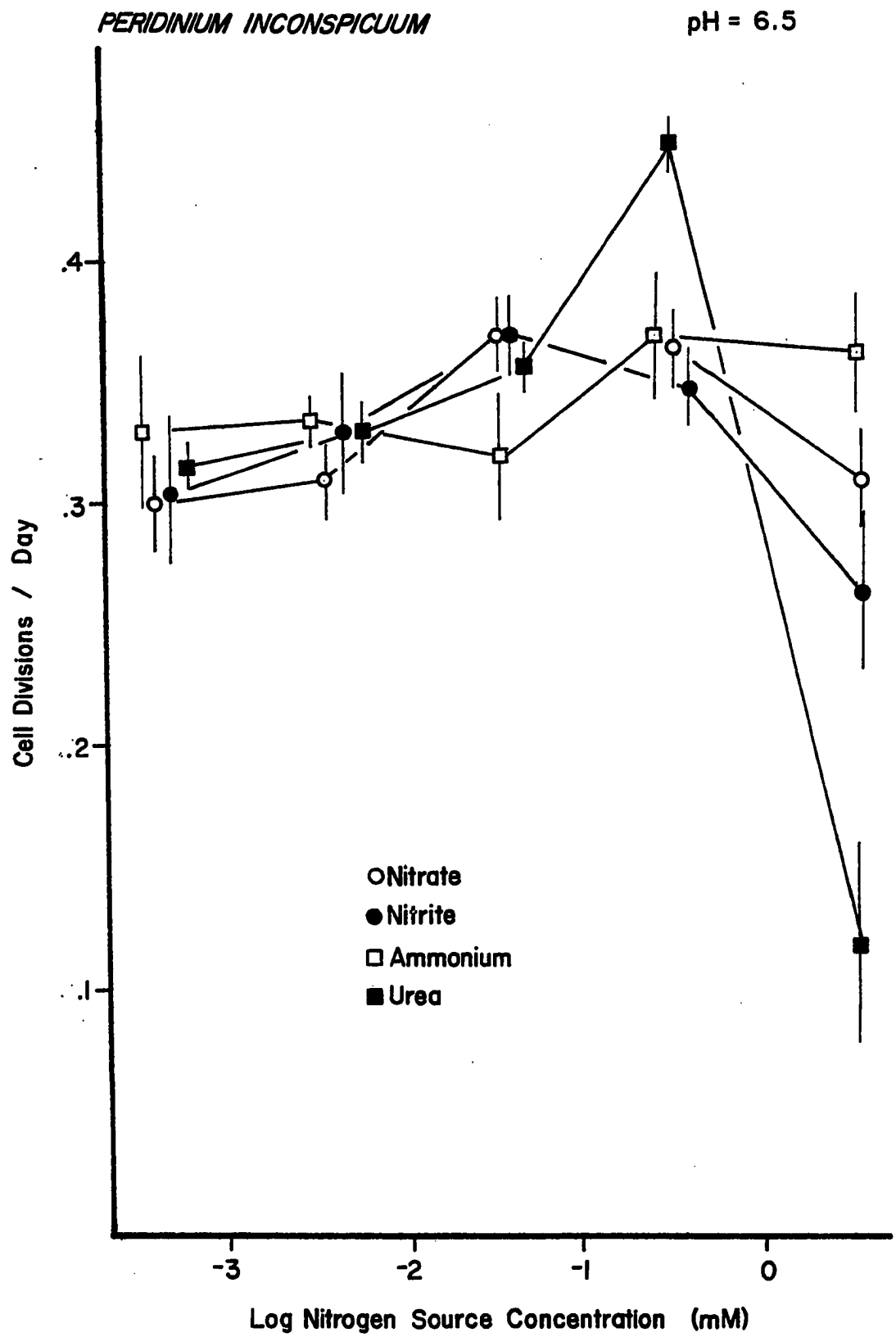

PERIDINIUM INCONSPICUUM
pH = 6.5
Cell Divisions / Day
.4
.3
.2
.1
-3
-2
-1
0
Log Nitrogen Source Concentration (mM)
Nitrate
Nitrite
Ammonium
Urea

Figure 11

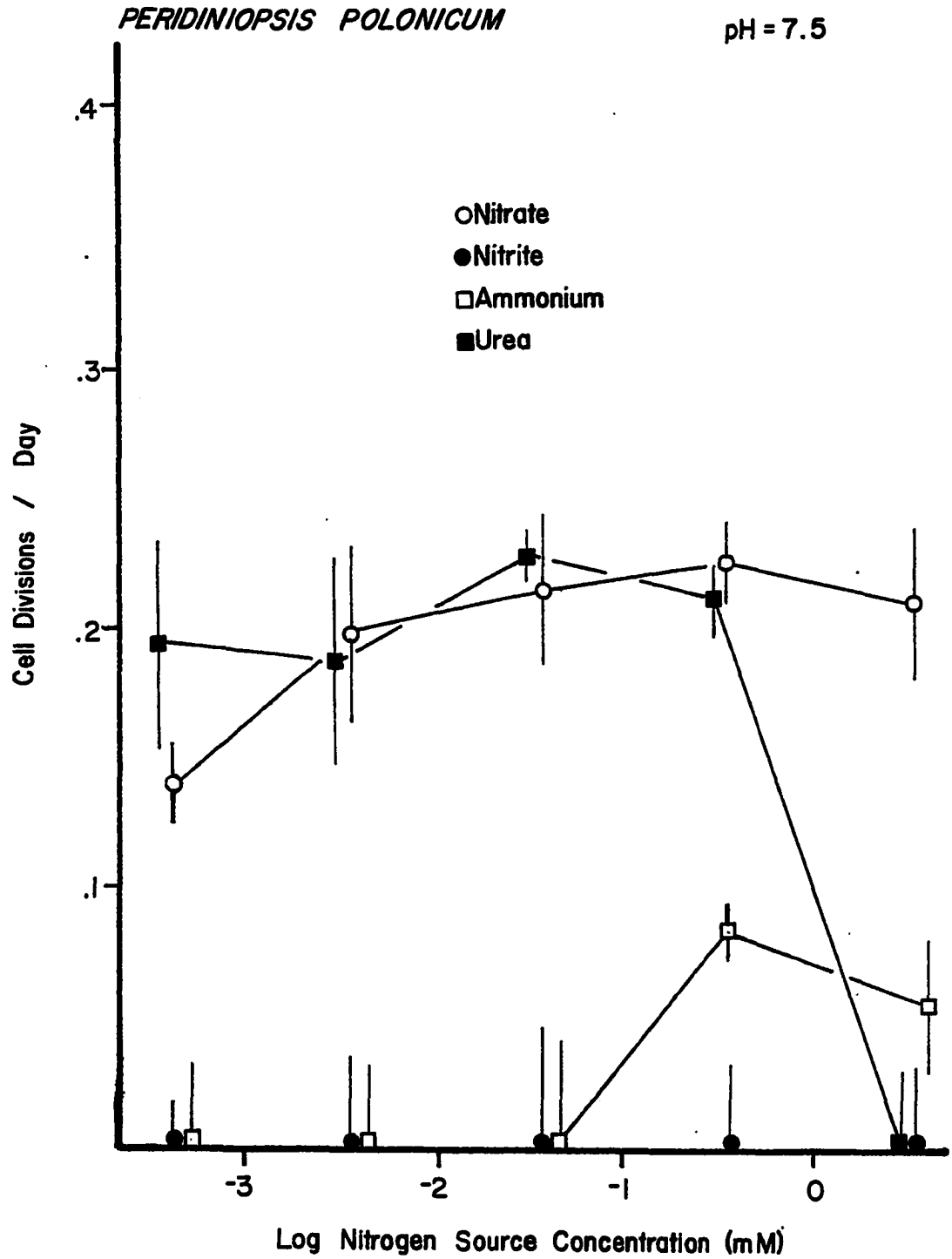

PERIDINIOPSIS POLONICUM
pH = 7.5
ONitrate
●Nitrite
□Ammonium
■Urea
Cell Divisions / Day
.4
.3
.2
.1
-3
-2
-1
0
Log Nitrogen Source Concentration (mM)

Figure 12

PAPER IV

A TECHNIQUE FOR COUNTING CHROMOSOMES OF ARMORED DINOFLAGELLATES, AND CHROMOSOME NUMBERS OF SIX FRESHWATER DINOFLAGELLATE SPECIES

A TECHNIQUE FOR COUNTING CHROMOSOMES OF ARMORED DINOFLAGELLATES, AND CHROMOSOME NUMBERS OF SIX FRESHWATER DINOFLAGELLATE SPECIES

ABSTRACT

A technique to stain, squash, and enumerate thecate dinoflagellate chromosomes is presented using a cellulose incubation and propionocarmine stain. Chromosome numbers for six freshwater armored dinoflagellates (*Peridinium cinctum* (O.F.M.) Ehrenberg, *P. inconspicuum* Lemm., *P. limbatum* (Stokes) Lemm., *P. volzii* Lemm., *P. willei* Huit.-Kaas, and *Peridiniopsis polonicum* (Wolosz.) Bourrelly) range from 41 *(P. inconspicuum)* to 210 *(P. cinctum)*. Evidence is presented to indicate dinoflagellate aneuploidy in culture.

