

AN ABSTRACT OF THE THESIS OF

EMERY ALLEN SUTTON for the DOCTOR OF PHILOSOPHY
(Name) (Degree)

in OCEANOGRAPHY presented on July 17, 1970
(Major) (Date)

Title: THE PHYSIOLOGY AND LIFE HISTORIES OF SELECTED
CRYOPHYTES OF THE PACIFIC NORTHWEST

Abstract approved: Redacted for Privacy

The snow algae literature of the past 150 years is reviewed and a discussion of the possible biochemical reasons for cryophily are presented. The physiological responses to variation in light intensity, temperature, and nutrient media are presented for the following species: Chromulina chionophila Stein, Chlamydomonas nivalis (Bauer) Wille, Stichococcus bacillaris Nag., Raphidonema nivale Lagerh., and Chlamydomonas yellowstonesis Kol. Only C. chionophila is truly cryophilic. The others appear to be only cryobiontic. The morphology of C. chionophila and Chlamydomonas nivalis changes with variation in culture conditions. The life cycle of C. nivalis in culture is illustrated.

Snow algae are found in the gut of the ice worm, Mesenchytraeus solifugus, and serve as food for rotifers in culture. The role of snow algae in the ecology of ice and snow fields is discussed.

The Physiology and Life Histories of Selected Cryophytes
of the Pacific Northwest

by

Emery Allen Sutton

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1972

APPROVED:

Redacted for Privacy

Professor of Oceanography

in charge of major

Redacted for Privacy

Chairman of Department of Oceanography

Redacted for Privacy

Dean of Graduate School

Date thesis is presented

July 13, 1970

Typed by Clover Redfern for

Emery Allen Sutton

ACKNOWLEDGMENT

The number of individuals who deserve to be acknowledged for their aid in the completion of this work would comprise a list whose length would rival the length of the thesis. These individuals are already aware of my gratitude and to place their names and titles in this section appears, to me, to be unnecessary. NASA Grant NGR-38 - 002 -013 provided financial support. Mrs. Carol Standley proofread the several revisions of the manuscript.

TABLE OF CONTENTS

	<u>Page</u>
REVIEW OF LITERATURE AND GENERAL INTRODUCTION	1
Literature Cited	17
PHYSIOLOGICAL RESPONSES OF SELECTED CRYOPHYTES TO VARIATION IN LIGHT, TEMPERATURE, AND GROWTH MEDIA	25
Introduction	25
Material and Methods	27
Experimental Organisms	31
Growth of the Organisms	35
Experimental Conditions	36
Analytical Methods	38
Results and Discussion	40
Light	40
Effect of Extended Dark Periods	49
Temperature	52
Media	67
Observations of Algae in Culture	70
Summary and Collation	85
Literature Cited	89
MISCELLANEOUS OBSERVATIONS FROM FIELD AND LABORATORY	92
The Development of Blooms in the Field	92
Species Composition of the Blooms	93
Summering and Wintering	93
Ecology of Snow Fields	95
Literature Cited	98

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Ingredients of modified Pringsheim's and Holm-Hansen's media.	29
2. Total spectral energy from 400-750 m μ , mW cm ⁻² in cubicles within the partitioned box.	38
3. pH variation with concentration of Holm-Hansen's medium.	70

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Cutaway drawing of Sherer-Gillett "environator."	37
2. Spectrum of "environator."	39
3. Light-temperature-photosynthesis response curves of <u>Chromulina chionophila</u> Stein.	41
4. Photosynthesis-light-temperature response curves of <u>Stichococcus bacillaris</u> Nag.	43
5. Temperature-light-photosynthesis response curves of the spores of a laboratory strain of <u>Chlamydomonas nivalis</u> (Bauer) Wille.	45
6. Light-temperature-photosynthesis response curves of <u>Raphidonema nivale</u> Lagerh. which had been grown in Holm-Hansen's medium but which was tested in Pringsheim's medium.	46
7. Light-temperature-photosynthesis response curves of a collection of assorted spores from the field.	47
8. Temperature-light-photosynthesis response of <u>Raphidonema nivale</u> Lagerh., grown and tested in Holm-Hansen's medium.	48
9. Light-temperature-photosynthesis curves for <u>Chlamydomonas yellowstonensis</u> Kol.	50
10. This represents the change in relative photosynthesis with an extended period in the dark at 0°C for <u>Chromulina chionophila</u> Stein.	51
11. Relative photosynthesis in <u>Stichococcus bacillaris</u> Nag. as a function of time spent in the dark at 0°C.	53
12. Temperature response of <u>Chromulina chionophila</u> Stein under similar conditions but with different prehistories.	55
13. This is a composite of seven experiments using <u>Chromulina chionophila</u> Stein.	56

<u>Figure</u>	<u>Page</u>
14. Growth curves of <u>Chromulina chionophila</u> Stein at different temperatures, light intensities, and nutrient conditions.	57
15. This is the relative photosynthesis curve for <u>Stichococcus bacillaris</u> Nag. as it varies with temperature.	60
16. The above graph shows the relative photosynthesis of <u>Stichococcus bacillaris</u> Nag. as a function of temperature.	61
17. Growth curve for <u>Stichococcus bacillaris</u> Nag.	62
18. Temperature-photosynthesis relationship in <u>Chlamydomonas yellowstonensis</u> Kol.	64
19. This graph represents the relative photosynthesis of a collection of a variety of spores from the field at different temperatures.	65
20. This graph represents photosynthetic response of a collection of spores from the field as a function of temperature.	66
21. This figure shows the anomolous behavior of <u>Raphidonema nivale</u> Lagerh.	68
22. Spores of laboratory grown <u>Chlamydomonas nivalis</u> (Bauer) Wille were suspended in Holm-Hansen's medium of varying dilution.	71
23. In this experiment <u>Chlamydomonas nivalis</u> was suspended in various concentrations of Holm-Hansen's medium in which the pH had been adjusted to 4.7 in all cases.	72
24. In this experiment a collection of spores from the field were suspended in Holm-Hansen's medium, ranging from 100% of the normal to 0.1% of the normal strength.	73

<u>Figure</u>	<u>Page</u>
25. In this experiment a field collection of spores was suspended in various dilutions of Holm-Hansen's medium.	74
26. Morphological variation found in <u>Chromulina chionophila</u> Stein grown in laboratory culture.	76
27. Asexual reproduction in <u>Chlamydomonas nivalis</u> (Bauer) Wille.	81
28. The life cycle of <u>Chlamydomonas nivalis</u> (Bauer) Wille in culture.	83
29. A comparison of the amount of carbon fixed per μg of cellular carbon present.	87
30. Spore form frequently found in the field and assigned to <u>C. nivalis</u> , although the identification is uncertain.	96

THE PHYSIOLOGY AND LIFE HISTORIES OF SELECTED
CRYOPHYTES OF THE PACIFIC NORTHWEST

REVIEW OF LITERATURE AND GENERAL INTRODUCTION

"And yet, why it should prefer to make the snow its habitat, or how it can find its way into those regions of frost and infertility, remains a question which still perplexes the naturalist and philosopher."

F. C. Clark MD
The American Naturalist (1875)
9:129-135

"Until the method of propagation of this plant is more satisfactorily established . . . it will be impossible to fix its systematic position."

Romyn Hitchcock (1885)
Bulletin of the Torrey Botanical
Club
12:131-132

"While at present there appears no possibility of distinguishing these 'species' of Chlamydomonas, discovery of a method of keeping them in pure culture would open the path to a solution of the problem."

E. Kol (1942)
Smithsonian Miscellaneous
Collection
101:1-36

Anyone who has ever skied, climbed or hiked early in the year in mountainous areas of the world has probably observed a phenomenon known either as "watermelon snow" or colored snow. The color of the snow may vary from red (watermelon snow), to orange, yellow, green and even black depending on the causative agent. Black snow is usually caused by a mixture of dust and industrial air pollutants. The agents responsible for the other coloration are microscopic algae found growing in the interstitial water among the ice crystals of the snow.

The study of colored snow is not of recent origin. According to F. C. Clark (1875), Aristotle was aware of red snow. Clark also points out that De Saussure found red snow on the slopes of Mt. Breven, Switzerland, in 1760, which he classed as the pollen of some plant. While exploring the Arctic in 1818, Sir John Ross found that an expanse of the cliffs overlooking Baffin Bay was colored red for an eight-mile distance and a depth of up to 12 feet. Captain Ross collected some of the red material and presented it to Francis Bauer upon his return. Bauer (1819) examined the sample and later inoculated a jar of fresh snow with the, by then, dried powder remaining from the sample. He stirred this into the snow and observed that the coloration of the snow increased. He also noted an increase in bulk of a part of the sample which had been placed on the snow in the open. Bauer concluded that the organism went from red to green and back to

red with maturity and classified it as Uredo nivalis, a fungus.

Robert Brown, who had also received some of the original sample from Captain Ross, classified the plant as an alga. A taxonomic controversy began, with a number of people even giving the causative agent an animal status (Clark, 1875).

The research which followed in the remainder of the 19th century and the first half of the 20th century was mainly of a taxonomic and biogeographical nature: collect and classify. George Gibbs (1871) reported the discovery of red snow in the Cascade Mtns. of the Washington Territory in 1865. With the aid of a lens he identified the causative agent as "tadpole-like bodies with perhaps two tails." Professor H. C. Woods (1871) reported red snow along the 40th parallel in North America in the Sierras, East Humboldt and Clover Mt. of Nevada, and the Uintahs of Utah. In the last two decades of the 19th century there were a number of review articles written on the subject of red snow. Romyn Hitchcock (1885a, 1885b) was the author of the early works and in one such paper (1885b) reported that Mr. Eugene Manler of Switzerland had furnished him with some green cells from the snow and offered the explanation that the red and green were different stages of the same organism. He postulated that it takes a year to go from green to red. Charles Hallock (1886) discussed the source of red snow and reported the sighting of an iceberg off Labrador with a red streak in it. A. M. Edwards (1894) published a microscopic

study of red snow concerned mainly with the phylum of the causative organism.

Fritsch (1912) reported on the fresh water algae collected in the Antarctic by R. N. Rudmose Brown which dealt primarily with the cryoflora of the region. In the 1920's Erzsebet Kol began her work on the cryophytes. In 1928 she published a paper dealing exclusively with cryovegetation. This paper was followed by studies of the snow and ice flora from many parts of the world (Kol, 1935, 1938, 1939, 1941, 1942, 1944, 1949, 1957, 1961, 1964, 1968). Since 1950 other investigators have been compiling information on the snow and ice flora and the list of organisms found in these habitats continues to grow. Fukushima (1963) published the results of ten years of study on cryophytes in Japan. This work reports on distribution of cryoflora of several regions, investigations into the ecology of the organism, life cycles of some of the organisms found, and the culture of cryophytes. Janet Stein authored and co-authored a number of papers dealing with snow flora and the addition of new organisms to the list of cryophytes (Stein, 1963; Stein and Brooke, 1964; Stein and Amudsen, 1967). Garric (1965) compiled a list of cryoflora of the Pacific Northwest, including many algae which had previously been reported.

Fukushima's work (1963) in Japan, and Hardy's work (1966) in this country, appear to signal the end of purely taxonomic work. Both workers were able to culture some of the snow algae. Thus,

Hitchcock's and Kol's plea for direct observation of growing material as a means of assigning taxonomic position approaches realization.

The last few years have become a period of diminishing returns for new taxa added to the list of cryophytes. Hardy and Curl (1968) described a member of the Euglenophyta, Trachelomonas kolii, a genus not previously included in the snow algae, as a new addition to the list of cryophytes. Since then it has been found in a number of locations in Oregon.

Recently, considerable interest has been shown in marine "terrestrial" cryophytes, that is, algae growing in sea ice (Bunt, 1963, 1964; Bunt et al., 1966, 1967; Burkholder and Mandelli, 1963; Meguro, Ito, and Fukushima, 1966). It is noteworthy that this flora is almost exclusively diatom, whereas diatoms are extremely rare in snow and may be entirely accidental.

There is a possibility that a large number of species have been classified as Chlamydomonas nivalis (Kol, 1942). It is also possible that a number of algae have different names because different stages of their life cycles were described and named. Most of the studies on snow algae have been on preserved material which is subject to discoloration, shrinkage and distortion. Fukushima (1963) has stated, "The study of living specimens both in the field and in the low temperature laboratory is necessary for the investigation of these characters but the most important is pure culture."

The literature on the physiology of cryobionts is meager. Hardy (1966) showed that Chromulina chionophila has an optimum temperature of 10°C for photosynthesis when grown and examined in Bristol's medium (Bristol, 1919a, b, 1920, 1923) and that temperatures greater than 20°C were lethal. Hindák and Komarek (1968) have shown that Koliella tatrae produces the greatest biomass at 4°C and that temperatures above 10°C are lethal in Bourelly's L-C medium (Bourelly, 1951). Fukushima (1963) observed that Ochromonas smithii has an optimum growth temperature of 5°C and that temperatures above 15°C are lethal when grown in Benecke's medium (Benecke, 1898). Brock (1967a, b) makes the point, while referring to thermophilic blue-green algae, that one must distinguish between organisms which are optimally adapted to the extremes and those which grow best in less extreme conditions; that is, the organism should be examined in the natural environment. Fogg (1967) attempted to measure $\text{H}^{14}\text{CO}_3^-$ uptake in the field in the South Orkney Islands and observed rather low values for carbon fixation. I know of no other field measurements of productivity save those carried out by our group in the Oceanography Department of Oregon State University and J.S. Bunt's group on ice algae in Antarctica. The laboratory work, although not directly convertible to field situations, must therefore remain as the starting point for our physiological studies.

Field observations of the life cycles of the organisms responsible

for the coloration of the snow are very few. Stein and Amudsen (1967) made observations on the life histories of some chlorophytes classed as snow algae under near-natural conditions. Fukushima (1963) was able to observe under nearly natural conditions some facets of the life cycles of Chlamydomonas nivalis (Bauer) Wille var. Kobayashi and other chlorophytes, as well as the Crysophyte Ochromonas smithii. The probability of encountering all stages of the life cycle of an organism in the field appears extremely low. The generation time of snow algae is not known, and probably differs from species to species. Fukushima (1963) reports that colored snow appears when the daily minimum air temperature is above zero. Stein and Amudsen (1967) report that 24 hours of wet snow are required for germination of spores and vegetative reproduction to take place. However, in the laboratory I have found that a period of one hour is required after exposure to fresh nutrients, for the germination of some laboratory grown spores.

For many years the physiology of snow algae had remained only an academic curiosity. After October 4, 1957 the feasibility of space exploration became apparent. Interest in the possibility of extraterrestrial life has always been centered on Mars. To us the Martian environment (Salisbury, 1962; Glasstone, 1968) appears to be a very harsh one indeed. This is a geocentric point of view, forcing terrestrial adaptations into Martian locality (Vallentyne, 1963).

Nevertheless, we are confined in our studies to earthbound organisms. Our present knowledge of the atmosphere of Mars would indicate that the organisms occurring there would lead an anoxic mode of existence. Glasston (1968) offers some suggestions for possible patterns of energy metabolism which might be found there. He suggests that there may have evolved organisms which utilize a prebiotic carbohydrate as both a carbon and an energy source with an inorganic electron acceptor other than oxygen. Another possibility is that the hypothetical carbohydrate might be fermented by an organism or group of organisms. Photosynthesis is another form of energy conversion which might be found on Mars. This form of photosynthesis would be that found in the photosynthetic bacteria of earth since it is highly improbable that the electron donor for the reduction of CO_2 would be H_2O . It is possible that this kind of photosynthesis has evolved in organisms with some resemblance to the snow algae. They must be able to withstand large fluctuations in temperature, low available moisture, and high ultraviolet dosage. The terrestrial organisms which most closely approach this description are the algae, and in particular, the snow algae. The snow algae of the north temperate zone of moderate altitude are faced with winter freezing and desiccation, spring and summer freezing and thawing cycles, high light intensity and finally, drying and high late-summer temperatures.

Among the autotrophs, the algae are remarkably well equipped

to survive extreme conditions. Lipman (1941) reports on the successful revival of a herbarium specimen of Nostoc commune 87 years old. Friedman, Liphin, and Reamp-Paus (1967) reported on a vegetation of non-aquatic, non-lichenized algae on the Negev Desert of Israel. These algae are thought to obtain their water mainly from dew. Other reports of desert algae come from the Atacama in Chile (Forest and Weston, 1966) and the Sonoran Desert in Arizona (Cameron, 1962). Blue-green algae inhabit hot springs with temperatures of greater than 70°C as well as the ice and snow fields of various parts of the world. The snow algae are clearly able to withstand sub-zero temperature, cycles of freezing and thawing, growth at zero degrees centigrade, desiccation, and summer temperatures of 35°C or more while in the soil. These extremes are not as great as those found on Mars but they represent the terrestrial approximation of a Martian environment.

It is generally agreed that organisms having temperature optima under 20°C may be termed cryophilic (psychrophilic). It is also generally agreed that organisms having temperature maxima under 20°C should be called obligate cryophils. Since little is known about the life histories and physiology of the snow algae the term cryophyte or cryoplankton is used to describe any of the algae collected from the ice or snow substratum. This will change as life histories and physiology are elucidated.

Most of the information concerning the biochemical basis for cryophily has been achieved through the use of bacteria and yeast as research organisms. These findings and proposed causes may be arranged in the following categories:

1. Extremely thermolabile enzymes:

Edwards and Rettger (1937) found that the maximum growth temperature was related to the minimal destruction temperature of respiratory enzymes. Militzer et al. (1949) compared the stability of malic dehydrogenase from a thermophilic bacterium, a mesophilic bacterium and kidney homogenate. They found that the enzyme from the thermophil was stable at 65° C while the mesophilic enzyme was inactivated after ten minutes at 65° C. Militzer et al. (1951) investigated the stability of an unbound enzyme, aparase (activity of enzyme[s] responsible for the hydrolysis of two phosphate groups of ATP). They found that aparase from a mesophil was less stable than that from a thermophil and that aparase from a potato was easily inactivated. Hagen and Rose (1962) studied the growth of the cryophilic yeast Cryptococcus. The low maximum temperature was thought to result from certain exceptionally thermolabile enzymes involved in the formation or synthesis of keto glutarate. Upadhyay and Stokes (1963a) found that formic hydrogenlyase (in a

cryophilic bacterium) was active at 30°C but inactive at 45°C while in Eschericia coli, on the other hand, was most active at 45°C. The hydrogenlyase forming system was extremely heat sensitive, being inactivated at 20°C whereas E. coli, Salmonella oranienburg, and Proteus vulgaris formed the enzyme at 45°C. Upadhyay and Stokes (1963b) found that hydrogenase was not formed at temperatures above 20°C in a psychophilic bacterium whereas it was formed at 45°C in E. coli and other mesophils. The psychophilic hydrogenase was 50 percent destroyed after two hours at 60°C and the mesophil hydrogenase was 25 percent destroyed upon receiving the same treatment. Burton and Morita (1963) proposed that heat lability of malic dehydrogenase and increased cell permeability were factors which relegated the marine bacterium Vibrio marinus strain PS207 to temperatures below 39°C and that malic dehydrogenase instability contributed to the inability of Vibrio marinus strain MP-1 to grow above 20°C. Langridge and Morita (1966) further implicated malic dehydrogenase thermolability as being partially responsible for the low maximum temperature in the marine psychophilic bacterium Vibrio marinus MP-1. Evison and Rose (1965) did a comparative study on the biochemical basis of the maximum temperature of growth of cryophilic

organisms and concluded that succinate metabolism was responsible in the bacterium Arthrobacter sp. and the yeast Candida sp.

2. Cellular or biochemical organization:

Ingraham and Bailey (1959), after studying the effects of temperature on the metabolism of mesophilic and psychrophilic bacteria and the homogenates from these bacteria, suggested that the differences between the mesophils and the psychrophils in their response to temperature may be a result of the biochemical organization of the organism and not be attributable to the properties of the individual enzymes.

3. Energy of activation of enzymes:

Morita and Burton (1965) found that the energy of activation of malic dehydrogenase from Vibrio marinus MP-1 was one-half the energy of activation of malic dehydrogenase from Eschericia coli. This led to the suggestion that a possible explanation of the rapid growth of obligate psychrophils at low temperatures is an overall lower energy of activation for cellular reactions.

The above hypotheses pertain to bacteria and yeast and their direct application to photosynthetic organisms should be with reservation. Sorokin (1960) has discussed the complication of analysis of temperature affects on photoautotrophic organisms by the

complimentary effect of light. So far as the snow algae are concerned, only three species have been reported to behave as obligate cryophils: Chromulina chionophila (Hardy, 1966), Ochromonas smithii (Fukushima, 1963), and Koliella tatrae (Hindák, 1968). Of these three, only Chromulina chionophila has had any physiological studies performed on it. Hardy (1966) indicates that the apparent cryophilic nature of this organism is due to a breakdown in the Krebs cycle which leads to disruption of the cell membrane. This organism appears to be a true cryophil in that it will not grow at or above 15°C but does grow well at 0°C.

The physiological problems of growth at lowered temperatures has been investigated to only a limited extent, and some of the other responses to environmental phenomena such as (1) freezing-thawing cycles, (2) desiccation and (3) exposure to high light intensity have had no more attention.

1. Freezing-thawing cycles:

Holm-Hansen (1963) has done some work on the survival of some blue-green and some green algae when subjected to rapid cooling. Holm-Hansen's work does not allow one to evaluate the relative contribution of the rate of cooling and the temperature to the decrease in survival rate. Little is known of the cyclic freezing and thawing cycles in the areas where snow algae are common, and the mechanism of "frost

resistance" of the common members of the snow algae flora is not known nor has it been investigated.

2. Desiccation:

The manner in which the snow algae resist desiccation is not known. Desiccation becomes a problem which the cell must face during periods of freezing and during the times of exposure to the hot and dry summer months. Although some cells have heavy-walled spores there are others for which spores have not been found and in neither case has the effect of drying been investigated.

3. Exposure to high light intensity:

The high-altitude snow fields and glaciers upon which snow algae are found are subject to high light intensity. Often the snow algae are found in the upper few centimeters of the snow exposed to nearly full intensity sunlight. Many of the snow algae are red or orange pigmented and this has been thought of as a protective adaptation. The fact remains that the protective nature of this pigmentation has yet to be proven. Brown et al. (1967) have shown that the concentration of red pigment in Chlorococcum wimmeri, a non-cryophilic alga, is a primary function of light intensity although it is enhanced by near red illumination and also influenced by CO₂ concentration. Bold and Parker (1962)

suggested that, in addition to high light intensity, desiccation and age were factors in the increase in red pigment in C. wimmeri. There was no clear separation between the effects of light and desiccation. Goodwin and Jamikorn (1954) attributed the intermediate coloration between green and red stages of Haematococcus pluvialis to quantitative differences in astaxanthin content. Droop (1955) supported the hypothesis of the photosynthetic origin of astaxanthin in H. pluvialis and also pointed out the discrepancy between the laboratory grown cells, which are red as resting spores and green during flagellated stages, while in nature all stages are red. Since a large proportion of the snow algae possess a red pigmentation it seems necessary to investigate further the possible relationship between pigment production and exposure to high light intensity. It is therefore believed that research along the above lines could offer some insight into the different physiological and biochemical mechanisms which have developed in the snow algae to meet the challenge of their environment. The differences, if they occur, between cryophils and algae with higher temperature optima should be explored.

It is fully realized that the conclusions drawn from this work are necessarily valid only under the conditions described in the procedure

for each experiment. The results will, however, increase the meager amount of knowledge which is now available concerning the physiological ecology and biochemistry of the snow algae.

Literature Cited

- Bauer, F. (1819)
 Microscopic Observations on the Red Snow
 The Quarterly Journal of Literature, Science and Arts
 7:222-229
- Benecke, W. (1898)
 Uber Culturbedingungen einiger Algen
 Botanische Zeitschrift 56:83-96
- Bold, H. C. and B. C. Parker (1962)
 Some Supplementary Attributes in the Classification of Chlorococcum
 Species
 Archiv fur Mikrobiologia 42:267-288
- Bourelly, P. (1951)
 "Catalogues des Collections Vivants, Herbiers et Museum"
 Nationale D'Histoire Naturelle Laboratoire de Cryptogamie. Paris
- Bristol, B.M. (1919a)
 On a Malay Form of Chlorococcum sinicola (Nag.) Rabenh.
 Journal of the Linnean Society of Botany 44:473-482
- _____ (1919b)
 On the Retention of Vitality by Algae from Old Stored Soils
 New Phytologist 18:92-104
- _____ (1920)
 On the Algal Flora of Some Desiccated English Soils: An Important
 Factor in Soil Biology
 Annals of Botany 34:35-80
- _____ (1923)
 Alga in "The Microorganisms of the Soil"
 E. J. Russel editor
- Brock, T.D. (1967a)
 Microorganisms Adapted to High Temperatures
 Nature 214:882-885
- _____ (1967b)
 Life at High Temperatures
 Science 158:1012-1019

Brown, T. E. , F. L. Richardson and M. L. Vaughn (1967)
Development of Red Pigmentation in Chlorococcum wimmeri
(Chlorophyta: Chlorococcales)
Phycologia 6(4):167-184

Bunt, J.S. (1963)
Diatoms of Antarctic Sea Ice as Agents of Primary Production
Nature 199:1255-1257

_____ (1964)
Primary Production Under Sea Ice in Antarctic Waters
Antarctic Research Series 4:13-26

_____ (1967)
Some Characteristics of Microalgae Isolated from Antarctic Sea Ice
Antarctic Research Series 7:1-14

Bunt, J.S. , O. V.H. Owens, and G. Hoch (1966)
Exploratory Studies on Physiology and Ecology of Marine
Psychrophilic Diatoms
Journal of Phycology 2:96-100

Burkholder, P. R. and E. F. Mandelli (1963)
Productivity of Microalgae in Antarctic Sea Ice
Science 149:872-874

Burton, S. D. and R. Y. Morita (1963)
Denaturation and Renaturation of Malic Dehydrogenase in Cell-Free
Extract from a Marine Psychrophile
Journal of Bacteriology 86:1019-1024

_____ (1965)
Kinetics of Enzyme Reactions and Synthesis in an Obligate
Psychrophilic Bacterium
Bacteriological Proceedings of the Society of American Bacteriologists
p. 20 only

Cameron, R. E. (1962)
Species of Nostoc Vaucher Occurring in the Sonoran Desert of Arizona
American Microscopical Society Transactions 81(4):379-384

Clark, F. C. (1875)
Red Snow
American Naturalist 9:129-135

- Droop, M. R. (1955)
Cartenogenesis in Haematococcus pluvialis
Nature 175(4444):42
- Edwards, A. M. (1894)
Red Snow as Seen by Means of a Microscope
American Monthly Microscopical Journal 15:70-74
- Edwards, O. F. and L. F. Rettger (1937)
The Relation of Certain Respiratory Enzymes to the Maximum
Growth Temperature of Bacteria
Journal of Bacteriology 34:489-515
- Evison, L. M. and A. H. Rose (1965)
A Comparative Study on the Biochemical Bases of Maximum Tem-
perature for Growth of Psychrophilic Organisms
Journal of General Microbiology 40(3):349-364
- Fogg, G. E. (1967)
Observations on the Snow Algae of the South Orkney Islands
Philosophical Transactions of the Royal Society of London
Series B, vol. 252:p. 279-287
- Friedman, I. Y., R. Reampo-Paus Lipkin (1967)
Desert Algae of the Negev (Israel)
Phycologia 6(4):185-201
- Forest, H. S. and C. R. Weston (1966)
Blue Green Algae from the Atacoma Desert of Northern Chile
Journal of Phycology 2:163-164
- Fritsch, F. E. (1912)
Freshwater Algae Collected in the South Orkney's
by R. N. Rudmose Brown B. Sc. of Scottish National Antarctic
Expedition (1902-1904)
Journal of Linnean Society of Botany 40:293-338
- Fukushima, H. (1963)
Studies on Cryophytes in Japan
Journal of Yokohama Municipal University
(Series C) 43(144):1-46
- Garric, R. K. (1965)
The Cryoflora of the Pacific Northwest
American Journal of Botany 52(1):1-8

- Gibbs, G. (1871)
Red Snow in Washington Territory
American Naturalist 5:166-117
- Glasstone, S. (1968)
"The Book of Mars"
National Aeronautics and Space Administration
Washington, D. C.
- Goodwin, T. W. and M. Jamikorn (1954)
Studies in Carotenogenesis II. Carotenoid Synthesis in the Alga
Haematococcus pluvialis
Biochemical Journal 57:376-381
- Hagen, P. O. and A.H. Rose (1962)
Studies on the Biochemical Basis of the Low Maximum Temperature
in a Psychrophilic Cryptococcus
Journal of General Microbiology 27:89-99
- Hallock, C. (1886)
Occurrence of Red Snow
American Monthly Microscopical Journal 7:42-43
- Hardy, J. T. and H. C. Curl, Jr. (1968)
New Species of Trachelomonas
Journal of Phycology 4:9-12
- Hardy, J. T. (1966)
Identification, Culture, and Physiological Ecology of Cryophilic Algae
M. S. Thesis
Oregon State University, Corvallis, Oregon 97331
- Hindák, F. and J. Komárek (1968)
Cultivation of the Cryosestonic Alga Koliella tatrae
(Kol) Hind.
Biologia Plantarum 10(2):95-97
- Hitchcock, R. (1881a)
Red Snow
American Monthly Microscopical Journal 2:74
- _____ (1881b)
Red Snow in Colorado
American Miscoscopical Journal 2:155

- _____ (1885a)
Red Snow
Bulletin of the Torrey Botanical Club 12:131-132
- _____ (1885b)
The Red Snow
American Monthly Microscopical Journal 6:221-224
- Holm-Hansen, O. (1963)
Viability of Blue Green and Green Algae After Freezing
Physiologia Plantarum 16:531-540
- Ingraham, J. L. and G. F. Bailey (1959)
Comparative Study of the Effect of Temperature on the Metabolism
of Psychrophilic and Mesophilic Bacteria
Journal of Bacteriology 77:609-613
- Kol, E. (1928)
Uber die Kryovegetation der Hohen Tatra I.
Folia Cryptogamica 1(6):613-622
- _____ (1935)
Uber die Kryovegetation des Retvzat and der umliegenden
Gebirge in Transsylvanien Verhandlungen der Internationale
Vereinigung fur Limnologie 7:475-486
- _____ (1938)
Some New Snow Algae from North America
Journal of Washington Academy of Sciences 28:55-58
- _____ (1939)
Zur Schneevegetation Patagoniens
Arkove fur Botanik 29 A(20):1-4
- _____ (1941)
Green Snow of Yellowstone National Park
American Journal of Botany 28(3):185-191
- _____ (1942)
The Snow and Ice Algae of Alaska
Smithsonian Miscellaneous Collections 101(16):1-36
- _____ (1944)
Vergleich der Kryovegetation der Nordlichen und Sudlichen
Hemisphare
Arkove fur Hydrobiologie 40:835-846

- _____ (1949)
 Vergleich der Kryovegetation der Alpen und der Karpaten
 Verhandlungen der Internationale Vereinigung für
 Limnologie 10:243-246
- _____ (1957)
 Über die Verbreitung der Schnee und eisbewohnenden
 Mikroorganismen in Europa I.
 Arkiv für Hydrobiologie 53(4):574-582
- _____ (1959)
 Red Snow of Greenland I. West Greenland
 Acta Botanica 5:55-70
- _____ (1961)
 Über roten und grünen Schnee der Alpen
 Verhandlungen der Internationale Vereinigung für
 Limnologie 14:912-917
- _____ (1964)
 Cryobiological Research in the Rocky Mountains
 Arkiv für Hydrobiologie 60(3):278-285
- _____ (1968)
 Die Binnengewässer Band XXIV
 E. Schweizerbart'sche Verlagsbuchhandlung
 Stuttgart 1968, 216 pgs., 16 Tables
- Kol, E. and E. A. Flint (1968)
 Algae in Green Ice from the Balleny Islands, Antarctica
 New Zealand Journal of Botany 6:249-261
- Langridge, P. and R. Y. Morita (1966)
 Thermolability of Malic Dehydrogenase from the Obligately
 Psychrophile Vibrio marinus
 Journal of Bacteriology 92(2):418-423
- Lipman, C. B. (1941)
 The Successful Revival of Nostoc commune from a Herbarium
 Specimen Eighty-seven Years Old
 Bulletin of Torrey Botanical Club 68:664-667
- Meguro, H., K. Ito, and H. Fukushima (1966)
 Diatoms and the Ecological Conditions of Their Growth in Arctic Sea Ice
 Science 152:1089-1090

Militzer, W., T. B. Soderegger, L. C. Tuttle, and C. E. Georgi
(1949)

Thermal Enzymes

Archives of Biochemistry 24:75-82

_____ (1951)

Thermal Enzymes III Aparase from a Thermophilic Bacterium

Archives of Biochemistry and Biophysics 24:416-423

Morita, R. Y. and S. D. Burton (1965)

Influence of Moderate Temperature on Growth and Malic
Dehydrogenase of a Marine Psychrophile

Journal of Bacteriology 86:1025-1029

Salisbury, F. B. (1962)

Martian Biology

Science 136:17-26

Sorokin, C. (1960)

Kinetic Studies of Temperature Effects on the Cellular Level

Biochemica et Biophysica Acta 38(2):197-204

Stein, J. (1963)

A Chromulina (Chrysophyceae) from Snow

Canadian Journal of Botany 41:1367-1370

Stein, J. and C. C. Amudsen (1967)

Studies on Snow Algae and Fungi from the Front Range of Colorado

Canadian Journal of Botany 45:2033-2045

Stein, J. and J. C. Brooke (1964)

Red Snow from Mt. Seymour, British Columbia

Canadian Journal of Botany 42:1183-1188

Upadhyay, J. and J. L. Stokes (1963a)

Temperature Sensitive Formic Hydrogenlyase in a Psychrophilic
Bacterium

Journal of Bacteriology 85:177-185

_____ (1963b)

Temperature Sensitive Hydrogenase and Hydrogenase Synthesis in
a Psychrophilic Bacterium

Journal of Bacteriology 86(5):992-998

Vallentyne, J.R. (1963)
Environmental Biophysics and Microbial Ubiquity
New York Academy of Sciences 108:342-352

Woods, H.C. (1871)
Algae in "U. W. Geological Exploration of the 40th Parallel" p. 415

PHYSIOLOGICAL RESPONSES OF SELECTED CRYOPHYTES TO
VARIATION IN LIGHT, TEMPERATURE, AND GROWTH MEDIA

Introduction

Although the phenomenon of colored snow has been known for some time (Clark, 1875) and the nature of the causative agents has been argued since the early days of investigation (Hitchcock, 1885) little work has been done to elucidate the physiological or biochemical characteristics of members of this cryophytic flora. More recent cryophycological research (circa Bauer, 1819, to the present) has been devoted to problems of distribution and taxonomy, a logical result of the difficulty of growing these organisms in culture. This section is devoted to a comparison of the behavior of selected cryophytes to changes in their environments as simulated in the laboratory.