INTRODUCTION

To date chromosome numbers of only 71 of the more than 1,000 dinoflagellate taxa have been reported (Loper et al., 1980; Shyam and Sarma, 1978; and Loeblich, 1976). Of the 44 freshwater *Peridinium* species described by Huber-Pestalozzi (1950), chromosome numbers of only three freshwater taxa are known (Shyam and Sarma, 1978). Clearly there is a need to know more chromosome numbers within this group in order to make comparisons of systematic relationships and life cycle characteristics.

There is also a need to investigate possible changes in chromosome number as cultures age. Loper et al. (1980) reported polyploidy within cultured *Ptychodiscus brevis* (Davis) Steidinger populations. A polyploid or aneuploid progression in *Peridinium* species was also suggested by Shyam and Sarma (1978). Indeed, Dodge (1963) reported 44 chromosomes for *Peridinium trochoideum* (Stein) Lemm. (Plymouth 104) while 11 years later Fine and Loeblich (1974) reported 80-90 chromosomes for the same cultured strain.

A major reason for the paucity of chromosome counts is that freshwater Peridinales are not easily cultured though there are several defined growth media for this group (Bruno and McLaughlin, 1980; Carefoot, 1968; and Lindström and Rodhe, 1980). With the recent culturing of five freshwater dinoflagellate species (*Peridinium willei, P. volzii, P. limbatum, P. inconspicuum,* and *Peridiniopsis polonicum*) by Pfiester (Pfiester, 1976; Pfiester and Skvarla, 1979; and Pfiester et al., in press), these are available for cytogenetic examination.

Standard chromosome spread and squash techniques are difficult

to apply to dinoflagellates because the nuclear membrane remains intact throughout nuclear division (Dodge, 1963; and Loeblich et al., 1974). Thus a method must address the problem of breaking the nuclear membrane but keeping the chromosomes together in a unified mass. Secondly, Perdinales species have armored plates which must be removed before a useful squash is made.

The purpose of this paper is to present a useful stain and squash technique for armored dinoflagellates, to report chromosome numbers of six freshwater dinoflagellates, and to examine possible aneuploidy in cultured dinoflagellates.

MATERIALS AND METHODS

Axenic cultures of five *Peridinium* species (*P. limbatum* [UTEX 2195], *P. inconspicuum* [UTEX 2255], *P. cinctum* [UTEX 1336], *P. volzii* [UTEX 2176], and *P. willei* [UTEX 2028], and one *Peridiniopsis* species (*P. polonicum* [UTEX 2257]) were obtained by the method of Droop (1967). Cultures were maintained in Modified Carefoot's Medium (Wynne and Berman, 1980) with vitamins added. Biotin and B_{12} were present in concentrations of 1 µg L^{-1}; thiamin was present at 1 mg L^{-1}. Stationary phase cells were harvested by gentle centrifugation for chromosome enumeration.

Cells were fixed, stained, and mounted as follows:

1. Cultures were incubated for two hours at 37°C and pH 5 with a surplus of cellulase (Endo-1,4,B-glucanose, 1,4-(1,3;1,4)-B-D glucan glucanohydrolase) (Sigma No. C-7502). To prevent destruction of chromosomes by microbial growth, an antibiotic mixture was added (Droop, 1967).

2. Cells were centrifuged and washed with distilled water three times. Cells were incubated in .01 mM Triton-X-100 for two-four hours at 37°C.

3. Cells were centrifuged and washed with distilled water until all detergent was gone. Cells were then fixed in Modified Carnoy's Solution (Cave and Pocock, 1951) for 24 hours.

4. Mixture was centrifuged and supernatant discarded. Pellet was stained in propionocarmine for one hour. Initially, stain was gently heated over an alcohol flame until stain just began to boil.

5. Stain-cell mixture was centrifuged and resulting pellet was rinsed in 45% acetic acid until no stain was seen in the supernatant. Cells were left in acetic acid solution one hour.

6. Cells were centrifuged and rinsed with distilled water until acetic acid was removed. (2-4 times).

7. Cells were centrifuged and rinsed in absolute methanol 2-4 times.

8. A drop of methanol-cell solution was added to a slide and squashed with a second slide.

9. Slides were pulled apart without sliding. Squash was allowed to dry.

10. One drop of permount was added to dried squash, and cover-glass (No. 1, 22x40 mm) was added.

11. Slide was then gently warmed on a slide warmer to harden mounting medium and enhance stain.

The squash was viewed with a Zeiss Standard microscope at 400X with phase optics. Photographs were taken with Kodak Panatomic-X film, and 20.5 X 25.4 cm prints were made with Kodabrome II RC F H paper. Chromosomes were marked with india ink to make a composite tracing of all optical sections (Figs. 1 and 2). The background of the composite tracing was then cleared with an I-KI solution.

RESULTS AND DISCUSSION

The stain and squash technique presented herein allowed a relatively precise enumeration of armored dinoflagellate chromosomes (Table 1). Mean counts ranged from 41.1 ± 2.2 for *Peridinium inconspicuum* to 209.7 ± 9.8 for *P. cinctum*. Though dinoflagellate chromosome numbers are often given as ranges (Dodge, 1963; Loeblich, 1976; Shyam and Sarma, 1978; and Loper et al., 1980), my counts yielded error ranges that are relatively low. Indeed, mean counts were all significantly even for species with nearly equivalent mean chromosome numbers. Low error was likely a consequence of making ten replicate counts for each species. This technique fosters the accumulation of counts because slides thus made are permanent and can be examined many times.

Though phase microscopy enhances chromosome resolution, chromosomes can easily be visualized using bright field microscopy. Since most dinoflagellates have high chromosome numbers (generally greater than 40 per cell), photography is necessary to achieve accurate counts. Also, since all chromosomes are rarely in the same plane of focus, a series of optical sections is often required. A composite tracing is thus prepared for each cell and counts accomplished with the tracing (Figs. 1 and 2).

A major drawback to this method is the length of time necessary to complete a count. Cells must be fixed, stained, photographed and counted. The entire procedure takes a minimum of three days.

Occasionally, I found cells with twice the apparent number of chromosomes as most cells counted. I assume these "diploids" are either zygotes or mitotically dividing nuclei. I suspect that these diploids

are zygotes in that all species counted exhibit diploid cells except *Peridinium volzii* and *P. inconspicuum*. *Peridinium volzii* is known to be heterothallic (Pfiester and Skvarla, 1979), and cells observed are from single strain cultures. Thus, zygotes could not have formed in the *P. volzii* cultures.

Similarly, *Peridinium inconspicuum* has been observed to undergo sexual reproduction only in the fall (Pfiester et al., in press) suggesting that the sexual cycle is governed by an endogenous rhythm. If true, cells fixed and stained in February should demonstrate no sexual reproduction or no diploids.

If these diploids are indeed zygotes, then the stain procedure must work on hypnozygotes. To my knowledge, armored dinoflagellate hypnozygote nuclei have not been routinely stained prior to this method. Perhaps, this method may aid in the study of meiosis which presumably occurs in the hypnozygote (Pfiester et al., in press).

When looking over the chromosome number data, I noted an apparent correlation: those species which had been in culture longest had the highest chromosome numbers (Fig. 3). Similarly, those species most recently brought into culture have the lowest chromosome numbers. Indeed, the correlation between time in culture and chromosome number is +.98 for the six species studied. I regret that there are no data following a single species through a number of years. I, however, believe that such a correlation is not merely coincidence. My chromosome squash and counting technique may aid in following future changes in chromosome numbers of armored dinoflagellates in culture.

The only other data for *Peridinium* chromosome numbers is for

P. trochoideum (Dodge, 1963; and Loeblich, 1976). In 1963 Dodge reported 44 chromosomes, but by 1976 Loeblich reported 88 chromosomes in the same cultured strain of *P. trochoideum (Scrippsiella trochoideum)*. Thus, evidence for polyploidy in a *Peridinium* species was inadvertently demonstrated but not recognized some years ago. Indeed, the regression line y-intercept of Figure 3 is between 40 and 50 chromosomes which corresponds well with the original *P. trochoideum* counts by Dodge (1963). I therefore believe that chromosome numbers herein given present an argument for an aneuploid series in cultured *Peridinium* species. I further suspect that the haploid chromosome number of *Peridinium* is about 44 chromosomes per cell.

My chromosome data also indicate that higher chromosome numbers are not only allowed in culture but may be selected for since variance does not noticably increase with chromosome numbers. If high numbers of chromosomes were not favored, one should find a wide range of counts in older cultures (e.g. *Peridinium cinctum*).

This phenomenon of dinoflagellate polyploidy in culture is not unique to *Peridinium*. Recently, Loper et al. (1980) clearly demonstrated polyploidy in an unarmored dinoflagellate, *Ptychodiscus brevis (Gymnodinium breve)*. They expressed the concern that with changes in chromosome number there may be corresponding changes in morphology, physiology and cell structure. Shyam and Sarma (1978) also suggest a possible polyploid or aneuploid series within the genus *Peridinium*.

At least in *Peridinium cinctum* we do not detect changes in morphology or cell structure though it has been in culture for over 10 years. Physiologically, *P. cinctum* responds to carbon sources in much

the same way as when it was first isolated (Carefoot, 1968; and Holt and Pfiester, in review a). Similarly, the growth response to nitrate, nitrite, ammonium, and urea has not changed in 8 years of culture (Pfiester, 1974; and Holt and Pfiester, in review b).

LITERATURE CITED

Bruno, S. F. and J. J. A. McLaughlin. 1977. The nutrition of the freshwater dinoflagellate *Ceratium hirundinella*. J. Phycol. 24:548-553.

Carefoot, J. R. 1968. Culture and heterotrophy of the freshwater dinoflagellate, *Peridinium cinctum* fa. *ovoplanum* Lindemann. J. Phycol. 4:129-131.

Cave, M. S. and M. A. Pocock. 1951. The aceto-carmine technic applied to the colonial Volvocales. Stain Technology 26:173-174.

Dodge, J. D. 1963. The nucleus and nuclear division in the Dinophyceae. Arch. Prostenk. 106:442-452.

Droop, M. R. 1967. A procedure for routine purification of algal cultures with antibiotics. Brit. Phycol. Bull. 3:295-7.

Fine, K. E. and A. R. Loeblich III. 1974. A comparison of *Scripsiella sweeneyae* (IUCC 1656) and *Peridinium trochoideum* (IUCC 1017). J. Phycol. 10(Suppl.):13-14.

Holt, J. R. and L. A. Pfiester. in review a. A survey of photo-heterotrophy in five freshwater dinoflagellates (Pyrrhophyta). J. Phycol.

_____. in review b. A survey of nitrogen source utilization and pH optima in five freshwater dinoflagellates (Pyrrhophyta). J. Phycol.

Huber-Pestalozzi, G. 1950. *Das Phytoplankton des Susswassers* Vol. XVI, part 3. In: Thienemann, A., ed. *Die Binnengewasser*. E. Schweizerbart'sche Verlagsbuchhandlung. Stuttgart. pp. 310.

Lindström, K. and W. Rodhe. 1978. Selenium as a micronutrient for the dinoflagellate *Peridinium cinctum* fa. *westii*. Mitt. Internat. Verein. Limnol. 21:168-173.

Loeblich, A. R. III. 1976. Dinoflagellate evolution: speculation and evidence. J. Protozool. 23:13-28.

_____, A. R. Klotz, T. M. Roberts, R. C. Tuttle, and J. R. Allen. 1974. The dinoflagellate nucleus. J. Phycol. 10(Suppl):14-15.

Loper, C. L., K. A. Steidinger, and L. M. Walker. 1980. A simple chromosome spread technique for unarmored dinoflagellates and implications of polyploidy in algal cultures. Trans. Amer. Soc. 99:343-346.

Pfiester, L. A. 1976. Sexual reproduction of *Peridinium willei* (Dinophyceae). J. Phycol. 12:234-238.

_____, and J. J. Skvarla. 1979. Heterothallism and thecal development in the sexual life history of *Peridinium volzii* (Dinophyceae). Phycologia 18:13-18.

_____, J. J. Skvarla, J. R. Holt, and D. Butler. in review. Structure and reproduction of *Peridinium inconspicuum* Lemmermann. American Journal of Botany.

Shyam, R. and Y. S. R. K. Sarma. 1978. Cytology of Indian freshwater Dinophyceae. Botanical Journal of the Linnean Society. 76:145-159.

Wynne, D. and T. Berman. 1980. Hot water extractable phosphorus — an indicator of nutritional status of *Peridinium cinctum* (Dinophyceae) from Lake Kinneret (Israel)? J. Phycol. 16:40-46.

Table 1. Chromosome count results of the six species investigated.

Species	Chromosome number ± 2 standard errors
Peridinium willei	114.8±8.1
Peridinium volzii	98.4±4.4
Peridinium cinctum	209.7±9.8
Peridinium limbatum	70.0±3.5
Peridinium inconspicuum	41.1±2.2
Peridiniopsis polonicum	56.2±2.7

LEGENDS TO FIGURES

Figure 1. Tracing of a haploid *Peridinium inconspicuum* chromosome squash.

Figure 2. Tracing of a diploid *Peridinium cinctum* chromosome squash.

Figure 3. Chromosome numbers versus years in culture for species studied. Correlation coefficient is $\pm.98$, and bars represent ± 2 standard errors.

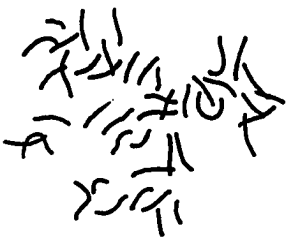


Figure 1

Figure 2

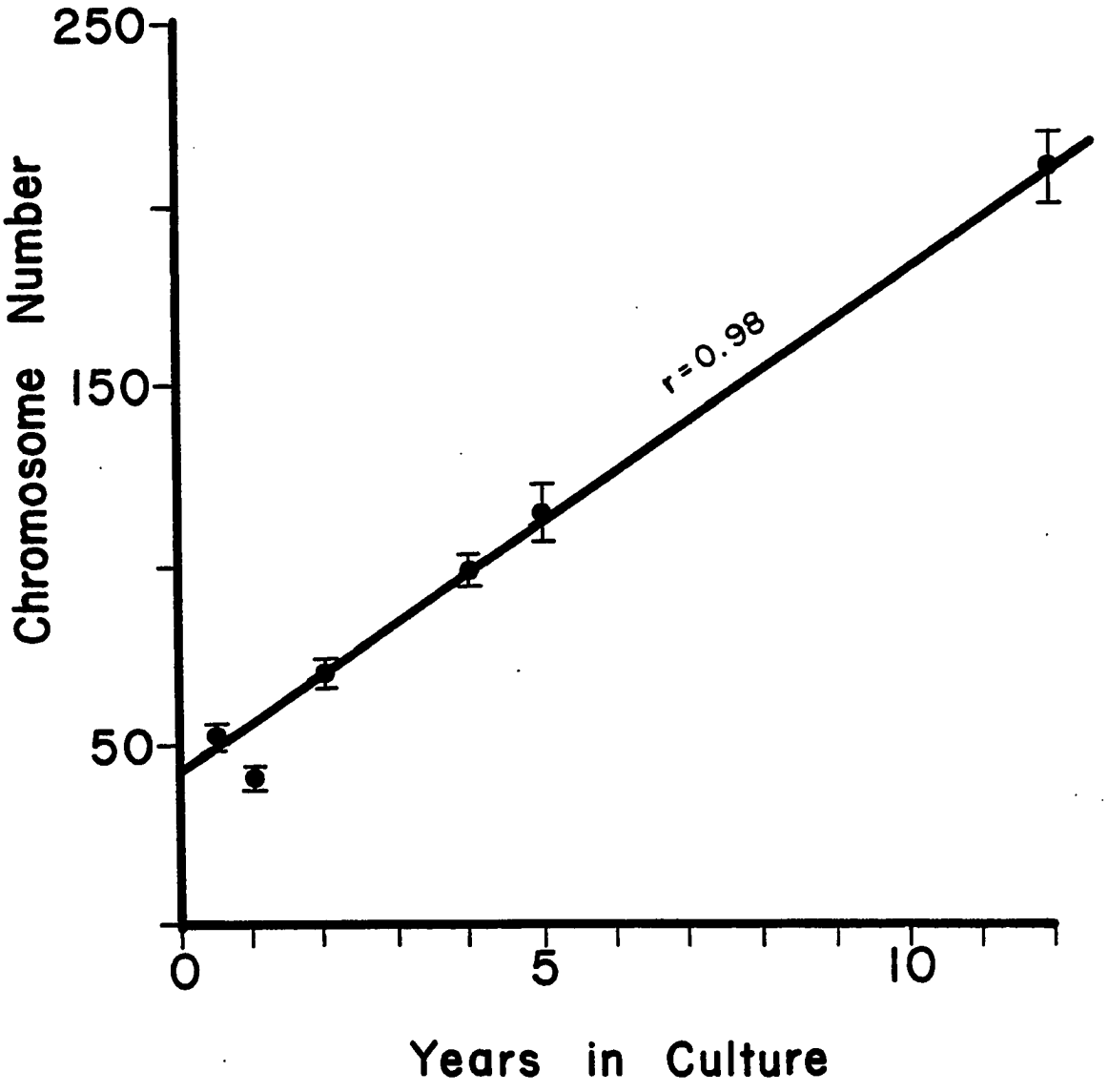


Figure 3

APPENDIX

Appendix A. Replicate cell counts of given species after 25 days exposed to Thiamin, Biotin, B_{12}, Thiamin/Biotin, Thiamin/B_{12}, Biotin/B_{12}, Thiamin/Biotin/B_{12}, and No Vitamin Controls. Correction factor for counts is 3.86 (135.48 for *Peridinium inconspicuum*).