Some members of the cryoflora are listed in the pure culture collections of both the University of Indiana and Cambridge University in England, but little is known of their behavior. Fukushima (1963) cultured Chlamydomonas nivalis, Stichococcus bacillaris, and Chodatella brevispina. He did not report on any physiological responses of these organisms. He did, however, report that Ochromonas smithii lysed when subjected to a rapid rise in temperature. He reported further, that upon very gradual warming the cells changed to globose cysts and lost their flagella. He observed that

when the medium is subsequently cooled to 4°C motile cells reappear. From this observation he concluded that the cysts change reciprocally into motile cells, although the process of change had not been observed. Fukushima judged the maximum living temperature for this organism to be 15°C with an optimum below 5°C.

The work of Hardy (1966) was concerned with the responses of another cryophyte to changes in temperature and light intensity. He observed that Chromulina chionophila had a photosynthetic maximum at 10°C for short term (two hours of incubation in the light) experiments. He also reported that cell disruption occurred between 15°C and 20°C, in Bristol's solution. Hardy further showed that Krebs cycle inhibitors would produce cell disruption even at low temperatures while inhibitors of glycolysis had no effect on cell integrity.

Hindák and Komarek (1968) stated that the green alga Koliella tatrae achieved maximum biomass when grown in L-C medium (Bourelly, 1951) at 4°C and that temperatures above 10°C were lethal to the organism.

The above examples comprise the bulk of the work which has been reported on the physiological responses of members of the cryoflora to changes in environmental factors that might affect the algae in the natural situation. Although the results achieved in the laboratory cannot be extrapolated directly to the field they nonetheless aid in understanding the physiological nature of the cryophytes and

perhaps lend some insight for answers to questions of why they are found in the ice and snow. However, the information gathered may, in some cases, be applicable only when referring to a given set of conditions such as those surrounding a particular laboratory experiment.

Material and Methods

The organisms used in the experimental work reported on this paper were collected from the snow fields on and around the base of Mount Bachelor, located at longitude 121°41'W and latitude 43°59'N in Central Oregon, U.S. A. Mount Bachelor has a basal elevation of 6,300 feet. The cells were collected in polyethylene vials and packed in snow in an insulated box for transport to the laboratory. The samples were held in the (Styrofoam^R) box for a minimum of three hours during transport to the laboratory. The samples could be kept in the snow-packed (Styrofoam^R) box for periods of up to four days with no apparent loss in viability.

The samples were examined microscopically, the species composition recorded, and then inoculated into selected medium. Over the period of investigation (1966-1970) changes in synthetic culture media were made. In the beginning, Bristol's (1919a, 1919b, 1920, 1923) medium was used for all snow algae in culture but growth was extremely slow (Hardy, 1966). The next medium was

E.G. Pringsheim's (1966) for Haematococcus pluvialis and related species. This medium is very dilute and is easily prepared. It was modified to fit the existing culture facilities of the laboratory, specifically the vitamins and trace metals are those used in the culture of marine phytoplankton. No difference was found in the growth of the organisms when this change was made. The ingredients of this modified medium are listed in Table 1A.

It was later found that certain algae could be induced to begin active growth in a medium proposed by Holm-Hansen (1964). A modified version of this medium appears in Table 1B. This medium has proven to be the superior medium for growing Chlamydomonas nivalis and Raphidonema nivale.

The concentration of the nutrients in these media is considerably higher than that found in snow melt water. Recently the concentration of nutrients and heavy metals found in snow melt has been examined, using the Auto-Technicon^R for PO_4^{\equiv} , NO_3^- , and SO_4^{\equiv} , and the Perkin-Elmer model 303 atomic absorption spectrophotometer for metals such as Mg, Ca, Zn, Fe and others (Curl, unpublished data).

These examinations have shown that the NO_3^- concentration of 44 snow melt samples ranged from 0.00 to 3.37 μM with a mean of 0.79 μM . The concentration of NO_3^- in Pringsheim's medium is 0.99 mM and 1.76 mM for Holm-Hansen's medium. The PO_4^{\equiv} concentration of 48 snow melt samples ranged from 0.00 to 0.95 μM with a

Table 1. Ingredients of modified Pringsheim's and Holm-Hansen's media.

A. Pringsheim's medium

KNO ₃	100 mg/l.
(NH ₄) ₂ HPO ₄	20 mg/l.
MgSO ₄ · 7H ₂ O	20 mg/l.
CaSO ₄	20 ml of saturated solution/l.
Fe Sequestrene	10 mg/l.
Vitamins	
Thiamine HCl	0.2 mg/l.
Biotin	1.0 mg/l.
B ₁₂	1.0 mg/l.
Trace metals	
CuSO ₄ · 5H ₂ O	0.196 mg/l.
ZnSO ₄ · 7H ₂ O	0.044 mg/l.
CoCl ₂ · 6H ₂ O	0.022 mg/l.
MnCl ₂ · 4H ₂ O	0.360 µg/l.
Na ₂ MoO ₄ · 2H ₂ O	0.030 µg/l.

B. Holm-Hansen's medium

K ₂ HPO ₄	39 mg/l.
MgSO ₄ · 7H ₂ O	20 mg/l.
NaNO ₃	150 mg/l.
NaSiO ₃ · 9H ₂ O	60 mg/l.
CaCl ₂	27 mg/l.
Na ₂ CO ₃	20 mg/l.
Fe Citrate	6 mg/l.
EDTA	1 mg/l.

Vitamins and trace elements are the same as in Pringsheim's medium

mean of $0.30 \mu\text{M}$. Pringsheim's and Holm-Hansen's media contain 0.15 mM and 0.39 mM , respectively. Also occurring in the snow but not used in Pringsheim's medium is SiO_3^- . This ion occurred in 41 samples of snow water with a concentration of from 0.00 to $17.0 \mu\text{M/L}$ and is added to Holm-Hansen's medium at $214 \mu\text{M/L}$. In this way Holm-Hansen's medium more closely approximates the mineral content of snow melt water. The NO_3^- and PO_4^- concentrations are three orders of magnitude greater in the media than in the snow but, when the concentration of Holm-Hansen's medium is diluted to the point where NO_3^- and PO_4^- concentrations approach those of snow, Chlamydomonas nivalis will not grow. The answer may lie in a complete analysis of the heavy metals found in the snow. This has not been completed, but preliminary results indicate that the proportions are far different in nature than in these culture media although the concentrations are of the same order of magnitude.

The organisms were isolated in unialgal cultures in the following way. When the samples arrived at the laboratory they were usually split and inoculated into Pringsheim's and Holm-Hansen's media. The organisms were incubated at 0° , 2° , 4° , or 10°C . The resulting cultures were then streaked onto nutrient agar plates which were prepared after the method of Allen (1968). The resulting colonies were then picked from the plate and placed in one or both media. In the case of some large spores it was also possible to extract one from a

microscope slide by means of a micropipette and place it in the desired nutrient solution. No attempt was made to obtain bacteria-free cultures. Stock cultures were maintained at 2-4°C under fluorescent lights at 2.0 mW cm^{-2} intensity in the most favorable medium.

Experimental Organisms

The organisms used in the following experiments were:

Stichococcus bacillaris Nag., Raphidonema nivale Lagerh., Chromulina chionophila Stein, Chlamydomonas yellowstonensis Kol, Chlamydomonas nivalis (Bauer) Wille, and a collection of spores from the field which included: Scotiella nivalis (Schuttleworth) Fritsch, Chodatella brevispina Fritsch, Trachelomonas kolii Hardy and Curl, and other spores which were not identified.

Stichococcus bacillaris is a very small green alga, $1-1.5 \times 4-12 \mu$, which is widely reported as part of the snow flora. It was first reported by Nageli (1849) and many species have been described in this genus which would probably best be placed in this same species. The morphology of this organism changes with culture conditions (Schroder, 1954) and similar behavior may be expected from this species in nature.

Stichococcus bacillaris can become a laboratory weed unless caution is used in culturing. The organism used in experiments was

isolated from dry, hot sand, at 7000 feet elevation on Mt. Bachelor, from which a snow melt pool had recently receded. Being non-motile S. bacillaris grows very slowly at the bottom of the culture vessel and remains in fairly low concentration unless means are provided for exposing the cells to more nutrients. When constant aeration was employed the cell concentration rose to more than 25×10^6 cells ml⁻¹ in 12 days from a small inoculum.

Raphidonema nivale Lagerh. is very often reported in samples collected from snow and ice. Although Hindák (1963) had divided the genus Raphidonema into two genera Koliella and Raphidonema, this distinction will not be made here since this pleomorphic genus will form a phenotype of either genus or a mixture of both depending on the condition of growth in the laboratory. This species grows very slowly under most conditions in the laboratory. Under constant light of 2.76 mW cm^{-2} at 10°C with constant aeration in Holm-Hansen's medium the organism reached a cell concentration of 1.8×10^6 cells ml⁻¹ after a long lag phase.

Chromulina chionophila was first described by Stein (1963) when it appeared in cultures which had been inoculated with snow. This organism previously had not been reported in nature but the author has seen a number of vegetative cells taken from (25-30 cm below the surface of the) snow near Todd Lake in the Mt. Bachelor area. Dr. H. C. Curl, Jr. (pers. comm.) has also observed this organism

in nature in this same region, near Todd Lake. The first physiological studies performed on C. chionophila were those of Hardy (1966) in which he showed that this organism behaved as a true cryophil, temperature in excess of 15°C being lethal. This organism is closely related to, and behaves much like, another crysophyte from the snow, Ochromonas smithii as described by Fukushima (1963).

Chromulina chionophila was examined for ^{14}C uptake when grown in different media, under different light and temperature regimes and when held in the dark at 0°C for varying length of time.

Chlamydomonas yellowstonensis was described from samples of green snow by Kol (1941) and was named as a new species, differing from C. nivalis in (1) shape of cells, (2) size of cells, (3) shape and size of zygospores, and (4) color of the cells. This organism remains green and does not produce the red pigment associated with C. nivalis. The taxonomy of the genus Chlamydomonas is very complicated (Ettl, 1965) and in many cases the assignment arbitrary. Our organism was compared with culture of C. yellowstonensis obtained from the University of Indiana collection and found to be morphologically the same.

Chlamydomonas nivalis (Bauer) Wille is most commonly reported as the causative agent of red snow. It does not occur in all collections but it is recorded in higher frequency than any other. Since little is known about the life history of this organism and since

it is seldom collected in the vegetative state but rather in the spore form, it is possible that more than one organism has been assigned this name. The spores collected in the field are extremely difficult to germinate. Once Chlamydomonas nivalis germinates it tends not to respond to attempted manipulation of its life cycle. One often has a mixture of several kinds of spores, vegetative non-motile cells, some motile cells and often times some newly-formed zygotes. It was decided, therefore, to look at the spore stages only (this being the only stage in which may occur as nearly 100 percent the same phase). These cells were not placed in the dark overnight as there is a tendency for many laboratory-grown spores to germinate in a very short period, 1-2 hours, after exposure to fresh nutrients. It would also be imprudent to assume that all the spores result from the same process or are in the same physiological state. A safer assumption would be that some of the spores were caused by a cessation of growth as described by Droop (1955) in Haematococcus pluvialis, and that others are perhaps the zygotes of the organism. The spores of C. nivalis tend to clump, causing difficulty in dispersing the cells equally into bottles for ^{14}C uptake measurement. The field collection of spores was collected on Dutchman's Flat along Century Drive near Mt. Bachelor from red snow patches in the open. The field collection of spores consisted of Chlamydomonas nivalis (Bauer) Wille, Scoteilla nivalis (Schuttleworth) Fritsch, Chodatella brevispina

Fritsch, Trachelomonas kolii Hardy and Curl, and others not identified. C. nivalis was the primary organism in the collection with T. kolii next in abundance. The cells which were to be used in ^{14}C uptake studies were kept at 0°C on a light-dark cycle of 15 hours light and nine hours dark. These cells were gently centrifuged and the supernatant saved for use as a medium in subsequent experiments.

Growth of the Organisms

The different organisms were grown under conditions which seemed to produce the best growth for each alga. However, the organisms may not have been grown at the absolute optima of nutrients, temperature, and light. The cells of the field collections were used directly. Spores of Chlamydomonas nivalis were obtained from laboratory cultures in which all cells had encysted, and spore counts revealed a constant number. The other organisms were used during log or early stationary phase.

Prior to experiments a portion of parent culture was withdrawn and placed in four liters of fresh medium at 0°C in the dark for at least 12 hours and up to a maximum of ten days. After the dark period the culture was stirred to produce a homogeneous distribution of cells. The culture was then dispensed into 60 ml light and dark bottles and each bottle was inoculated with $1.6 \mu\text{C}$ of H^{14}CO_3 and incubated in the light for a period of one-half hour.

Experimental Conditions

A Sherer-Gillet^R "environator" equipped with fluorescent and incandescent lights was used in all experiments (Figure 1). The temperature in the environator can be adjusted from -20°C to 50°C . A partitioned box, painted a flat black to minimize reflected light, with eight individual cubicles held the bottles. The temperature within the environator varied by $\pm 0.5^{\circ}\text{C}$ at any given point. The light intensity was varied through the use of neutral density filters made of layers of plastic window screen. Each filter was constructed to cover a single cubicle in the partitioned box. The neutrality of the filters was tested using a Bausch and Lomb Spectronic 505^R spectrophotometer. No variation in absorbance was found from $400\text{ m}\mu$ to $700\text{ m}\mu$. Since the light sources did not furnish uniform intensity it was determined for each cubicle in the partitioned box (Table 2) and appropriate filters were chosen so that a more or less linear increase in intensity was obtained.

The spectrum of the environator (Figure 2) was recorded using an ISCO^R model SR Spectroradiometer. The spectral energy curve was integrated to give the total energy.

The maximum energy available (filtered), 8.37 mW cm^{-2} , is greater than the spectral energy available at the surface of the snow during overcast or stormy weather; 2.66 mW cm^{-2} (Gessner, 1955),

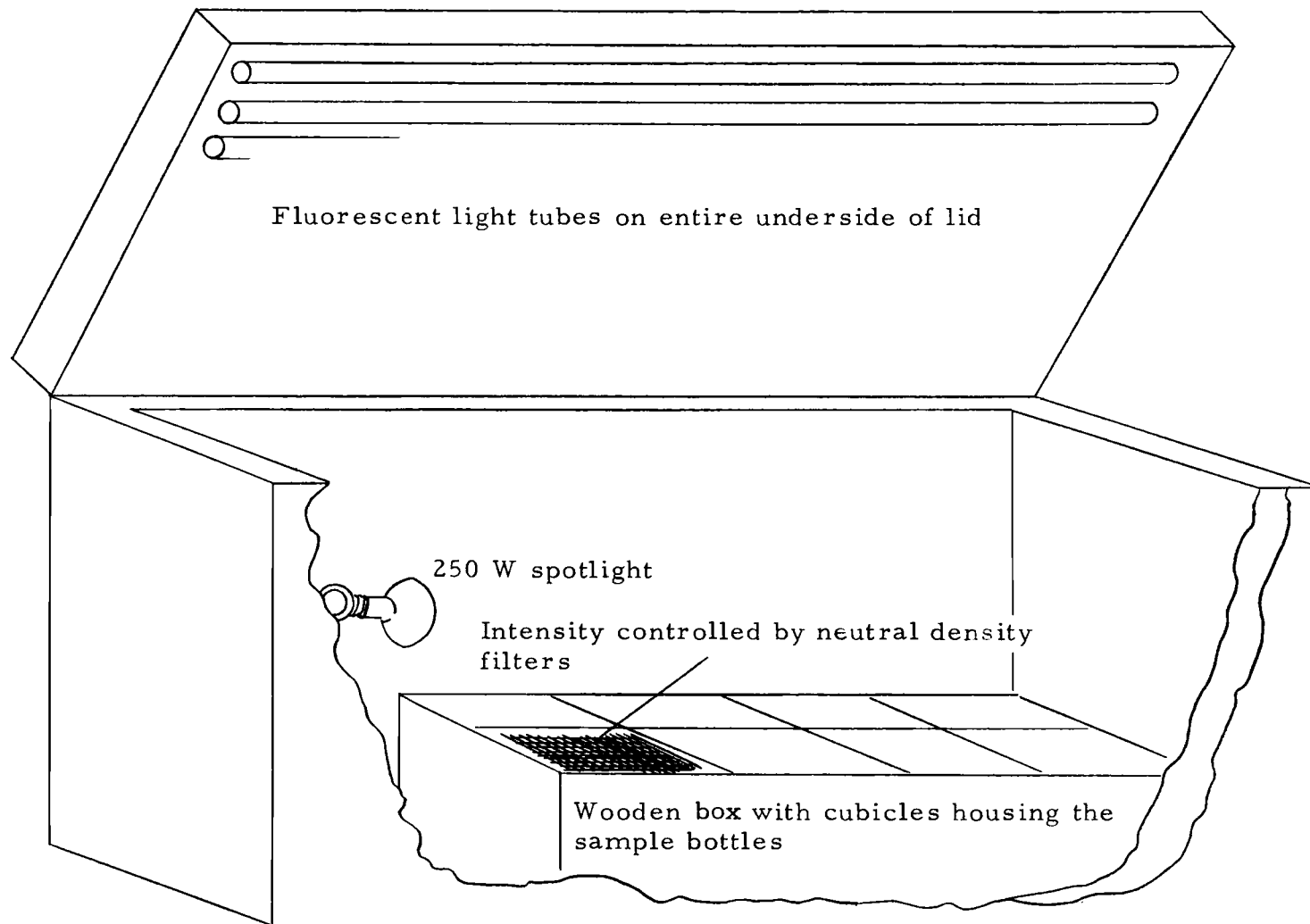


Figure 1. Cutaway drawing of Sherer-Gillett "environator."