Appendix A-1. *Peridinium willei*.

VITAMINS	REPLICATES 1	2	3	4
Thiamin	2011	1715	1992	2301
Biotin	2297	1785	1991	2088
B_{12}	1840	2029	1284	1627
Thiamin/Biotin	1780	2261	2198	1986
Thiamin/B_{12}	1269	2215	2002	1883
Biotin/B_{12}	2460	2010	1559	1991
All Vitamins	1644	2193	1814	1945
No Vitamin Control	2206	2373	1614	1923

Initial concentration = 21.9 cells/ml

Appendix A-2. *Peridinium volzii*

VITAMINS	REPLICATES			
	1	2	3	4
Thiamin	262	415	565	257
Biotin	281	203	151	262
B_{12}	273	297	416	325
Thiamin/Biotin	255	217	141	222
Thiamin/B_{12}	576	300	491	216
Biotin/B_{12}	59	234	163	259
All Vitamins	226	201	299	246
No Vitamin Control	1047	425	376	491

Initial concentration = 30.9 cells/ml

Appendix A-3. *Peridinium cinctum*

VITAMINS	REPLICATES 1	2	3	4
Thiamin	284	198	120	169
Biotin	132	216	197	106
B_{12}	293	210	199	133
Thiamin/Biotin	104	219	147	122
Thiamin/B_{12}	161	103	264	215
Biotin/B_{12}	229	291	102	177
All Vitamins	152	227	205	173
No Vitamin Control	123	157	155	207

Initial Concentration = 22.2 cells/ml

Appendix A-4. *Peridinium limbatum*

VITAMINS	REPLICATES 1	2	3	4
Thiamin	212	143	162	133
Biotin	9	1	2	5
B_{12}	8	3	5	3
Thiamin/Biotin	76	179	96	87
Thiamin/B_{12}	147	153	199	136
Biotin/B_{12}	9	6	5	1
All Vitamins	159	171	200	196
No Vitamin Control	7	3	5	6

Initial Concentration = 17.9 cells/ml

Appendix A-5. *Peridinium inconspicuum*

VITAMINS	REPLICATES			
	1	2	3	4
Thiamin	2011	1414	1683	1215
Biotin	1359	1533	914	2006
B_{12}	1631	3014	1489	1316
Thiamin/Biotin	1582	1337	1903	1298
Thiamin/B_{12}	1198	1815	1639	2227
Biotin/B_{12}	1864	2181	1345	1555
All Vitamins	1453	1864	1783	1223
No Vitamin Control	1728	1473	1921	1114

Initial Concentration = 122.6 cells/ml

Appendix A-6. *Peridiniopsis polonicum*

VITAMINS	REPLICATES 1	2	3	4
Thiamin	17	3	10	8
Biotin	11	8	6	4
B_{12}	93	129	74	63
Thiamin/Biotin	7	5	5	8
Thiamin/B_{12}	69	84	152	96
Biotin/B_{12}	86	96	58	93
All Vitamins	57	109	53	75
No Vitamin Control	9	2	5	7

Initial Concentration = 22.0 cells/ml

Appendix B. Response of *Peridinium volzii* to varying concentrations of Biotin at pH 6.5 after 25 days.

CONCENTRATION BIOTIN (ug/l)	REPLICATES 1	2	3	4
0	785	316	592	857
0.1	541	258	614	315
1.0	125	265	107	288
10	14	21	65	13

Initial Concentration = 22.1 cells/ml

Appendix C. Replicate cell counts of given species after 25 days exposed to 2.5 mM concentrations of 17 given organic substrates and controls (no carbon substrates added) at pH 5.5, 6.5, 7.5 and 8.5. Correction factor for counts is 3.86 (135.48 for *Peridinium inconspicuum*). All experiments run in a 12:12 LD photoregime.

Appendix C-1. *Peridinium willei*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	29	37	49	33
a-Ketoglutarate	59	22	27	65
Citrate	0	1	0	3
Fructose	37	39	49	62
Galactose	35	21	56	22
Glucose	52	58	21	34
Glycerol	52	67	82	73
Lactate	62	49	35	45
Malate	15	22	35	16
Malonate	15	32	49	27
Maltose	41	32	39	61
Mannose	0	0	0	0
Propionate	0	3	2	0
Pyruvate	66	52	59	32
Rhamnose	23	25	33	5
Succinate	52	69	33	47
Sucrose	100	86	73	93
Control	46	52	39	69

Initial Concentration = 19.6 cells/ml

Appendix C-2. *Peridinium willei*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	1761	1952	1087	1543
a-Ketoglutarate	1352	798	815	1895
Citrate	1007	527	705	926
Fructose	1287	959	742	1595
Galactose	1486	1009	843	1259
Glucose	2843	1857	1988	2643
Glycerol	2847	2000	1643	2516
Lactate	0	5	4	2
Malate	987	873	*	764
Malonate	3227	3778	3516	2010
Maltose	1209	1257	1485	615
Mannose	0	18	5	7
Propionate	5	6	0	10
Pyruvate	2124	2610	3315	2517
Rhamnose	3790	3510	3963	2199
Succinate	10	8	0	2
Sucrose	1001	517	843	2009
Control	1201	864	957	1816

Initial Concentration = 19.6 cells/ml

*experimental tube contaminated

Appendix C-3. *Peridinium willei*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	2916	1487	*	1545
a-Ketoglutarate	1415	927	1247	1817
Citrate	1600	*	1294	853
Fructose	1201	916	1865	1345
Galactose	1927	2016	1427	2216
Glucose	2955	2419	2642	2773
Glycerol	2817	3259	2999	3087
Lactate	0	18	4	20
Malate	2001	1261	1596	1465
Malonate	3465	3933	4092	3210
Maltose	2065	2716	1543	1749
Mannose	0	33	15	8
Propionate	8	5	10	8
Pyruvate	3695	4003	3012	3516
Rhamnose	3127	4096	4257	3815
Succinate	5	27	0	18
Sucrose	1384	1007	1248	1764
Control	1468	1519	2007	1953

Initial Concentration = 19.6 cells/ml

*experimental tube contaminated

Appendix C-4. *Peridinium willei*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	607	952	888	723
a-Ketoglutarate	601	523	223	417
Citrate	207	326	404	573
Fructose	203	264	201	507
Galactose	601	235	317	305
Glucose	607	1010	723	647
Glycerol	1201	423	701	566
Lactate	6	14	0	3
Malate	826	643	515	702
Malonate	953	787	305	601
Maltose	527	159	101	286
Mannose	17	21	0	5
Propionate	5	17	0	6
Pyruvate	1016	1129	1572	852
Rhamnose	1216	843	225	862
Succinate	0	15	6	4
Sucrose	526	185	199	316
Control	407	926	295	206

Initial Concentration = 19.6 cells/ml

Appendix C-5. *Peridinium volzii*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	3	3	4	2
a-Ketoglutarate	0	0	0	1
Citrate	7	0	6	5
Fructose	9	12	0	8
Galactose	5	0	0	1
Glucose	81	93	64	153
Glycerol	92	86	51	125
Lactate	19	8	9	3
Malate	41	19	57	36
Malonate	73	89	81	109
Maltose	0	2	0	1
Mannose	9	3	5	2
Propionate	0	0	3	1
Pyruvate	5	3	7	4
Rhamnose	19	5	15	21
Succinate	14	54	47	26
Sucrose	142	109	136	101
Control	101	137	85	73

Initial Concentration = 19.5 cells/ml

Appendix C-6. *Peridinium volzii*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	5	7	2	5
a-Ketoglutarate	0	2	1	0
Citrate	8	0	13	14
Fructose	0	12	13	8
Galactose	*	2	1	0
Glucose	103	149	63	99
Glycerol	159	207	106	133
Lactate	9	6	17	5
Malate	19	5	53	35
Malonate	123	162	153	147
Maltose	1	1	3	3
Mannose	15	0	2	0
Propionate	0	2	3	0
Pyruvate	2	8	3	5
Rhamnose	5	17	25	6
Succinate	25	37	15	29
Sucrose	201	109	152	111
Control	136	101	87	152

Initial Concentration = 19.5 cells/ml

*experimental tube contaminated

Appendix C-7. *Peridinium volzii*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	2	0	1	1
a-Ketoglutarate	5	0	0	0
Citrate	0	0	3	0
Fructose	3	10	0	2
Galactose	0	0	1	5
Glucose	8	15	23	8
Glycerol	47	31	49	36
Lactate	5	5	4	0
Malate	1	1	5	0
Malonate	39	42	62	43
Maltose	3	1	2	2
Mannose	2	1	0	1
Propionate	3	0	1	0
Pyruvate	12	5	7	0
Rhamnose	0	3	6	2
Succinate	0	5	3	2
Sucrose	42	65	47	82
Control	53	41	37	69

Initial Concentration = 19.5 cells/ml

Appendix C-8. *Peridinium volzii*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	3	0	4	0
a-Ketoglutarate	0	2	0	0
Citrate	5	1	0	2
Fructose	0	0	0	2
Galactose	9	13	6	5
Glucose	15	22	9	12
Glycerol	19	23	41	25
Lactate	0	3	0	0
Malate	1	2	0	1
Malonate	27	19	56	12
Maltose	0	0	1	0
Mannose	0	0	0	0
Propionate	0	0	0	3
Pyruvate	1	0	1	0
Rhamnose	2	0	5	0
Succinate	10	15	12	22
Sucrose	53	69	42	73
Control	43	25	33	39

Initial Concentration = 19.5 cells/ml

Appendix C-9. *Peridinium cinctum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	32	21	12	29
a-Ketoglutarate	2	0	0	0
Citrate	8	24	3	22
Fructose	5	17	12	14
Galactose	19	35	13	23
Glucose	21	25	38	22
Glycerol	61	57	82	43
Lactate	6	3	14	12
Malate	39	26	33	46
Malonate	37	41	29	39
Maltose	14	37	27	15
Mannose	24	47	24	18
Propionate	0	2	0	0
Pyruvate	39	28	46	35
Rhamnose	32	46	25	35
Succinate	9	5	12	6
Sucrose	10	7	14	12
Control	31	26	33	26

Initial Concentration = 20.2 cells/ml

Appendix C-10. *Peridinium cinctum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	210	194	179	283
a-Ketoglutarate	271	256	233	165
Citrate	27	55	35	38
Fructose	5	3	10	6
Galactose	265	297	309	286
Glucose	206	223	214	181
Glycerol	573	547	653	376
Lactate	5	1	2	0
Malate	285	382	271	295
Malonate	306	298	211	367
Maltose	28	31	10	17
Mannose	195	249	151	186
Propionate	0	1	3	0
Pyruvate	301	351	405	228
Rhamnose	251	196	118	287
Succinate	253	293	206	232
Sucrose	218	306	261	216
Control	260	232	251	194

Initial Concentration = 20.2 cells/ml

Appendix C-11. *Peridinium cinctum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	288	383	360	242
a-Ketoglutarate	12	5	4	6
Citrate	27	25	6	10
Fructose	51	59	101	35
Galactose	255	207	174	296
Glucose	307	328	367	233
Glycerol	516	786	657	498
Lactate	2	1	0	3
Malate	416	356	449	573
Malonate	692	972	556	709
Maltose	59	54	98	61
Mannose	233	116	176	153
Propionate	2	3	0	0
Pyruvate	286	251	194	328
Rhamnose	53	76	41	48
Succinate	364	264	202	257
Sucrose	301	231	174	327
Control	321	365	280	228

Initial Concentration = 20.2 cells/ml

Appendix C-12. *Peridinium cinctum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	49	66	30	55
a-Ketoglutarate	0	0	0	0
Citrate	0	3	2	0
Fructose	0	5	3	7
Galactose	87	39	85	70
Glucose	49	83	32	25
Glycerol	92	198	83	135
Lactate	0	0	0	0
Malate	88	43	59	54
Malonate	102	86	135	185
Maltose	15	6	7	12
Mannose	12	19	1	8
Propionate	0	0	0	0
Pyruvate	49	66	31	48
Rhamnose	0	5	2	0
Succinate	27	58	39	73
Sucrose	77	37	70	52
Control	38	93	79	47

Initial Concentration = 20.2 cells/ml

Appendix C-13. *Peridinium limbatum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	307	201	166	251
a-Ketoglutarate	23	10	0	7
Citrate	32	47	23	5
Fructose	261	201	153	286
Galactose	101	172	125	83
Glucose	521	436	505	302
Glycerol	816	607	517	692
Lactate	0	3	0	1
Malate	73	173	87	99
Malonate	247	317	186	205
Maltose	95	65	88	117
Mannose	97	101	62	123
Propionate	0	4	0	2
Pyruvate	87	98	128	65
Rhamnose	769	487	506	621
Succinate	5	0	6	2
Sucrose	327	261	407	287
Control	85	107	52	96

Initial Concentration = 10.6 cells/ml

Appendix C-14. *Peridinium limbatum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	73	84	41	63
a-Ketoglutarate	15	32	7	5
Citrate	49	57	31	86
Fructose	157	206	173	111
Galactose	53	65	32	49
Glucose	206	147	201	106
Glycerol	307	201	126	356
Lactate	0	1	5	0
Malate	65	62	31	87
Malonate	106	136	87	101
Maltose	64	33	49	52
Mannose	50	62	39	76
Propionate	0	0	3	6
Pyruvate	53	42	68	*
Rhamnose	283	204	307	166
Succinate	0	1	3	0
Sucrose	97	136	207	82
Control	48	67	85	53

Initial Concentration = 10.6 cells/ml

*experimental tube contaminated

Appendix C-15. *Peridinium limbatum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	101	59	63	104
a-Ketoglutarate	0	2	2	3
Citrate	*	32	101	29
Fructose	83	92	61	51
Galactose	41	63	24	34
Glucose	86	123	92	93
Glycerol	136	106	83	101
Lactate	0	0	1	0
Malate	46	73	31	52
Malonate	102	62	82	72
Maltose	37	58	31	47
Mannose	22	18	38	49
Propionate	8	0	0	0
Pyruvate	22	29	56	35
Rhamnose	106	83	101	129
Succinate	8	0	0	1
Sucrose	106	36	89	74
Control	37	31	59	26

Initial Concentration = 10.6 cells/ml

*experimental tube contaminated

Appendix C-16. *Peridinium limbatum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	14	6	9	8
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	9	9	6	7
Galactose	9	12	7	5
Glucose	10	10	3	17
Glycerol	29	18	37	16
Lactate	0	0	0	0
Malate	5	3	9	13
Malonate	25	37	15	36
Maltose	11	3	*	14
Mannose	15	6	8	5
Propionate	0	0	0	0
Pyruvate	10	2	7	*
Rhamnose	21	7	22	15
Succinate	0	0	1	0
Sucrose	5	0	7	9
Control	10	16	5	10

Initial Concentration = 10.6 cells/ml

*experimental tube contaminated

Appendix C-17. *Peridinium inconspicuum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	155	206	133	137
a-Ketoglutarate	193	147	132	159
Citrate	137	106	197	123
Fructose	823	897	615	735
Galactose	115	143	126	125
Glucose	519	587	693	406
Glycerol	612	901	1262	823
Lactate	0	4	6	0
Malate	412	317	205	459
Malonate	816	567	778	614
Maltose	132	101	197	199
Mannose	61	123	145	132
Propionate	0	5	3	7
Pyruvate	725	432	517	699
Rhamnose	135	172	138	97
Succinate	132	159	142	196
Sucrose	437	516	516	823
Control	206	163	107	219

Initial Concentration = 101 cells/ml

Appendix C-18. *Peridinium inconspicuum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	107	136	135	62
a-Ketoglutarate	217	135	130	263
Citrate	159	310	126	147
Fructose	979	997	873	632
Galactose	129	122	271	219
Glucose	799	822	687	621
Glycerol	1166	895	984	643
Lactate	5	2	1	1
Malate	511	525	654	525
Malonate	587	892	426	654
Maltose	62	210	125	154
Mannose	129	125	135	122
Propionate	0	1	2	0
Pyruvate	775	987	559	687
Rhamnose	87	101	61	251
Succinate	139	123	129	97
Sucrose	592	877	889	942
Control	146	153	139	152

Initial Concentration = 101 cells/ml

Appendix C-19. *Peridinium inconspicuum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	197	95	133	156
a-Ketoglutarate	86	65	102	159
Citrate	201	135	156	188
Fructose	1002	914	417	675
Galactose	133	101	153	197
Glucose	523	599	436	915
Glycerol	1032	939	517	897
Lactate	5	0	1	0
Malate	637	501	416	492
Malonate	427	359	816	532
Maltose	159	205	166	123
Mannose	122	169	143	162
Propionate	1	0	1	1
Pyruvate	615	526	707	493
Rhamnose	152	101	216	187
Succinate	197	164	147	157
Sucrose	901	827	615	994
Control	125	139	86	197

Initial Concentration = 101 cells/ml

Appendix C-20. *Peridinium inconspicuum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	135	197	164	225
a-Ketoglutarate	116	186	156	97
Citrate	153	184	102	128
Fructose	296	407	499	565
Galactose	145	69	129	209
Glucose	426	601	323	526
Glycerol	573	711	510	493
Lactate	1	1	0	0
Malate	419	297	534	355
Malonate	449	998	495	612
Maltose	126	165	156	162
Mannose	116	187	319	254
Propionate	2	0	1	0
Pyruvate	386	536	288	316
Rhamnose	166	171	111	286
Succinate	133	297	152	166
Sucrose	853	421	359	699
Control	169	206	153	125