5.22 mW cm⁻² (Hardy and Curl, unpublished data). It is far less than that available on clear days; 19.92 mW cm⁻² (Gessner, 1955) and 20.38 mW cm⁻² (Hardy and Curl, unpublished). The lowest energy level which was used in the environator was 0.217 mW cm⁻². This approximates the level which might be found in the snow at a depth of from 8-30 cm, depending on the weather and density of the snow.

Table 2. Total spectral energy from 400-750 m μ , mW cm⁻² in cubicles within the partitioned box.

Cubicle	Without Filter	With Filter
1	7.72	7.72
2	5.28	5.28
3	11.31	8.37
4	6.15	6.15
5	6.05	1.33
6	5.56	3.33
7	4.32	0.22
8	4.36	2.39

Analytical Methods

Chlamydomonas nivalis, Chlamydomonas yellowstonensis and the field collections were retained by 0.8 μ membrane filters and the remaining organisms by 0.45 μ filters. The filters were glued to planchettes and allowed to dry. They were then counted for Beta activity using a Nuclear Chicago^R Model 181 B counter with an efficiency of 25 percent. During the earlier experiments the organisms

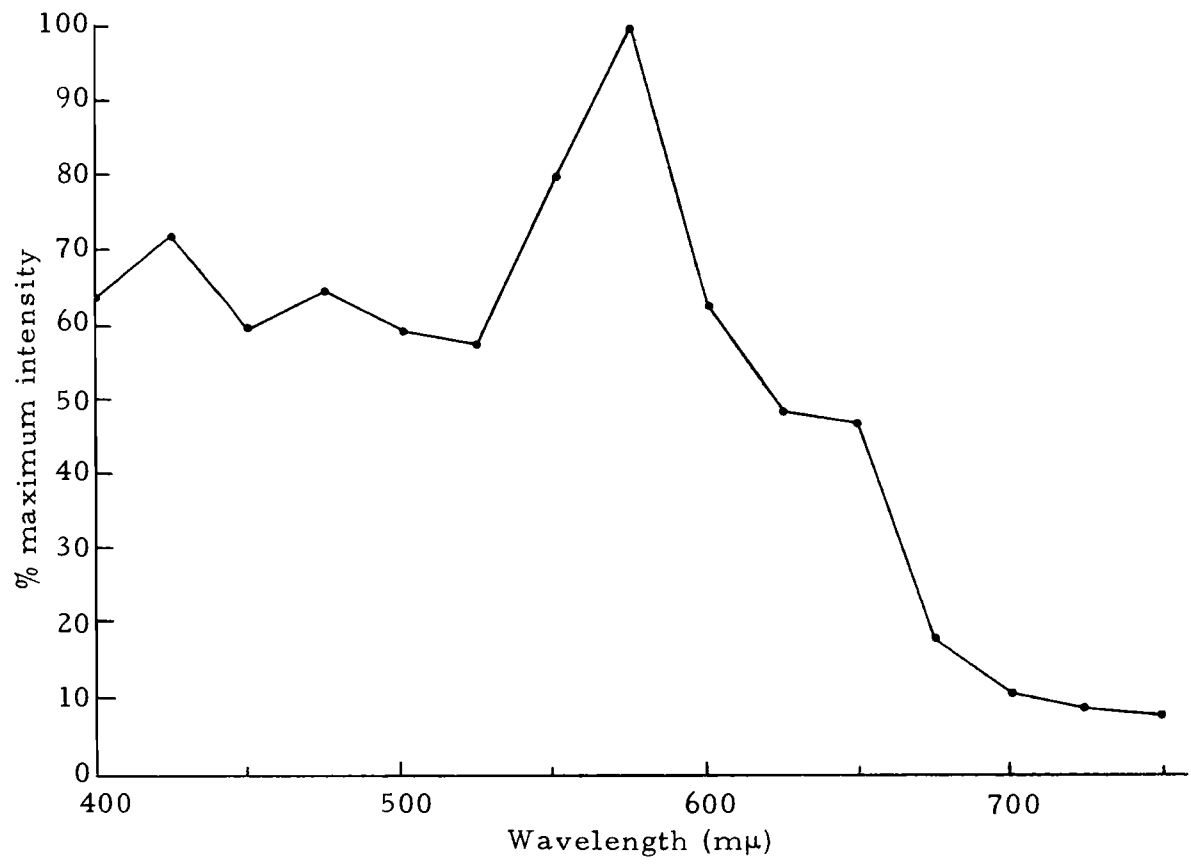


Figure 2. Spectrum of "environator." The spectrum is shown as % maximum intensity. The total spectral energy, 400-750 mμ, for each cubicle appears in Table 2.

were compared on a basis of counts per minute per cell. In later work the basis of comparison was changed to counts per minute per μg of carbon. For the carbon analysis an aliquot of cell suspension was retained by a Whatman GFA glass filter and, after drying, burned in a Hewlett-Packard model 185 gas chromatograph to obtain the total carbon per unit volume of cell suspension.

Results and Discussion

Light

The responses of the organisms tested to variation in light intensity fall into three general categories. The first type of response is illustrated by Figures 3 and 4. There appears to be an inhibition of photosynthesis at low light levels when the cells were tested at low temperatures. The inhibition is more pronounced at higher temperatures in Chromulina chionophila and less so in Stichococcus bacillaris.

Chromulina chionophila. From personal experience it has been found that Chromulina chionophila appears either in samples taken from 20-30 cm below the surface of the snow or from lesser depths in shaded areas as when found beneath Pinus contorta. It would appear that C. chionophila can tolerate low light. Figure 3 shows the light-temperature-photosynthesis relationship in one experiment using C. chionophila, but is representative of six similar experiments using

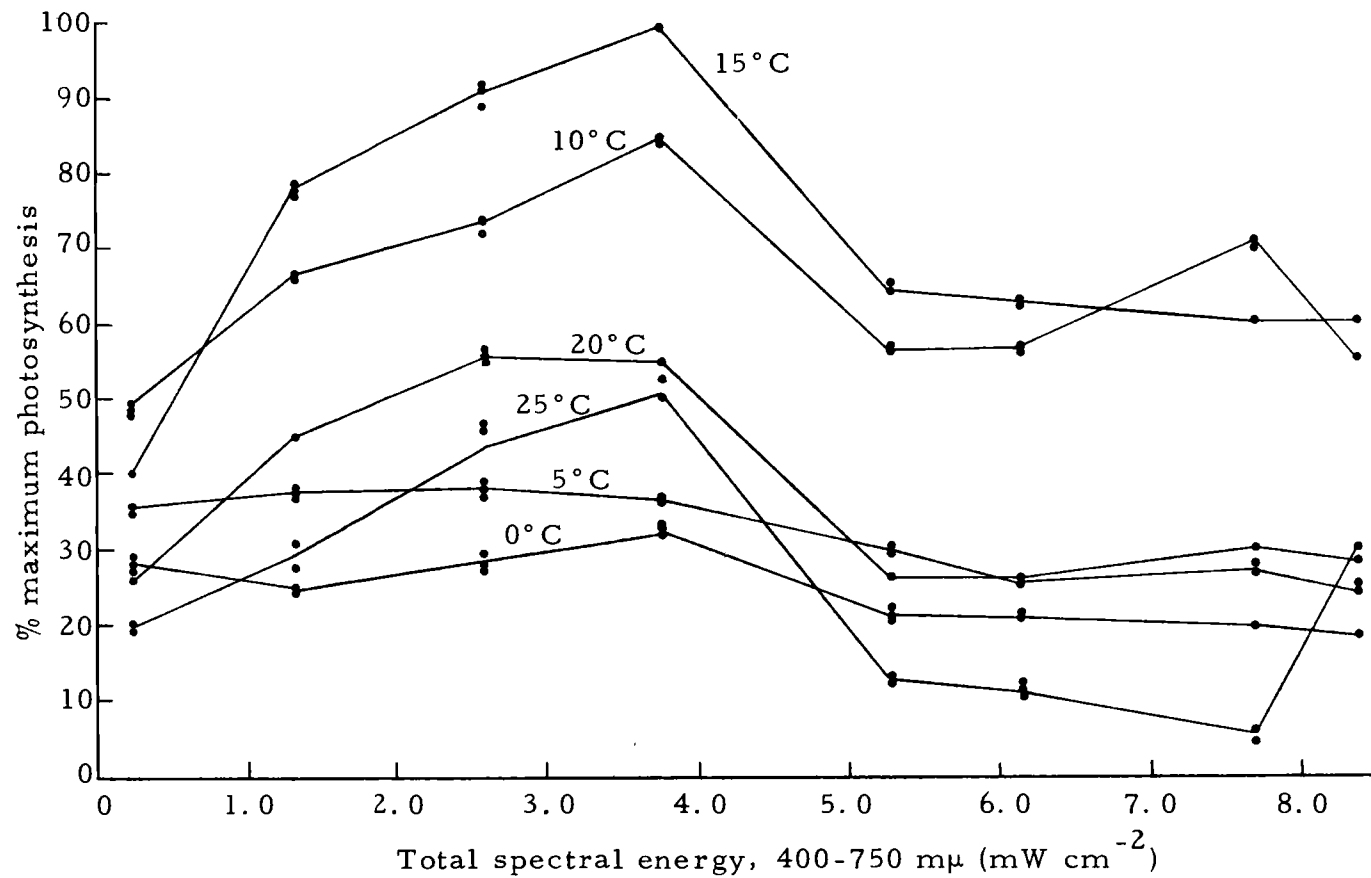


Figure 3. Light-temperature-photosynthesis response curves of *Chromulina chionophila* Stein. This is a random selection of one of the experimental series but is typical for this species under the experimental conditions.

this organism. At 0°C, photosynthesis is light saturated at 0.217 mW cm⁻² and appears to be inhibited at intensities above 5.0 mW cm⁻².

With increased temperature the point of light saturation increases to 3.77 mW cm⁻², above which there is a sharp decrease in photosynthesis when the temperature is greater than 5°C. The low light intensity, 0.2-0.7 mW cm⁻², found at 30 cm in corn snow (Hardy and Curl, unpublished) does not represent a limiting factor in photosynthesis of C. chionophila.

Chromulina chionophila does not tolerate high light intensity at low temperatures, an intensity of 13.73 mW cm⁻² at 2.2° is lethal: cells round up and settle to the bottom of the culture vessel after dropping their flagellum and decrease in number. At an intensity of 6.28 mW cm⁻² and an identical temperature the cells grow very well. In comparison, this is a higher light intensity than that listed by Hardy (1966) as being the maximum tolerated by C. chionophila at these temperatures (5.0 mW cm⁻² at 0-5°C). It should be noted, however, that he used Bristol's solution as a culture medium.

Stichococcus bacillaris. Measurements were made of the relative ¹⁴C uptake, with respect to temperature and light gradients. Light intensities above 1.33 mW cm⁻² are sufficient for light saturation. At temperatures of 0°, 5° and 10°C, light intensities above this figure were slightly inhibitory (Figure 4). These data would indicate that S. bacillaris should be found in the snow where the light intensity

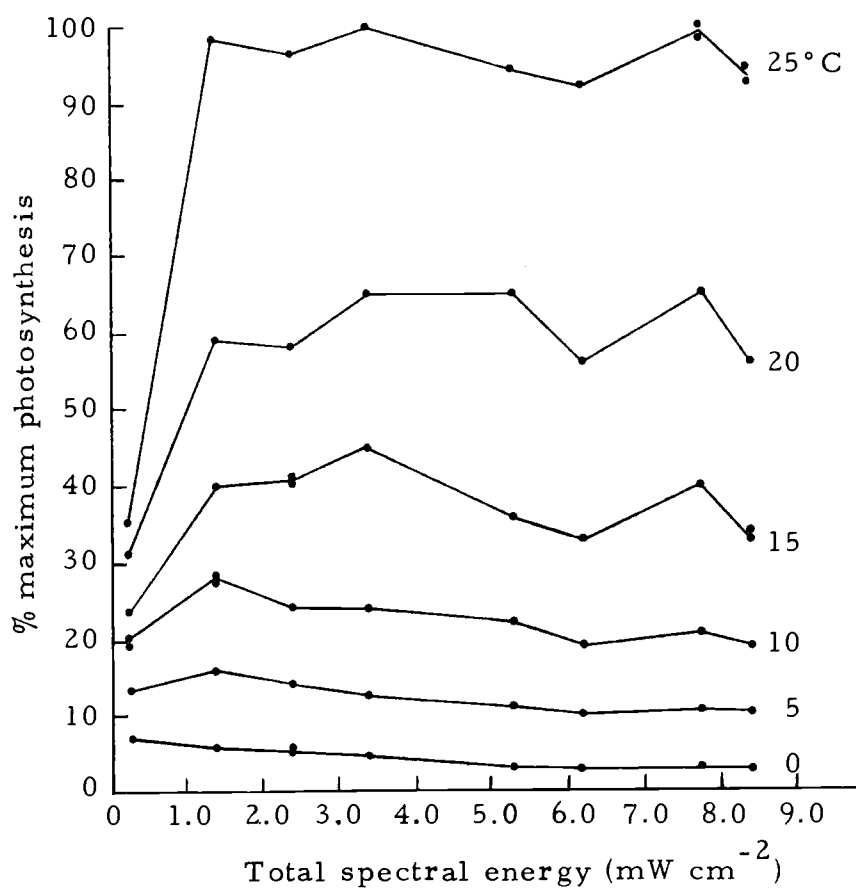


Figure 4. Photosynthesis-light-temperature response curves of *Stichococcus bacillaris* Nag. These cells had been grown at 2.2°C under fluorescent light with a total spectral energy level of 3.69 mW cm⁻²; 400-750 mμ.

was greatly reduced, but this has not been substantiated.

The second general type of response to a change in light intensity is illustrated by Chlamydomonas nivalis (Figure 5), Raphidonema nivale (Figure 6), and the collection of spores from the field (Figure 7). This type of response is characterized by a lack of photoinhibition and a low saturation point which remains fairly constant with increasing temperature.

Chlamydomonas nivalis. The spores of laboratory-grown C. nivalis did not show a photoinhibition and reached light saturation at 1.33 mW cm^{-2} regardless of the temperature. The great difficulty in obtaining a homogeneous cell suspension shows up as erratic photosynthetic rates at a given temperature.

Raphidonema nivale. This organism shows a response which is essentially the same as that of C. nivalis, a low saturation point (Figure 6). This series of curves represents an experiment in which the stock culture was growing in Holm-Hansen's medium and the cells for the experiment were suspended in Pringsheim's medium. Figure 8 represents an identical experiment with the exception that the experimental cells were suspended in Holm-Hansen's medium. The stock culture was 24 hours older. A third experiment, identical to the first, was performed and the resulting curves are as in Figure 6.

Collection of spores. The series of curves in the experiments with the spores from the field is very similar to that which was found

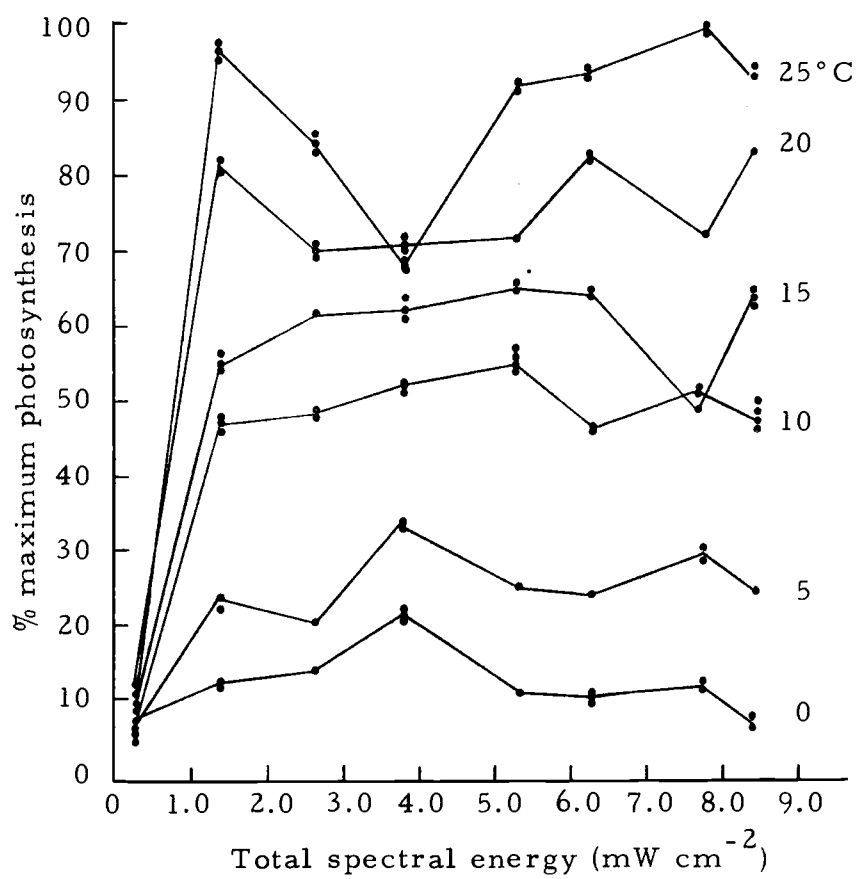


Figure 5. Temperature-light-photosynthesis response curves of the spores of a laboratory strain of Chlamydomonas nivalis (Bauer) Wille.

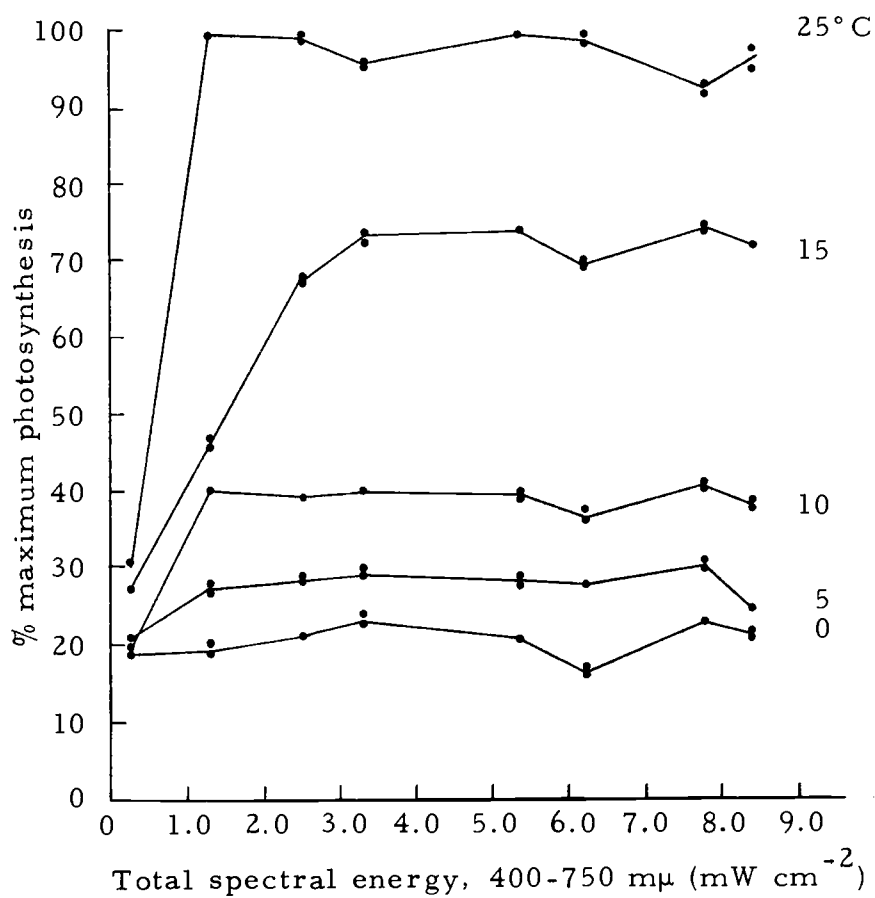


Figure 6. Light-temperature-photosynthesis response curves of *Raphidonema nivale* Lagerh. which had been grown in Holm-Hansen's medium but which was tested in Pringsheim's medium.

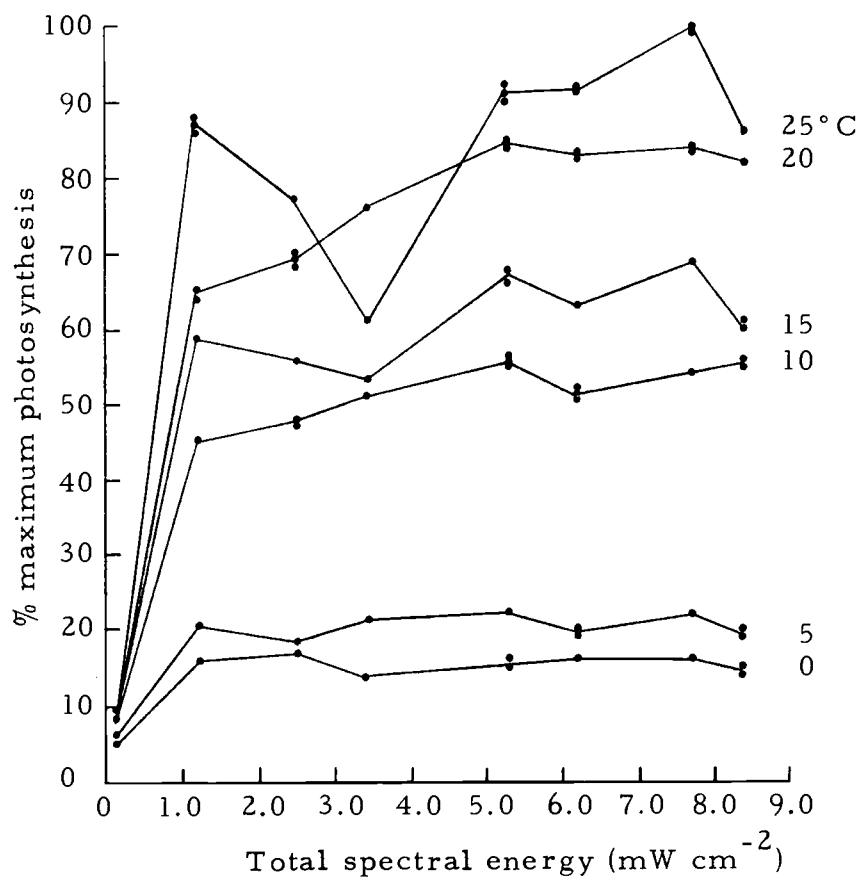


Figure 7. Light-temperature-photosynthesis response curves of a collection of assorted spores from the field. The primary species was Chlamydomonas nivalis (Bauer) Wille.

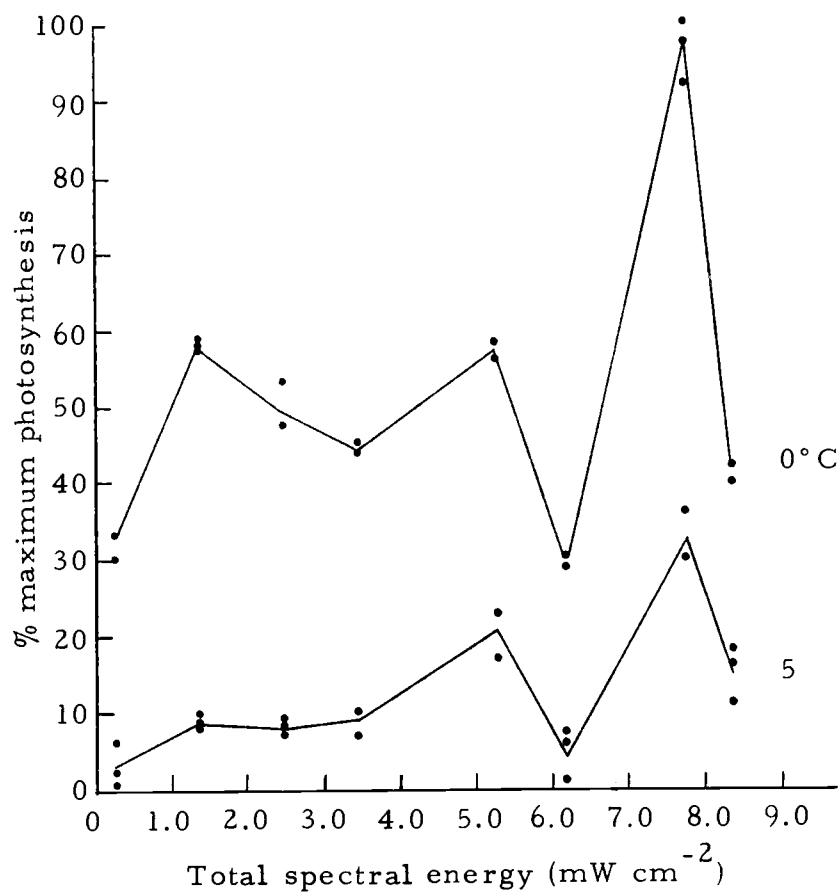


Figure 8. Temperature-light-photosynthesis response of Raphidonema nivale Lagerh., grown and tested in Holm-Hansen's medium. The stock culture from which these cells were taken was 24 hours older than that shown in the previous figure.

in C. nivalis and may reflect the influence this organism had on the physiology of the collection. Figure 7 also shows the same erratic behavior as seen in Figure 5.

The third general type of behavior is shown in the series of curves involving Chlamydomonas yellowstonensis (Figure 9). In this organism the saturation point increases with an increase in temperature from 0° C through 25° C.

Effect of Extended Dark Periods

Chromulina chionophila. The effect of extended dark periods on C. chionophila was investigated. An attempt was made to expose C. chionophila to fresh medium in the dark at 0° C for an identical amount of time before beginning each photosynthesis experiment. This was not always possible and caused some concern as to the effect on photosynthesis experiments. Chromulina chionophila was inoculated into fresh medium and placed in the dark at 0° C. Identical samples were withdrawn at various intervals and tested for ^{14}C uptake at 0° C. It is clear that the length of the dark period has a marked influence on relative photosynthesis (Figure 10). The cell count at the beginning of the experiment was 10,000 cells ml⁻¹ and at the end of the ten day period the cell count was 400-500 cells ml⁻¹.

Hardy (1966) showed that the integrity of the cellular membrane depended upon a supply of energy and that cytochrome and Krebs cycle

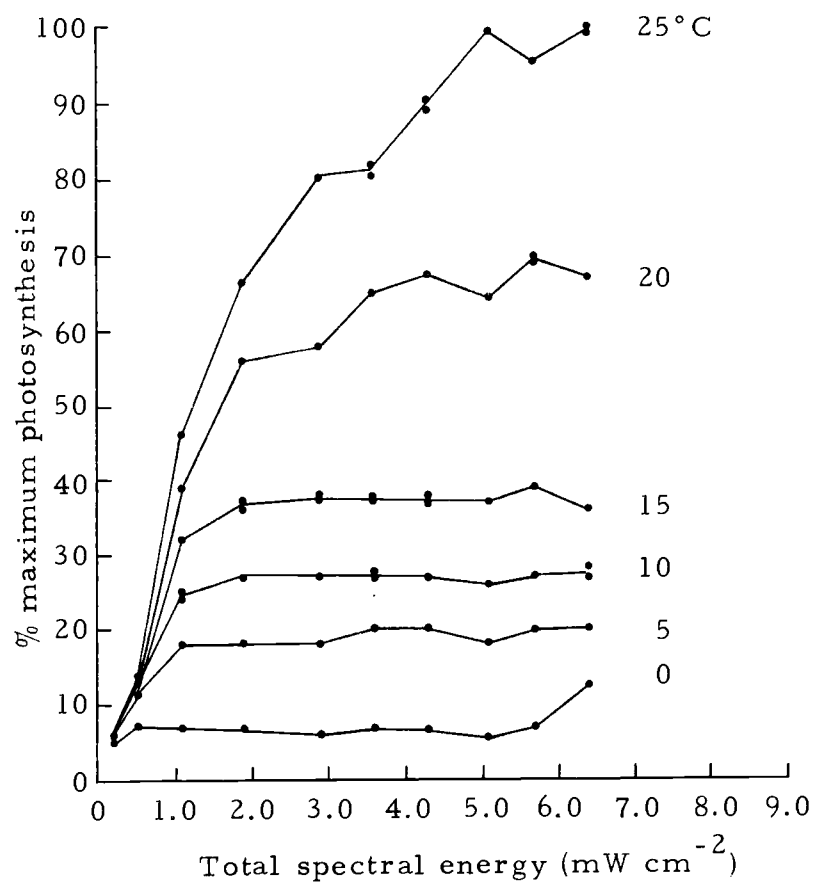


Figure 9. Light-temperature-photosynthesis curves for Chlamydomonas yellowstonensis Kol.

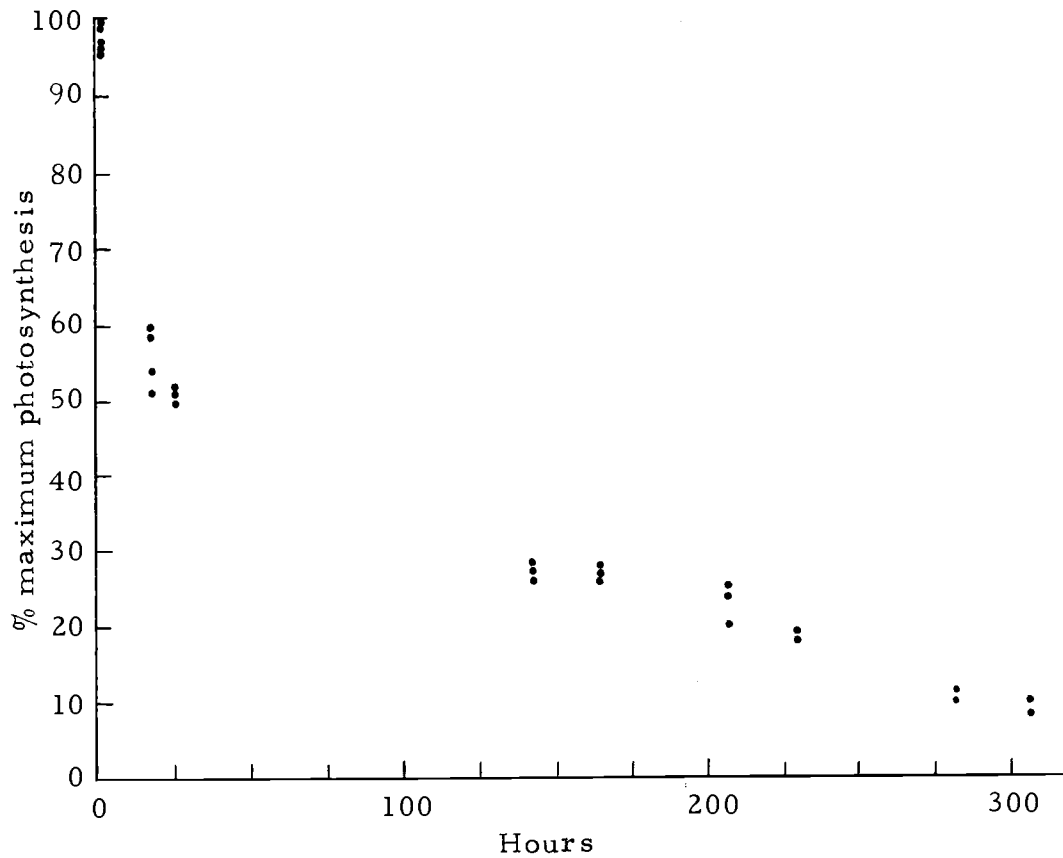


Figure 10. This represents the change in relative photosynthesis with an extended period in the dark at 0°C for Chromulina chionophila Stein. During this period the cell density was reduced from 10,000 cells/ml to approximately 500 cells/ml.

inhibitors caused lysis even at lower temperatures. There is the possibility that with the depletion of endogenous energy sources and the inability to photosynthesize, the cell lacks energy to maintain its integrity. From the shape of the curve it may be inferred that, if this is correct, the majority of cells had little in the way of cellular reserves. Since these cells were taken from a log phase culture, one in which the cells are rapidly reproducing, it would seem that this is the case.

Stichococcus bacillaris. This alga was treated in the same manner as C. chionophila and the results are shown in Figure 11. Stichococcus bacillaris behaves in quite a different manner from C. chionophila; uptake continues to increase with time spent in the dark. The different behavior of this organism in the dark can be attributed to the fact that cytokinesis continues with a corresponding diminution of cellular dimensions until after ten days the cells are near spheres on the order of 1.5-2.0 μ in diameter. The increased surface to volume ratio could account for the increase in uptake rate.

Temperature

There are two general classes of behavior among the organisms tested. The first is illustrated by Chromulina chionophila.