Initial Concentration = 10l cells/ml

Appendix C-21. *Peridiniopsis polonicum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	36	34	23	19
a-Ketoglutarate	16	12	14	12
Citrate	20	17	15	25
Fructose	25	21	16	19
Galactose	20	22	25	25
Glucose	15	23	20	27
Glycerol	33	34	46	37
Lactate	0	0	0	1
Malate	*	26	19	25
Malonate	34	25	39	29
Maltose	17	23	31	16
Mannose	26	25	27	22
Propionate	0	0	0	0
Pyruvate	27	19	26	33
Rhamnose	30	32	27	33
Succinate	22	25	27	21
Sucrose	42	36	15	33
Control	22	17	27	21

Initial Concentration = 20.1 cells/ml

*experimental tube contaminated

Appendix C-22. *Peridiniopsis polonicum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	5	19	15	23
a-Ketoglutarate	23	6	19	21
Citrate	12	41	31	20
Fructose	27	17	13	29
Galactose	29	32	15	27
Glucose	9	14	27	19
Glycerol	30	25	33	52
Lactate	0	3	8	5
Malate	29	21	15	22
Malonate	34	26	47	39
Maltose	22	17	19	15
Mannose	22	34	29	15
Propionate	0	0	1	0
Pyruvate	29	16	21	20
Rhamnose	32	47	22	37
Succinate	14	29	22	27
Sucrose	32	41	30	26
Control	25	15	33	29

Initial Concentration = 20.1 cells/ml

Appendix C-23. *Peridiniopsis polonicum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	198	106	217	159
a-Ketoglutarate	211	212	251	132
Citrate	112	105	228	154
Fructose	554	255	523	851
Galactose	406	353	487	319
Glucose	262	223	322	104
Glycerol	564	688	882	520
Lactate	7	2	5	14
Malate	605	419	597	662
Malonate	587	644	594	665
Maltose	107	238	205	172
Mannose	122	223	338	257
Propionate	4	9	7	1
Pyruvate	542	523	655	885
Rhamnose	568	812	886	923
Succinate	201	109	156	182
Sucrose	363	514	407	619
Control	205	107	362	192

Initial Concentration = 20.1 cells/ml

Appendix C-24. *Peridiniopsis polonicum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	1	0
Citrate	0	2	1	1
Fructose	12	2	6	4
Galactose	1	0	1	1
Glucose	3	8	7	5
Glycerol	7	12	5	9
Lactate	0	0	0	0
Malate	5	3	7	4
Malonate	0	6	2	5
Maltose	0	0	0	0
Mannose	1	0	0	1
Propionate	0	1	0	0
Pyruvate	2	3	5	0
Rhamnose	9	12	5	17
Succinate	2	0	3	5
Sucrose	6	2	5	8
Control	0	5	10	2

Initial Concentration = 20.1 cells/ml

Appendix D. Replicate cell counts of given dark-grown species after 25 days exposed to 2.5 mM concentrations of 17 given organic substrates and controls (no carbon substrates added) at pH 5.5, 6.5, 7.5, and 8.5. Correction factor for counts is 3.86 (135.48 for *Peridinium inconspicuum*).

Appendix D-1. *Peridinium willei*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	2	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	1	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	1	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 20.1 cells/ml

Appendix D-2. *Peridinium willei*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	1	0
Galactose	0	1	0	0
Glucose	1	1	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	1
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	1	0	0	*
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 19.2 cells/ml

*experimental tube contaminated

Appendix D-3. *Peridinium willei*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	*	1	1
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	1	*
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	1	1	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	1	0	0
Propionate	1	0	1	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	1	0	1	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 19.2 cells/ml

*experimental tube contaminated

Appendix D-4. *Peridinium willei*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 19.2 cells/ml

*experimental tube contaminated

Appendix D-5. *Peridinium volzii*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	*	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	1	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	*	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 22.1 cells/ml

*experimental tube contaminated

Appendix D-6. *Peridinium volzii*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	*
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	1	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	1
Control	0	0	0	0

Initial Concentration = 22.1 cells/ml

*experimental tube contaminated

Appendix D-7. *Peridinium volzii*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	*	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 22.1 cells/ml

*experimental tube contaminated

Appendix D-8. *Peridinium volzii*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	1
Control	0	0	0	0

Initial Concentration = 22.1 cells/ml

*experimental tube contaminated

Appendix D-9. *Peridinium cinctum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	2	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	2	0	0
Glucose	0	0	*	0
Glycerol	0	1	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	1	0	0
Maltose	0	0	0	1
Mannose	0	0	0	1
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	1	0	0	0
Control	0	0	0	0

Initial Concentration = 25.2 cells/ml

*experimental tube contaminated

Appendix D-10. *Peridinium cinctum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	1	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	*	0
Galactose	0	0	0	1
Glucose	0	0	0	0
Glycerol	1	1	0	0
Lactate	0	0	0	0
Malate	0	*	0	0
Malonate	1	1	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	1	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	2	0
Control	0	0	0	0

Initial Concentration = 25.2 cells/ml

*experimental tube contaminated

Appendix D-11. *Peridinium cinctum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	2	0
a-Ketoglutarate	0	0	0	0
Citrate	1	0	1	0
Fructose	0	0	0	0
Galactose	0	0	0	*
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	1	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	1
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	1	0
Control	0	0	0	0

Initial Concentration = 25.2 cells/ml

*experimental tube contaminated

Appendix D-12. *Peridinium cinctum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	2	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	1	1	0	0
Maltose	0	0	1	0
Mannose	0	0	0	0
Propionate	0	1	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	*	0	0	0
Control	0	0	0	0

Initial Concentration = 25.2 cells/ml

*experimental tube contaminated

Appendix D-13. *Peridinium limbatum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	1
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	1
Maltose	0	0	0	0
Mannose	0	*	0	0
Propionate	2	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	1	0
Sucrose	0	0	0	0
Control	0	0	1	0

Initial Concentration = 21.5 cells/ml

*experimental tube contaminated

Appendix D-14. *Peridinium limbatum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	1	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	2	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	1	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	1	1	0	0
Control	0	0	0	0

Initial Concentration = 21.5 cells/ml

*experimental tube contaminated

Appendix D-15. *Peridinium limbatum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	*
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 21.5 cells/ml

*experimental tube contaminated

Appendix D-16. *Peridinium limbatum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	*	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 21.5 cells/ml

*experimental tube contaminated

Appendix D-17. *Peridinium inconspicuum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	1
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	1	0	0	0
Pyruvate	0	0	1	0
Rhamnose	0	0	0	0
Succinate	0	1	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 101.1 cells/ml

Appendix D-18. *Peridinium inconspicuum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	1	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	1
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 101.1 cells/ml

Appendix D-19. *Peridinium inconspicuum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	1	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	1
Control	0	0	0	0

Initial Concentration = 101.1 cells/ml

Appendix D-20. *Peridinium inconspicuum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	1	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 101.1 cells/ml

Appendix D-21. *Peridiniopsis polonicum*, pH 5.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	1	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 22.0 cells/ml

*experimental tube contaminated

Appendix D-22. *Peridiniopsis polonicum*, pH 6.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	1
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 22.0 cells/ml

*experimental tube contaminated

Appendix D-23. *Peridiniopsis polonicum*, pH 7.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	*	0	0	0
Galactose	0	0	1	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	1	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	*

Initial Concentration = 22.0 cells/ml

*experimental tube contaminated

Appendix D-24. *Peridiniopsis polonicum*, pH 8.5

SUBSTRATE	REPLICATES 1	2	3	4
Acetate	0	0	0	0
a-Ketoglutarate	0	0	0	0
Citrate	0	0	0	0
Fructose	0	0	0	0
Galactose	0	0	0	0
Glucose	0	0	0	0
Glycerol	0	0	0	0
Lactate	0	0	0	0
Malate	0	0	0	0
Malonate	0	0	0	0
Maltose	0	0	0	0
Mannose	0	0	0	0
Propionate	0	0	0	0
Pyruvate	0	0	0	0
Rhamnose	0	0	0	0
Succinate	0	0	0	0
Sucrose	0	0	0	0
Control	0	0	0	0

Initial Concentration = 22.0 cells/ml

*experimental tube contaminated

Appendix E. Replicate cell counts of given species after 25 days in Modified Carefoot's Medium at pH 5.5, 6.5, 7.5 and 8.5. Correction factor for counts is 3.86.

Appendix E-1. *Peridinium willei*

pH	REPLICATES 1	2	3	4
5.5	153	188	223	147
6.5	274	534	465	397
7.5	1002	750	837	899
8.5	163	149	159	165

Initial Concentration = 19.0 cells/ml

Appendix E-2. *Peridinium volzii*

pH	REPLICATES 1	2	3	4
5.5	381	432	407	359
6.5	621	516	653	592
7.5	242	231	291	252
8.5	196	170	207	221

Initial Concentration = 10.7 cells/ml

Correction Factor = 0.368

Appendix E-3. *Peridinium cinctum*

pH	REPLICATES 1	2	3	4
5.5	15	11	12	16
6.5	68	116	122	101
7.5	148	121	139	143
8.5	18	21	13	23

Initial Concentration = 10.7 cells/ml

Appendix E-4. *Peridinium limbatum*

pH	REPLICATES 1	2	3	4
5.5	216	193	246	201
6.5	133	94	106	122
7.5	120	88	93	58
8.5	13	6	9	10

Initial Concentration = 24.0 cells/ml

Appendix E-5. *Peridinium inconspicuum*

pH	REPLICATES 1	2	3	4
5.5	4923	4410	5264	5011
6.5	4584	4707	5035	4916
7.5	3435	5923	5527	5142
8.5	6816	5145	6361	5927

Initial Concentration = 99.4 cells/ml

Correction Factor = 3.68 cells/ml

Appendix E-6. *Peridiniopsis polonicum*

pH	REPLICATES 1	2	3	4
5.5	19	26	25	27
6.5	26	26	35	22
7.5	203	246	283	192
8.5	6	16	8	2

Initial Concentration = 24.0 cells/ml

Appendix F. Replicate cell counts of given species after 25 days exposed to serial dilutions of nitrogen substrates (2.94, $2.94X10^{-1}$, $2.94X10^{-2}$, $2.94X10^{-3}$, and $2.94X10^{-4}$ mM) and controls (with no nitrogen added) at pH 5.5, 6.5, 7.5, and 8.5. Nitrogen substrates tested are urea, ammonium, nitrite and nitrate. Correction factor for counts is 3.68 (135.48 for *Peridinium inconspicuum*).

Appendix F-1. *Peridinium willei*, pH 5.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	2	615	412	142	119
	1	785	206	144	83
	0	574	335	101	186
	0	401	472	264	152
Ammonium	224	259	352	153	106
	206	201	301	189	123
	149	316	216	206	101
	173	157	293	193	159
Nitrite	7	141	306	215	105
	6	163	321	243	76
	10	86	215	141	196
	4	201	279	197	123
Nitrate	326	601	497	106	106
	101	458	206	299	158
	252	521	359	251	216
	188	192	287	185	297

0 Nitrogen Control 12
8
5
15

Initial Concentration = 19.3 cells/ml

Appendix F-2. *Peridinium willei*, pH 6.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	5	1366	930	229	225
	0	1419	815	301	186
	4	1245	1142	266	297
	6	915	855	316	206
Ammonium	305	525	555	343	201
	406	324	504	216	121
	253	688	699	355	255
	351	459	603	326	226
Nitrite	6	153	226	347	211
	4	291	205	307	291
	19	247	282	488	152
	9	143	307	389	228
Nitrate	321	1003	883	161	319
	465	875	994	519	106
	259	1216	615	338	259
	392	597	436	619	182

0 Nitrogen Control 12
5
8
15

Initial Concentration = 19.3 cells/ml

Appendix F-3. *Peridinium willei*, pH 7.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	7	2553	2061	615	359
	9	2624	1623	843	406
	5	2011	1923	621	586
	0	1836	2215	472	421
Ammonium	0	12	6	5	0
	5	14	0	10	1
	17	3	0	14	7
	6	5	7	23	0
Nitrite	12	323	463	600	317
	14	407	527	565	216
	0	584	306	217	392
	23	289	391	386	201
Nitrate	927	2101	1643	1011	612
	818	1512	1291	263	126
	1135	1009	415	692	217
	860	2065	861	591	526

0 Nitrogen Control 4
1
3
3

Initial Concentration = 19.3 cells/ml

Appendix F-4. *Peridinium willei*, pH 8.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	543	325	157	123
	3	615	287	167	167
	0	201	562	201	83
	0	458	107	153	129
Ammonium	0	12	5	0	0
	5	6	5	1	0
	2	12	7	6	1
	0	0	2	3	1
Nitrite	0	151	253	226	107
	1	163	301	316	23
	1	103	475	102	151
	0	135	200	187	136
Nitrate	319	523	326	205	192
	121	614	115	215	286
	243	362	627	309	126
	197	201	296	147	152

0 Nitrogen Control 5
12
0
6

Initial Concentration = 19.3 cells/ml

Appendix F-5. *Peridinium volzii*, pH 5.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	103	131	206	26
	2	156	149	59	59
	0	96	110	101	37
	1	85	159	86	42
Ammonium	62	79	101	81	136
	53	83	149	102	101
	71	87	56	101	145
	69	64	72	62	23
Nitrite	0	12	56	29	11
	5	31	42	38	29
	2	16	49	22	21
	0	25	26	32	8
Nitrate	75	153	149	104	59
	102	206	157	192	92
	53	137	142	96	129
	73	116	100	111	62

0 Nitrogen Control 0
5
3
0

Initial Concentration = 10.3 cells/ml

Appendix F-6. *Peridinium volzii*, pH 6.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	146	263	217	52
	2	299	283	205	43
	3	201	204	399	75
	1	179	319	102	62
Ammonium	101	133	152	165	307
	96	162	249	209	112
	126	105	101	129	253
	84	129	149	203	115
Nitrite	1	25	98	49	22
	2	33	74	75	34
	2	65	85	43	39
	1	42	59	62	17
Nitrate	159	301	285	208	105
	209	153	206	186	193
	105	265	299	360	243
	93	201	310	229	117

0 Nitrogen Control 1
2
3
6

Initial Concentration = 10.3 cells/ml

Appendix F-7. *Peridinium volzii*, pH 7.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	52	64	77	26
	0	165	87	60	49
	0	69	95	125	53
	1	72	63	93	37
Ammonium	6	5	0	5	0
	15	12	3	2	1
	0	8	2	6	0
	9	8	1	4	3
Nitrite	0	8	31	21	7
	2	0	26	10	5
	0	5	15	15	2
	5	1	37	12	1
Nitrate	42	101	64	57	86
	65	79	35	85	10
	35	89	76	22	23
	71	93	116	95	39

0 Nitrogen Control 2
5
2
2

Initial Concentration = 10.3 cells/ml

Appendix F-8. *Peridinium volzii*, pH 8.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	44	59	36	5
	0	86	65	25	53
	0	62	37	87	36
	0	65	21	10	4
Ammonium	0	1	0	0	0
	0	2	0	1	5
	0	0	0	0	0
	1	0	0	0	2
Nitrite	0	2	23	15	6
	0	5	15	0	22
	0	3	26	5	15
	0	0	5	19	0
Nitrate	38	83	26	37	10
	26	52	15	14	0
	59	37	52	25	29
	26	91	65	39	15

0 Nitrogen Control 0
2
1
0

Initial Concentration = 10.3 cells/ml

Appendix F-9. *Peridinium cinctum*, pH 5.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	84	95	5	8
	2	63	59	18	0
	0	102	119	14	16
	1	78	64	12	4
Ammonium	10	5	7	19	21
	12	12	15	12	15
	6	5	8	6	0
	12	11	16	8	6
Nitrite	0	5	4	5	7
	2	6	15	15	12
	0	15	9	3	2
	1	12	7	6	5
Nitrate	31	85	23	25	21
	15	47	27	24	27
	29	51	35	37	11
	15	46	29	16	5

0 Nitrogen Control 0
0
5
0

Initial Concentration = 15.1 cells/ml

Appendix F-10. *Peridinium cinctum*, pH 6.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	6	.672	941	227	51
	10	726	547	124	70
	4	585	1116	361	62
	8	1261	895	257	81
Ammonium	88	35	48	147	332
	97	46	87	67	107
	63	96	125	123	216
	104	110	73	104	173
Nitrite	12	50	39	31	70
	15	42	145	56	88
	24	61	101	127	21
	10	47	83	63	37
Nitrate	153	841	216	207	198
	306	503	288	234	104
	159	461	333	148	85
	104	502	438	334	263

0 Nitrogen Control 6
12
10
8

Initial Concentration = 15.1 cells/ml

Appendix F-11. *Peridinium cinctum*, pH 7.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	11	525	812	301	109
	14	406	402	112	85
	26	315	671	187	236
	12	487	212	212	146
Ammonium	0	5	6	5	1
	1	7	0	0	1
	0	14	1	6	5
	0	0	1	0	1
Nitrite	5	59	79	26	71
	26	62	14	25	15
	17	47	49	45	42
	12	53	38	37	41
Nitrate	219	586	623	385	267
	307	601	502	452	201
	251	215	385	581	141
	185	785	419	201	187
0 Nitrogen Control	0				
	1				
	5				
	1				