Chromulina chionophila. Seven series of experiments were carried out using this alga. Prior to these experiments this organism

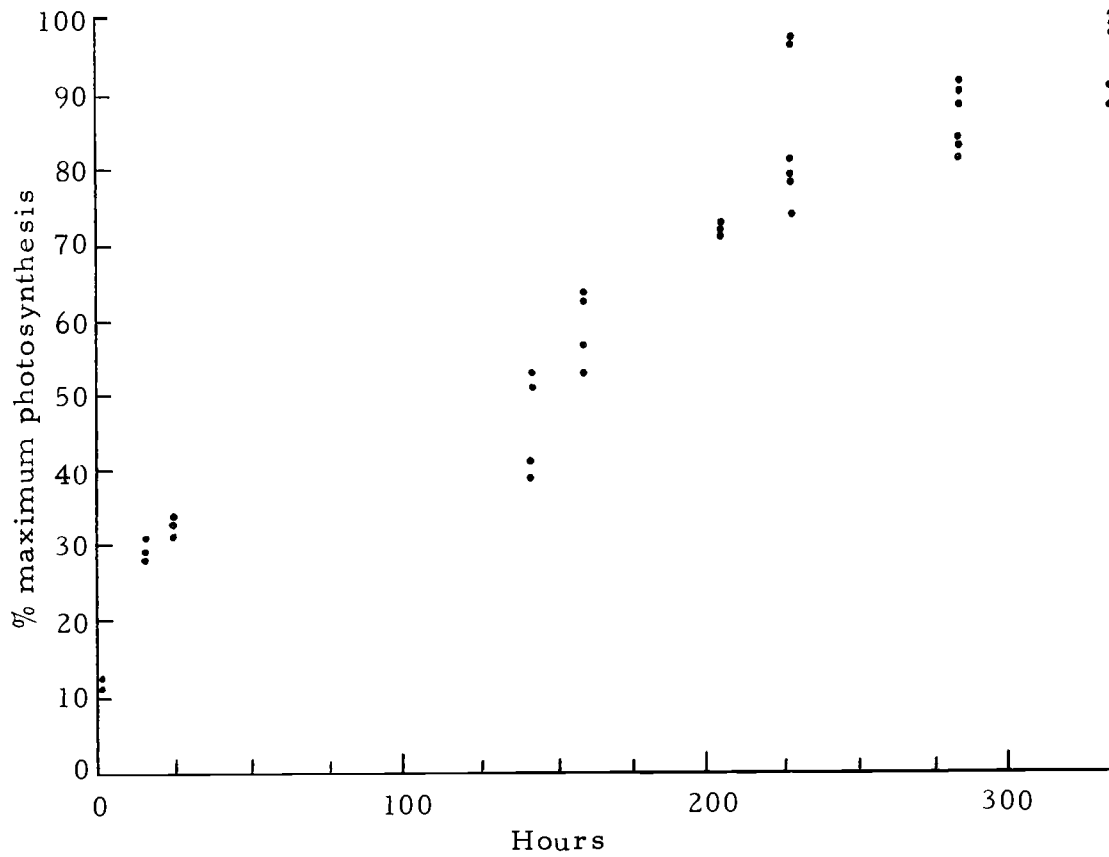


Figure 11. Relative photosynthesis in Stichococcus bacillaris Nag. as a function of time spent in the dark at 0°C. This organism will continue to divide in the dark with a decrease in the mean cell diameter.

was subjected to different light, temperature and medium regimes. The photosynthesis response curve was essentially the same regardless of pretreatment of the cells (Figure 12). Figure 13 is a composite of seven experiments using C. chionophila. From this composite it can be seen that C. chionophila increases in photosynthetic activity from 0° to 15°C and then decreases from 15° to 25°C. One might expect to find some cell growth accompanying photosynthesis in Chromulina chionophila at 20° and 25°C. However, C. chionophila has a temperature maximum for growth of less than 15°C. At 15° the fate of the organism is time and temperature dependent. At this temperature the cells round up and drop the flagellum, as Fukushima (1963) reported for Ochromonas smithii. At 20°C the cellular integrity is lost, although the amount of time required is not known. If the cells are held at 25°C for one-half hour, lysis occurs.

Chromulina chionophila was subjected to different light, temperature and nutrient regimes (Figure 14). The cultures were all grown in Pringsheim's medium under constant aeration. The highest growth rate of approximately one doubling per day occurred when cells were grown at 10°C and under constant illumination of 4.69 mW cm^{-2} (Figure 14A). Under these conditions the growth constant, k , was 0.0298 and the doubling time was 23 hours. The lowest growth rates occurred at 2.2°C with a light intensity of 3.69 mW cm^{-2} . These conditions gave a growth constant of 0.009 and a doubling time of

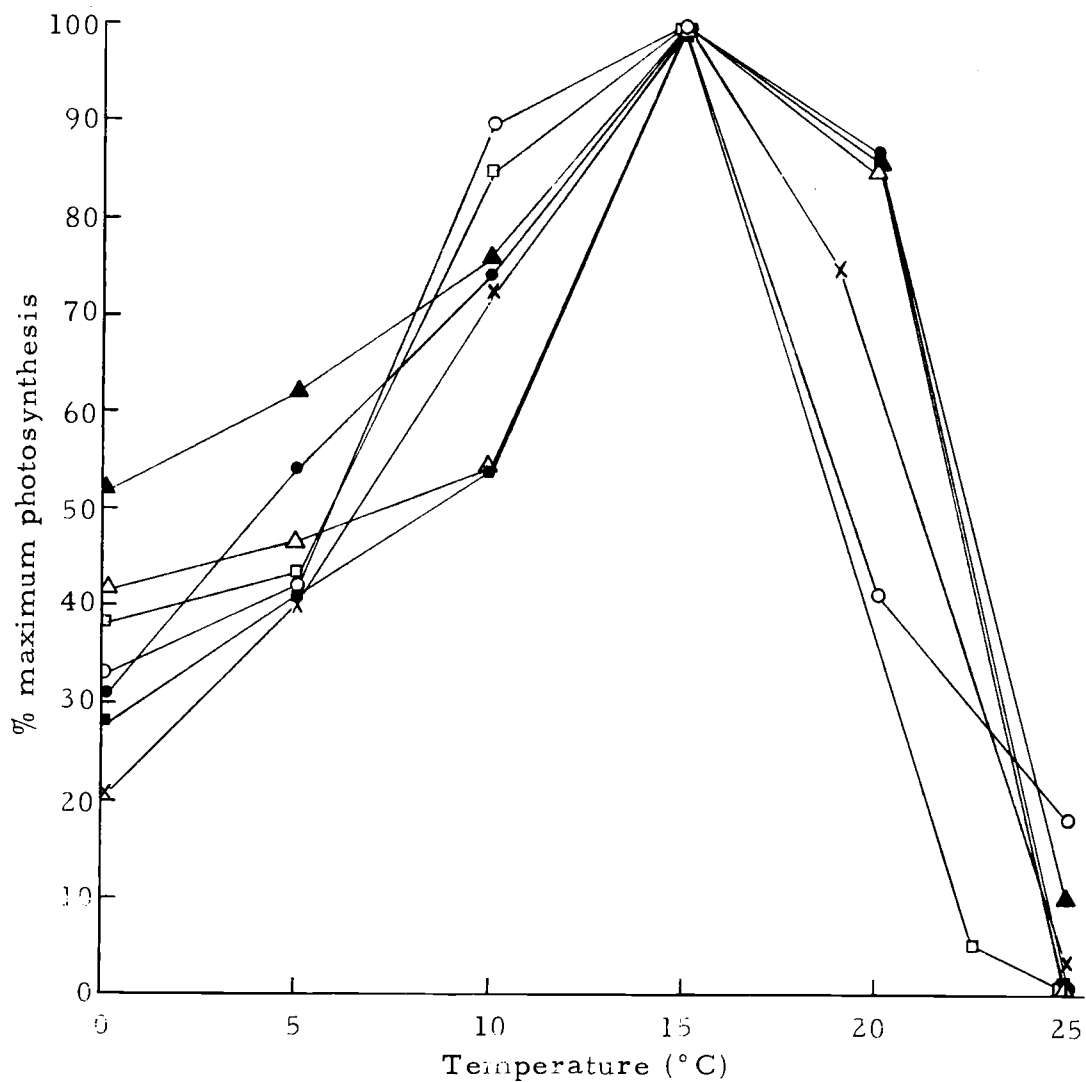


Figure 12. Temperature response of *Chromulina chionophila* Stein under similar conditions but with different prehistories.

- Grown at 5°C with neon light source.
- △ Grown at 5°C with constant fluorescent light.
- Grown at 0°C with light-dark period of 15 hr. light and 9 hr. dark, with fluorescent light source.
- x ● ▲ ■ Grown at 10-12°C under constant fluorescent light.

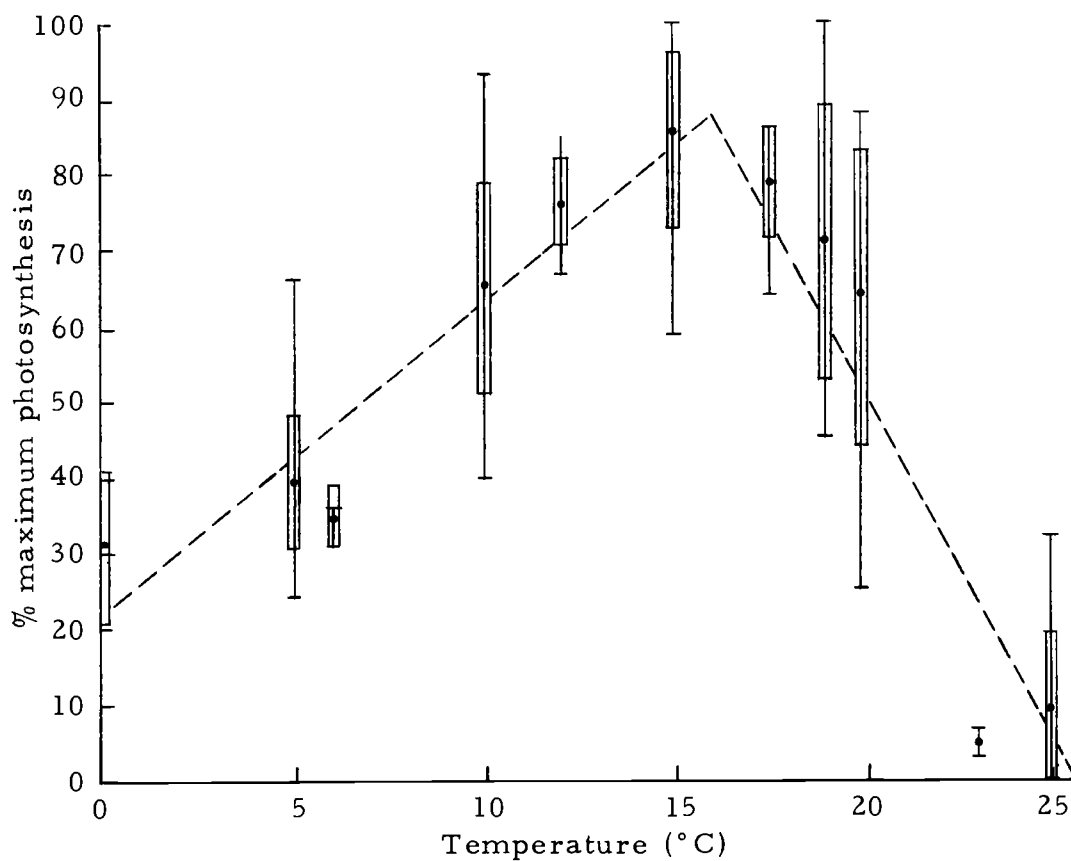
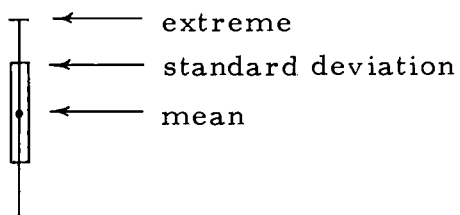


Figure 13. This is a composite of seven experiments using Chromulina chionophila Stein. The past histories of the subject differed with respect to growth temperature, light source and intensity, media, and lengths of exposure to 0°C in the absence of light. The dashed lines represent linear regressions of the means of the values from 0-15°C and from 15-25°C.



This figure at left is the key for this and the graphs which follow.

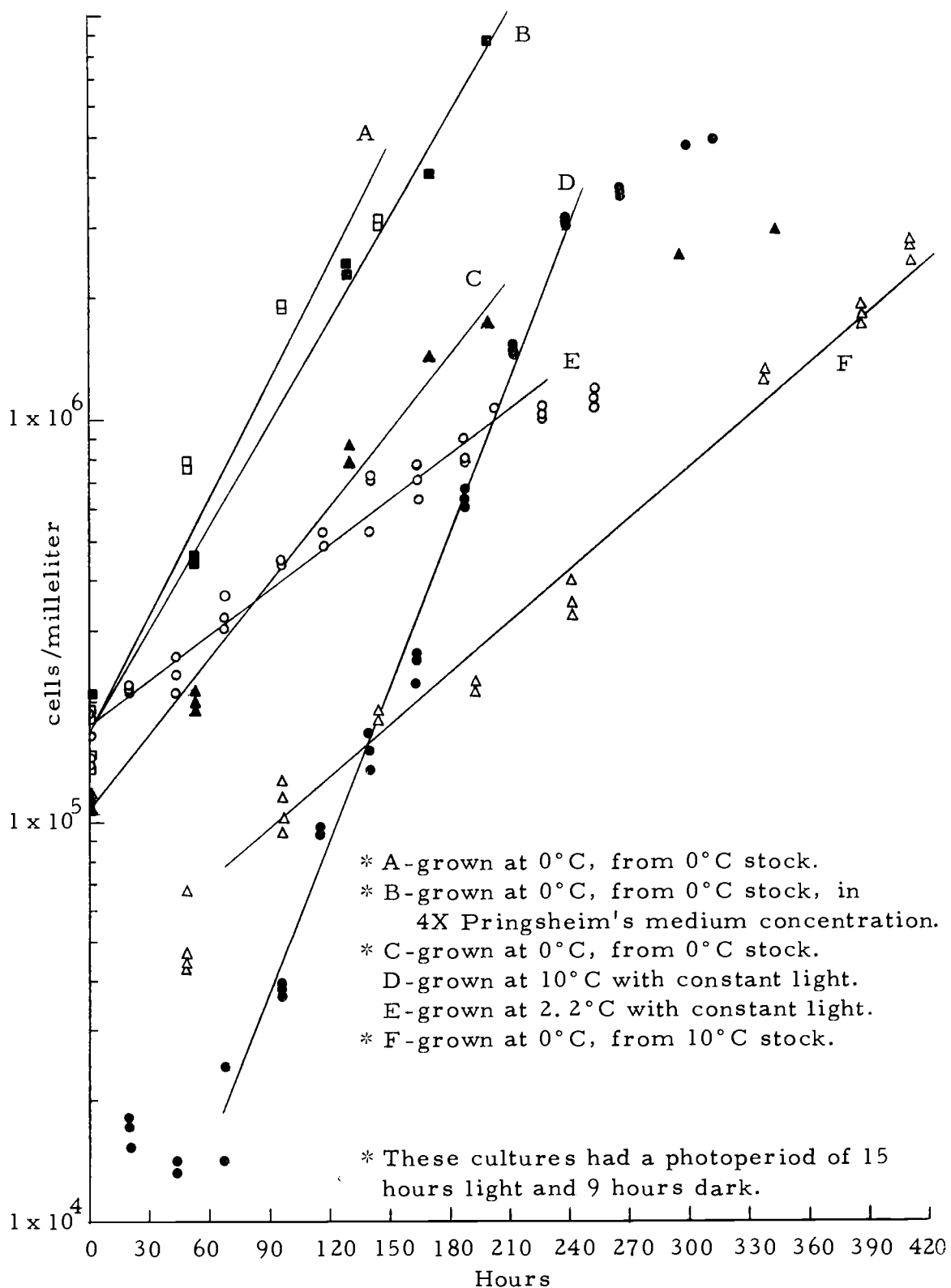


Figure 14. Growth curves of *Chromulina chionophila* Stein at different temperatures, light intensities, and nutrient conditions.

77 hours (Figure 14E).

These are both extremely long periods between doublings compared to many microbial organisms, but it must be kept in mind that Hardy (1966) found doubling times of approximately 140 hours under constant illumination of 0.35 mW cm^{-2} at 5°C in Bristol's solution. Between these extremes there are a number of intermediate values. Figure 14F is the growth curve for C. chionophila grown at 0°C with a light intensity of 3.79 mW cm^{-2} , and a 15 hour light, 9 hour dark cycle, originally from a stock culture grown at 10°C under constant illumination. This culture had a doubling time of 64 hours. The progeny of these cells were used to start another culture (Figure 14A) under the same light and temperature regime. The doubling time of this culture was 34 hours. From this culture two more experiments were carried out; one using normal strength Pringsheim's medium, (Figure 14C) which had a doubling time of 46 hours, and one using 4X Pringsheim's medium, (Figure 14B) which had a doubling time of 36 hours. The 4X culture continued to grow until the cell density was $30 \times 10^6 \text{ cells ml}^{-1}$ whereas normal strength cultures tend to reach a maximum cell density of $4.5\text{-}5.5 \times 10^6 \text{ cells ml}^{-1}$.

The second type of response to temperature variation is found in Chlamydomonas nivalis, Stichococcus bacillaris, Chlamydomonas yellowstonensis and the collection of spores from the field. In all these organisms the photosynthesis rate continues to increase

with temperature.

Chlamydomonas nivalis. Photosynthetic rate increased with temperature increase through 25°C. From culture work, I have observed that only palmelloid growth is observed at 20°C and above. At 20°C the C. nivalis is overrun by whatever bacteria and fungi are present after a period of two to three weeks.

Stichococcus bacillaris. This organism has an increase in relative photosynthesis over the temperature range at which it was tested. In both Figures 15 and 16 there is approximately a 20-fold increase in relative photosynthesis and yet this species is found on snow and ice in many parts of the world.