Initial Concentration = 15.1 cells/ml

Appendix F-12. *Peridinium cinctum*, pH 8.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	59	81	59	18
	0	48	60	43	22
	2	63	41	5	16
	0	40	52	10	5
Ammonium	0	4	1	1	3
	0	0	0	1	2
	0	1	5	0	0
	3	1	0	0	1
Nitrite	0	0	2	1	5
	0	3	1	0	0
	1	0	1	3	0
	0	0	0	0	0
Nitrate	46	81	28	27	25
	31	53	36	22	19
	25	45	14	59	33
	21	49	55	26	41

0 Nitrogen Control 0
0
0
0

Initial Concentration = 15.1 cells/ml

Appendix F-13. *Peridinium limbatum*, pH 5.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	206	316	152	211
	0	351	288	188	105
	1	210	359	107	83
	0	164	201	169	116
Ammonium	41	82	92	10	6
	65	121	104	18	9
	82	104	53	35	18
	53	62	62	12	4
Nitrite	1	28	82	52	38
	4	59	101	111	62
	2	33	52	65	26
	3	106	61	23	59
Nitrate	106	127	194	255	232
	112	146	116	261	143
	85	123	198	362	247
	129	132	201	297	199

0 Nitrogen Control 1
9
6
4

Initial Concentration = 10.0 cells/ml

Appendix F-14. *Peridinium limbatum*, pH 6.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	101	126	75	63
	0	119	101	89	41
	0	85	159	38	59
	0	80	63	62	39
Ammonium	25	41	26	14	0
	37	53	59	3	3
	51	27	18	10	8
	15	35	66	5	4
Nitrite	0	14	53	36	25
	0	29	29	22	16
	1	86	61	39	39
	0	42	18	14	15
Nitrate	91	62	95	115	73
	53	83	102	124	85
	65	52	51	26	62
	42	97	65	72	79

0 Nitrogen Control 0
0
2
0

Initial Concentration = 10.0 cells/ml

Appendix F-15. *Peridinium limbatum*, pH 7.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	83	102	58	106
	0	99	92	33	57
	0	54	38	81	85
	0	69	56	76	72
Ammonium	7	6	7	5	3
	8	2	0	6	9
	8	3	0	12	6
	0	1	5	12	5
Nitrite	0	10	31	15	12
	3	18	27	38	31
	0	12	20	23	9
	0	35	35	7	20
Nitrate	39	42	61	81	73
	48	36	56	64	51
	26	59	68	75	82
	33	31	35	93	65

0 Nitrogen Control 1
5
0
3

Initial Concentration = 10.0 cells/ml

Appendix F-16. *Peridinium limbatum*, pH 8.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	25	29	15	8
	0	21	2	0	11
	0	0	15	9	5
	0	15	6	26	3
Ammonium	0	0	1	0	0
	2	0	2	0	1
	0	0	0	0	0
	1	0	0	0	0
Nitrite	0	3	5	5	3
	0	12	19	0	10
	1	2	21	7	6
	1	11	4	5	2
Nitrate	11	15	25	19	25
	23	36	24	27	37
	5	12	16	30	22
	12	13	20	21	6

0 Nitrogen Control 0
0
0
0

Initial Concentration = 10.0 cells/ml

Appendix F-17. *Peridinium inconspicuum*, pH 5.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	5	2002	461	274	217
	8	1427	259	358	102
	7	2115	387	401	188
	12	1723	209	206	123
Ammonium	306	415	243	241	269
	497	298	123	278	296
	216	609	386	149	201
	358	391	216	301	233
Nitrite	167	319	409	257	291
	204	386	486	138	101
	105	201	236	309	244
	94	237	359	201	316
Nitrate	158	307	507	207	116
	169	387	229	223	187
	101	264	348	101	157
	285	502	526	216	161

0 Nitrogen Control 2
0
0
6

Initial Concentration = 101.3 cells/ml

Appendix F-18. *Peridinium inconspicuum*, pH 6.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	14	1930	363	209	176
	4	2247	399	264	195
	3	1794	282	291	153
	9	1565	356	162	174
Ammonium	338	649	267	262	261
	454	526	106	311	297
	693	688	187	198	332
	341	269	289	228	106
Nitrite	123	420	519	265	252
	87	315	314	291	87
	37	346	615	135	152
	59	228	444	315	207
Nitrate	206	440	509	147	201
	107	528	552	258	98
	212	316	321	132	152
	152	395	463	157	133

0 Nitrogen Control 5
1
8
7

Initial Concentration = 101.3 cells/ml

Appendix F-19. *Peridinium inconspicuum*, pH 7.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	2635	599	289	47
	2	1934	638	406	259
	3	1385	325	201	164
	0	2091	246	381	199
Ammonium	29	20	6	19	0
	26	16	0	3	0
	37	43	15	2	1
	14	106	6	5	0
Nitrite	86	253	363	198	263
	105	106	225	302	101
	64	346	291	201	387
	193	192	163	159	64
Nitrate	197	205	478	316	101
	64	117	299	201	179
	128	298	143	207	62
	219	186	562	87	95

0 Nitrogen Control 3
1
0
0

Initial Concentration = 101.3 cells/ml

Appendix F-20. *Peridinium inconspicuum*, pH 8.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	1926	597	417	165
	12	1006	322	120	23
	14	1538	428	326	217
	0	2203	265	301	205
Ammonium	16	15	17	4	0
	2	37	29	0	1
	25	4	0	0	1
	14	25	12	3	1
Nitrite	81	329	263	198	206
	217	205	597	264	117
	153	257	444	127	86
	106	184	327	315	264
Nitrate	284	294	263	186	139
	206	315	405	352	152
	252	249	361	151	166
	107	198	298	216	112

0 Nitrogen Control 0
0
1
0

Initial Concentration = 101.3 cells/ml

Appendix F-21. *Peridiniopsis polonicum*, pH 5.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	57	21	0	5
	2	25	0	6	7
	0	10	15	12	2
	5	12	8	8	3
Ammonium	2	0	0	0	2
	2	4	0	0	0
	0	0	1	0	0
	0	0	0	0	0
Nitrite	0	0	3	0	0
	0	0	0	0	1
	1	0	2	0	0
	0	0	0	2	0
Nitrate	5	2	5	17	5
	15	10	12	5	16
	0	5	11	2	2
	6	12	8	9	5

0 Nitrogen Control 0
0
2
0

Initial Concentration = 10.1 cells/ml

Appendix F-22. *Peridiniopsis polonicum*, pH 6.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	0	5	6	0
	0	4	7	2	2
	0	7	8	0	0
	1	9	3	0	5
Ammonium	4	5	9	7	4
	6	2	8	5	6
	3	6	3	3	7
	3	4	6	3	3
Nitrite	0	0	0	4	1
	3	0	2	0	2
	4	0	0	1	0
	0	0	6	1	0
Nitrate	26	15	15	31	13
	37	38	0	22	45
	21	25	8	28	12
	27	22	29	19	19

0 Nitrogen Control 3
2
0
0

Initial Concentration = 10.1 cells/ml

Appendix F-23. *Peridiniopsis polonicum*, pH 7.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	231	305	193	231
	2	275	255	107	278
	5	213	314	64	154
	2	162	220	293	61
Ammonium	14	25	2	4	6
	11	22	2	3	3
	28	18	5	5	5
	10	27	7	2	2
Nitrite	2	2	1	3	7
	4	4	1	2	4
	0	4	1	2	0
	6	8	5	7	6
Nitrate	188	295	357	286	107
	279	357	115	101	118
	301	205	207	95	205
	106	266	304	307	152

0 Nitrogen Control 4
5
6
6

·Initial Concentration = 10.1 cells/ml

Appendix F-23. *Peridiniopsis polonicum*, pH 8.5

SUBSTRATE	SUBSTRATE CONCENTRATION (mM)				
	2.94	$2.94X10^{-1}$	$2.94X10^{-2}$	$2.94X10^{-3}$	$2.94X10^{-4}$
Urea	0	18	38	27	42
	0	49	46	14	47
	2	27	25	15	18
	0	101	51	16	31
Ammonium	0	0	0	0	0
	0	1	0	0	0
	0	2	0	0	0
	1	0	0	0	0
Nitrite	0	6	8	0	0
	3	3	7	3	5
	5	5	2	0	3
	2	5	1	1	0
Nitrate	38	59	37	56	41
	41	37	21	46	62
	86	25	16	29	18
	27	16	38	38	25
0 Nitrogen Control	0				
	0				
	0				
	0				

Initial Concentration = 10.1 cells/ml

Appendix G. Replicate chromosome counts for the six species studied.

Peridinium willei	*Peridinium volzii*	*Peridinium cinctum*	*Peridinium limbatum*	*Peridinium inconspicuum*	*Peridiniopsis polonicum*
122	101	225	70	44	64
254*	95	205	138*	34	57
140	93	393*	64	42	105*
97	94	402*	144*	45	57
103	116	188	134*	40	54
234*	97	203	135*	42	57
103	95	397*	73	36	60
111	102	235	78	42	54
112	93	455*	61	44	54
116	98	217	157*	42	95*

*Counts treated as diploids in determination of chromosome number.