Growth and Survival. A growth curve of S. bacillaris was determined (Figure 17). The organism was in log phase growth from 72 hours until the end of sampling at 312 hours. During this time k is equal to 0.019 and the doubling time is 36.5 hours. During this time the cells were exposed to constant illumination of 3.59 mW cm^{-2} and 2.2°C accompanied by constant aeration. The apparent lag phase may be due in part to the stickiness of S. bacillaris, so that many of the cells adhered to the growth vessel. The occurrence of this organism in snow algae collections from around the world would indicate that it can tolerate adverse conditions which it faces even without the aid of a thick walled resistant stage. Stichococcus bacillaris has tolerated drying for three months and high (for a snow alga)

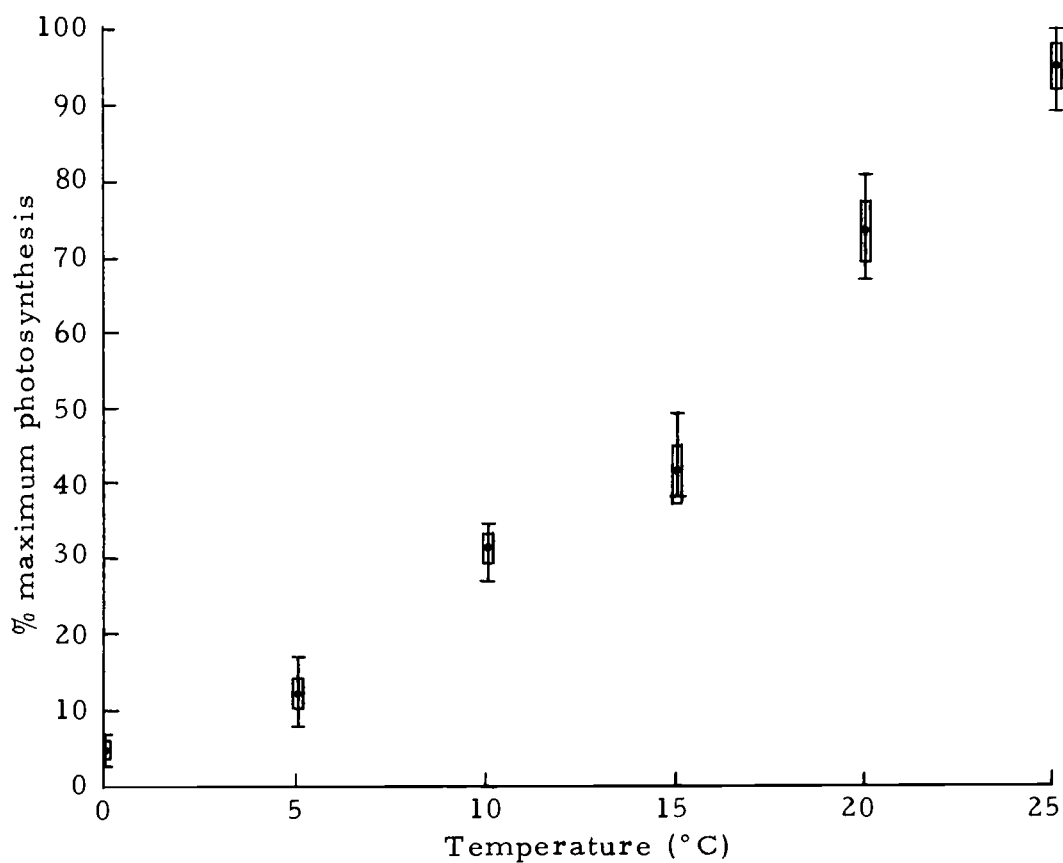


Figure 15. This is the relative photosynthesis curve for Stichococcus bacillaris Nag. as it varies with temperature. The cells were kept at 0° C, in the dark, for 46 hours prior to the beginning of the experiment.

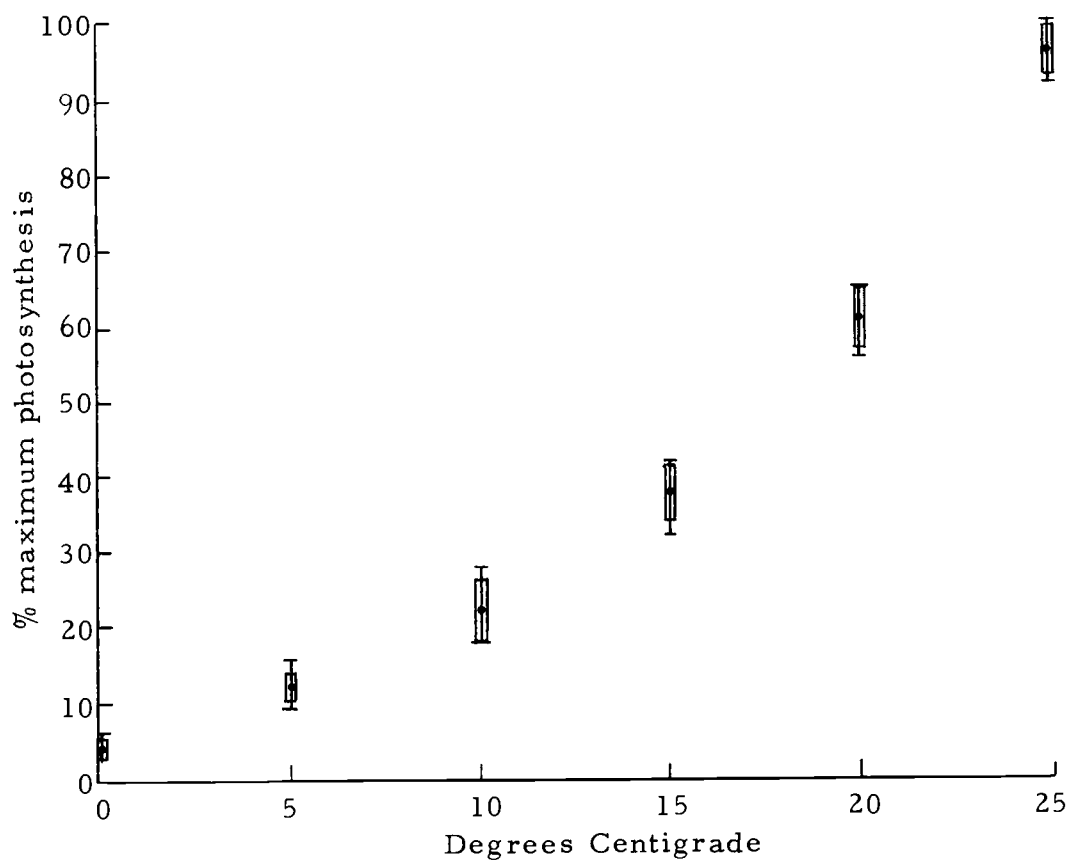


Figure 16. The above graph shows the relative photosynthesis of Stichococcus bacillaris Nag. as a function of temperature. These cells had been kept at 0°C in the dark for ten days prior to their exposure to light during the experiment.

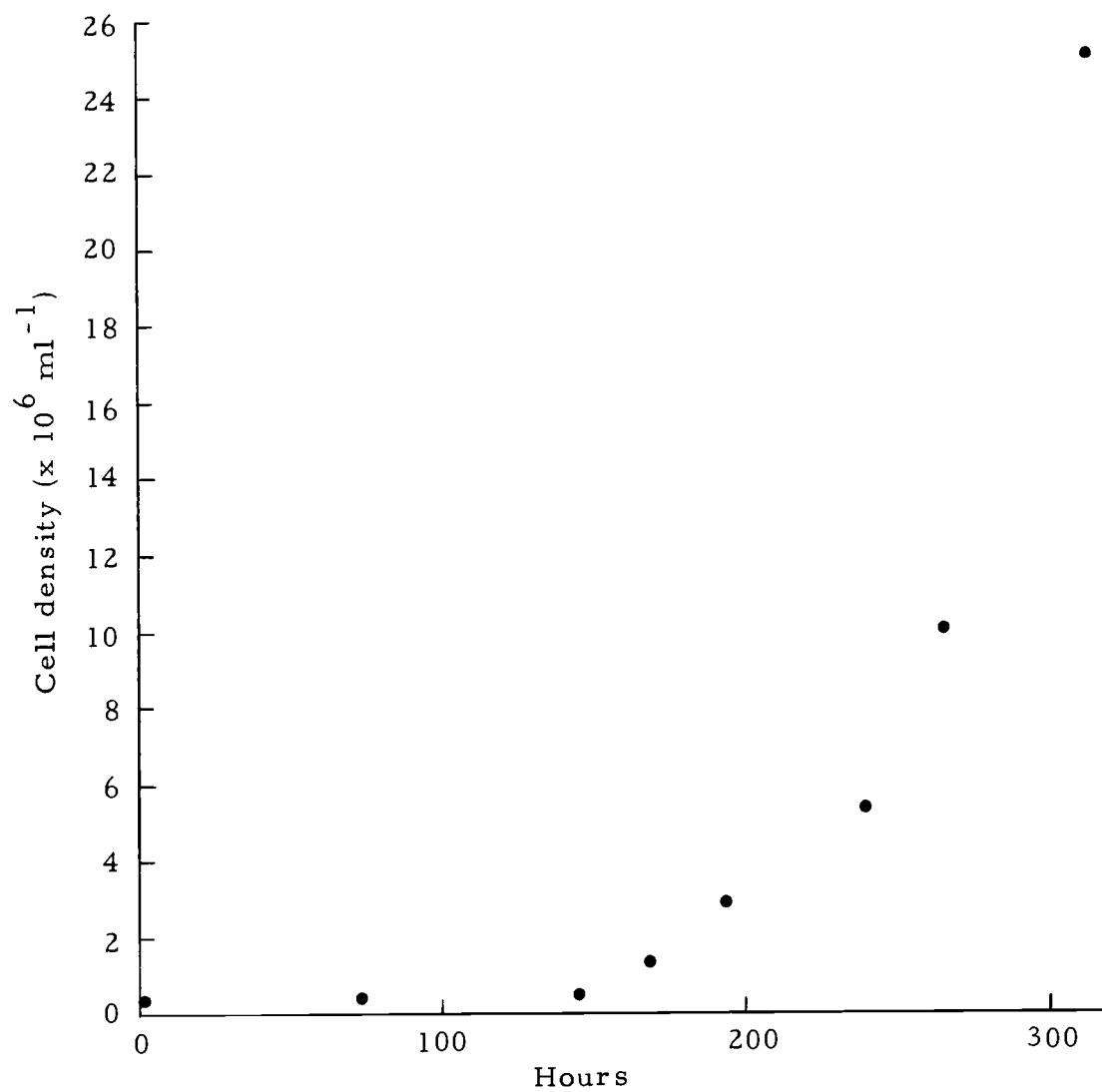


Figure 17. Growth curve for *Stichococcus bacillaris* Nag. This culture was grown at 2.2°C with constant agitation under fluorescent light with an intensity of 3.69 mW cm^{-2} .

temperatures, of greater than 35°C in the soil and still grows when placed in the nutrient medium. This organism grows in either Pringsheim's or Holm-Hansen's medium with seemingly equal vigor and has survived eight hours in a drying oven at 60°C, on a membrane filter, and grown when placed in nutrient medium at 2°C.

Chlamydomonas yellowstonensis. This alga was collected in the spring of 1968 from green colored snow in a shady area among Pinus contorta trees near Todd Lake in central Oregon. Chlamydomonas yellowstonensis grows well in culture in either Pringsheim's or Holm-Hansen's medium and does not seem under stress when grown at 0°C or 16°C but does grow more rapidly at 16°C. Thus, it is not surprising that there is an increase in the relative photosynthesis as the temperature is raised (Figure 18).

Collection of Spores. The first experiment was conducted in unenriched snow melt water at six temperatures (Figure 19). There is a steady increase in the fixation of carbon dioxide as the temperature is increased from 0 to 25°C. Cells from the field collection in which snow melt water was enriched with the salts and vitamins of Holm-Hansen's medium were tested for ^{14}C uptake (Figure 20). Enriched snow water gave considerably lower values (Figure 29F, G) when compared on a carbon basis.

There was yet a third type of behavior found when relating photosynthesis to the effect of temperature. This is the anomalous

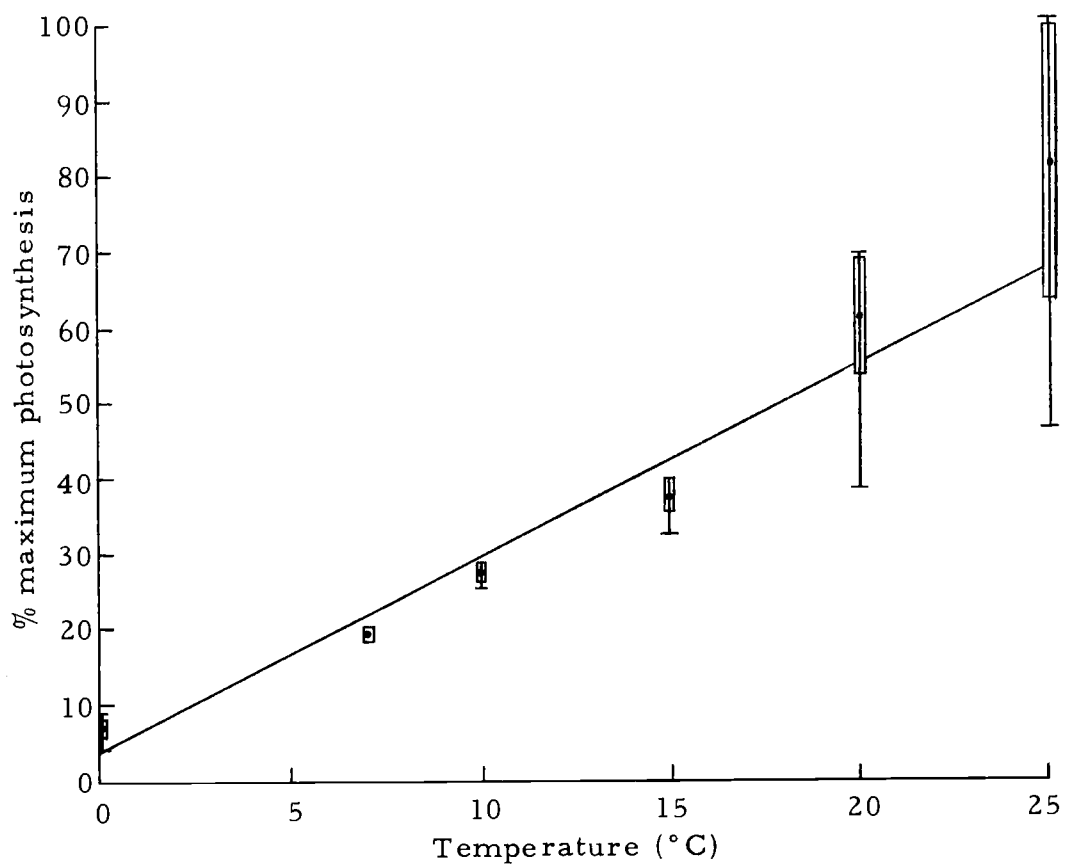


Figure 18. Temperature-photosynthesis relationship in Chlamydomonas yellowstonensis Kol. Variation in photosynthesis increases with temperature due to an increase in the light saturation point as temperature increases.

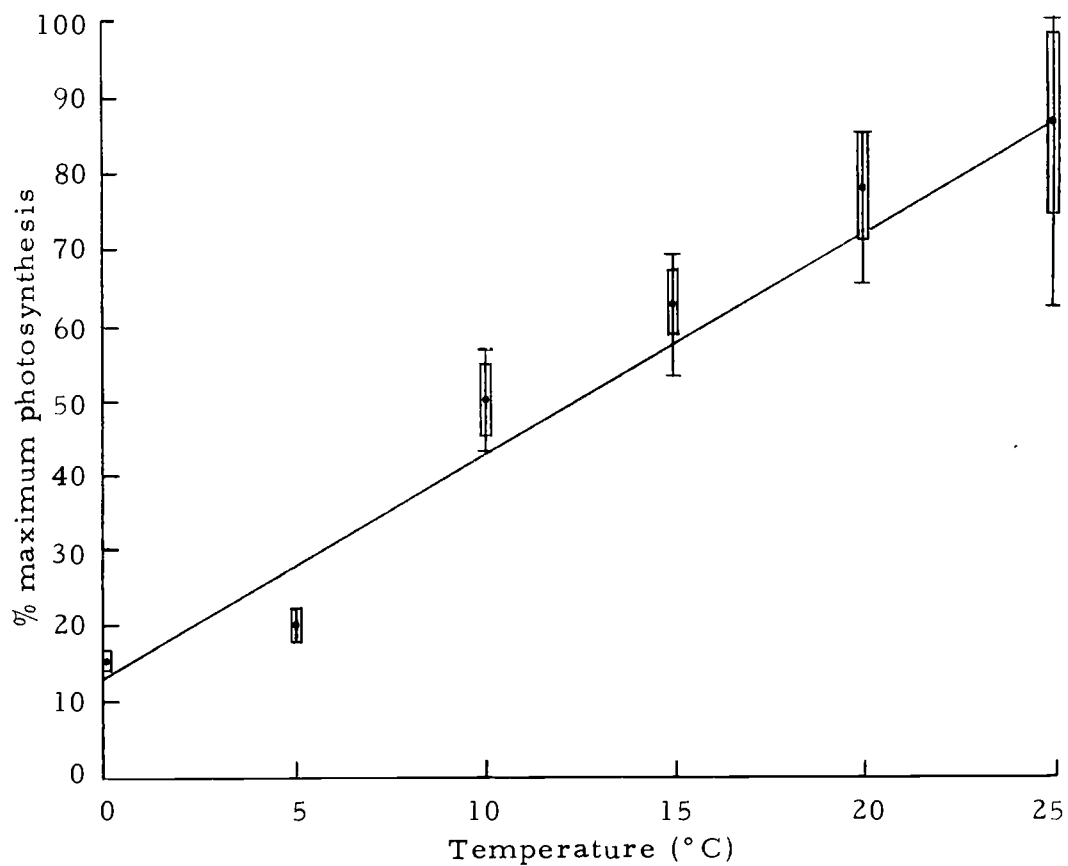


Figure 19. This graph represents the relative photosynthesis of a collection of a variety of spores from the field at different temperatures. For this experiment the spores were resuspended in melt water of the snow in which they were collected.

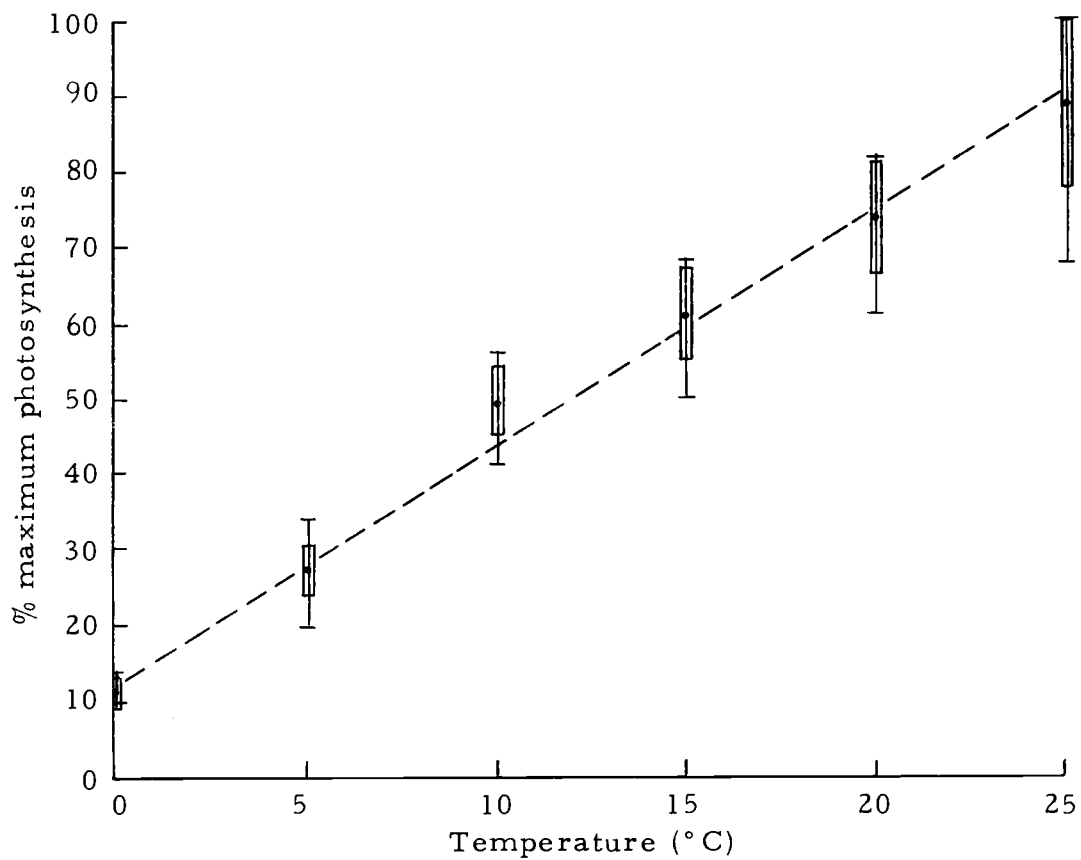


Figure 20. This graph represents photosynthetic response of a collection of spores from the field as a function of temperature. These spores were suspended in snow melt water which had been enriched with the inorganic and organic constituents of Holm-Hansen's medium.

behavior of Raphidonema nivale.

Raphidonema nivale. This alga, grown in Holm-Hansen's medium, was placed in Pringsheim's medium in the dark at 0°C and subsequently tested for ^{14}C uptake (Figure 21A), then 24 hours later the experiment was repeated using Holm-Hansen's medium (Figure 21C). The experiment was performed a third time using Pringsheim's medium as the suspending medium and a comparison of the change in relative photosynthesis with temperature as shown in Figure 21B. These results indicate that R. nivale behaves as a cryophil in Holm-Hansen's medium and a cryobiont in Pringsheim's medium. The R. nivale cultures were lost when the cooling system broke down and the temperature in the cultures in Holm-Hansen's medium reached 20°C.

Media

The effects of the nutrient medium on R. nivale were rather drastic. In working with C. nivalis and the collection of spores other effects were noted.

The medium used in the culture of C. nivalis is that of Holm-Hansen. This is approximately 1,000 times the concentration of snow water with respect to nitrate and phosphate. While using this medium to test the uptake of ^{14}C of spores from the field it was found that uptake was much lower than when snow melt water was used as the suspending medium. A concentration series was set up from distilled

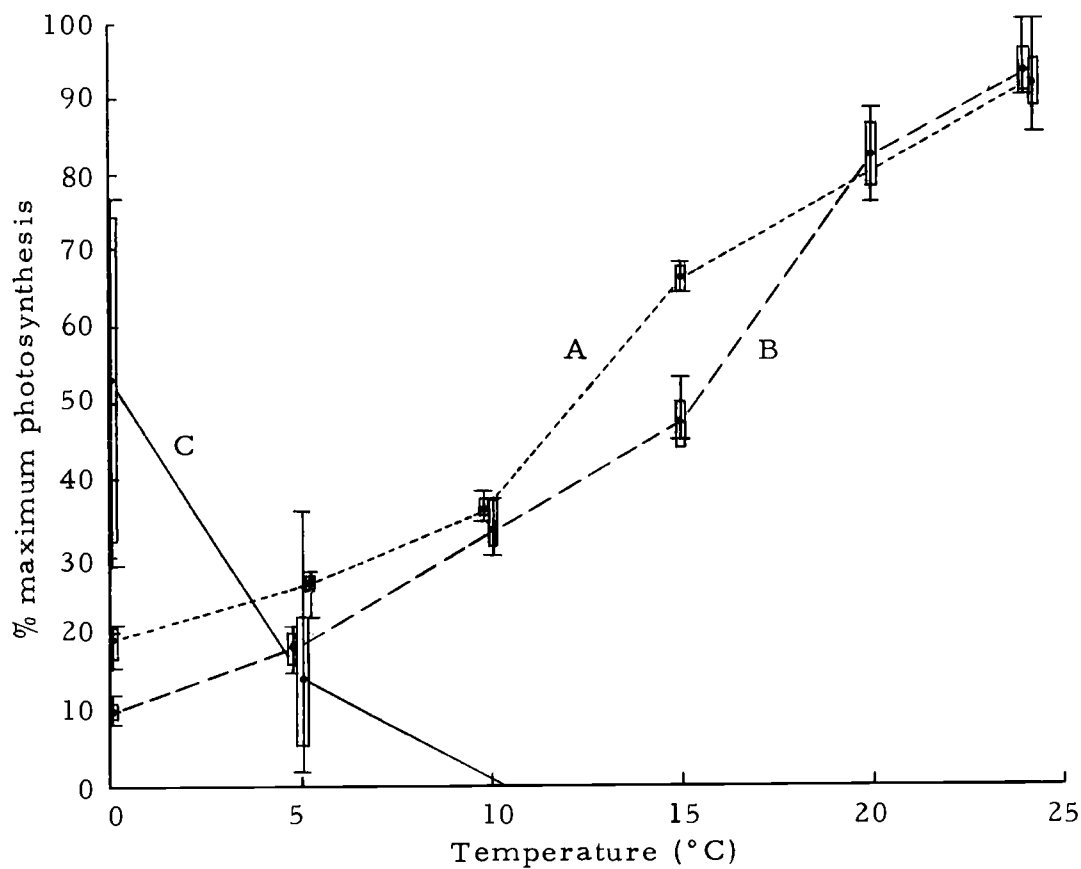


Figure 21. This figure shows the anomolous behavior of Raphidonema nivale Lagerh. An explanation of the curves is as follows.

- A - The stock culture was grown in Holm-Hensen's medium and experimental cells were placed in fresh Pringsheim's medium for photosynthesis measurements.
- B - Received the same treatment as A but the stock culture was six days older than A.
- C - Was grown and tested in Holm-Hansen's medium, 24 hours after A.

water to 100% Holm-Hansen medium (Table 3). A similar experiment was attempted with laboratory-grown C. nivalis (Figure 22). These spores had been grown in OHH (Osmund Holm-Hansen's) medium but full strength medium inhibits ^{14}C uptake under the conditions of the experiment. The experiment was repeated with pH adjusted to 4.7 (Figure 23). The full strength medium, even at reduced pH, is not conducive to $\text{H}^{14}\text{CO}_3^-$ uptake. However, in growth studies using these same concentrations at pH 4.7 it was discovered that cell division did not occur in snow water or in 0.1% OHH medium. When exposed to a range of pH from 3.0-8.5 the cells died in pH 3.0 medium but grew in all the others. Chlamydomonas nivalis grew in the same manner at pH 8.0-8.5 as when subjected to 20°C temperatures; i. e., there was only the palmelloid form of growth. Using 100% Holm-Hansen's medium with pH varying from 3.0-8.5 at approximately 0.5 pH intervals, it was found that there was no significant variation in $\text{H}^{14}\text{CO}_3^-$ uptake with pH. A pH of 5.0-5.5 was chosen as the level to which future (OHH) medium will be adjusted.

Collection of Spores. A series of dilutions of Holm-Hansen's medium were prepared. The dilutions ranged from full strength to a medium which contained only distilled water and snow melt water. Distilled water gave approximately the same amount of carbon dioxide fixation as 0.1% of OHH medium (Figure 24). The snow water gave a ^{14}C uptake rate which was between 33% and 41% of the maximum.

Uptake of H^{14}CO_3 was plotted against pH as in Figure 25. The ^{14}C uptake increased between pH 2.2 and 4.8 and then decreased.

Table 3. pH variation with concentration of Holm-Hansen's medium.

pH	Concentration (% of normal medium)
5.5	distilled water
4.8	0.1%
5.7	1.0
6.4	10.0
6.8	20.0
7.2	40.0
7.5	60.0
7.7	90.0
7.6*	100.0

* It was found later that the pH of Holm-Hansen's medium varied from 7.6-8.5 after autoclaving.

The findings of Fogg (1967) concerning the change of carbon dioxide fixation with temperature did not agree with what had been observed in this laboratory and subsequent experiments did not change the state of agreement. Fogg found that under light-limited conditions there was only a 15% increase in carbon dioxide fixation between samples at 0° and 15°C, but there was an increase in dark fixation of 105% over the same temperature range.

Observations of Algae in Culture

Chromulina chionophila Stein. This alga has been observed by the author in laboratory culture over a period of three years. In

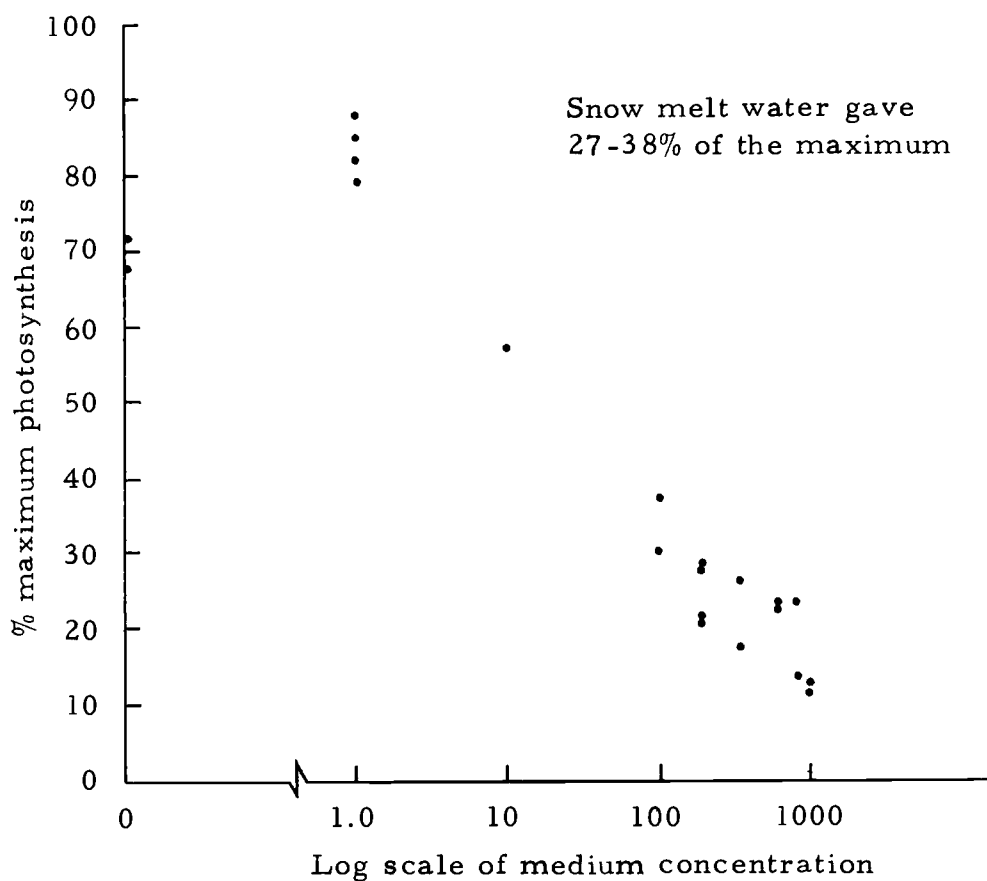


Figure 22. Spores of laboratory grown Chlamydomonas nivalis (Bauer) Wille were suspended in Holm-Hansen's medium of varying dilution. On the abscissa, the value 1.0 represents the point to which the medium has been diluted where it approximates the concentration of salts in snow water. The number 1000 corresponds to 100% Holm-Hansen's medium.

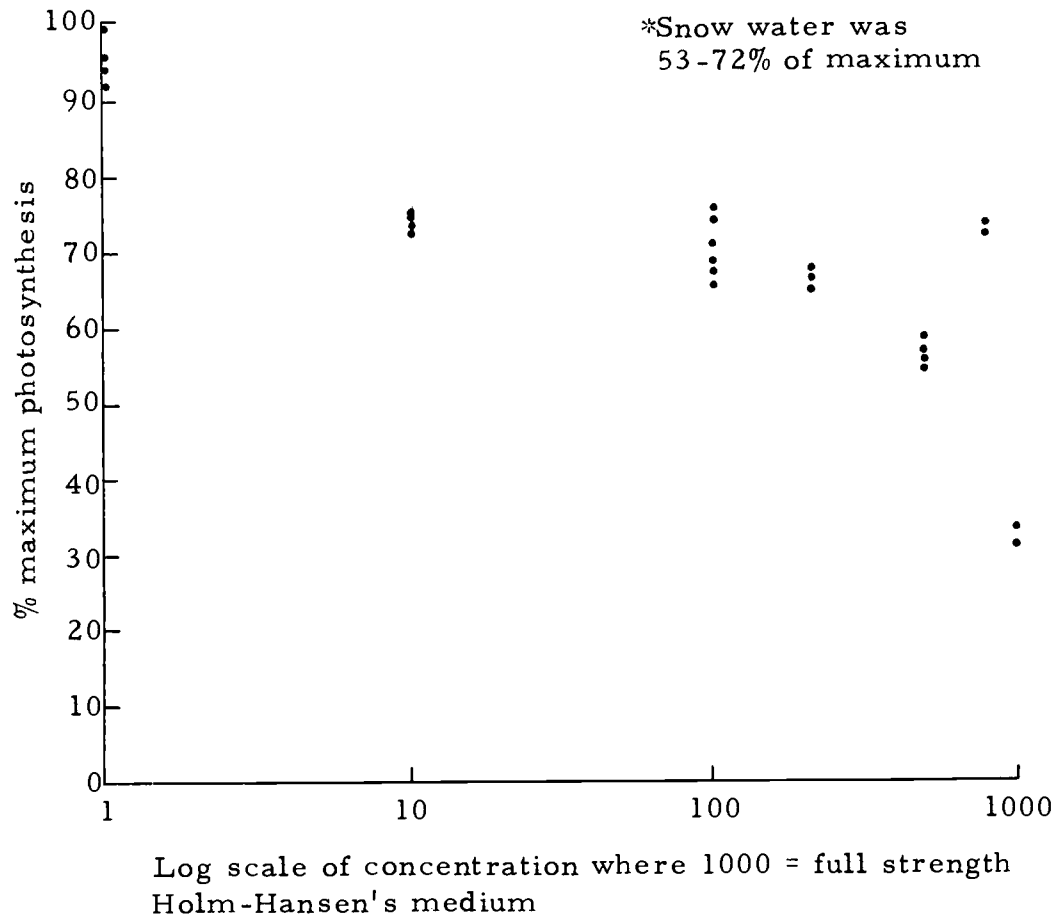


Figure 23. In this experiment Chlamydomonas nivalis was suspended in various concentrations of Holm-Hansen's medium in which the pH had been adjusted to 4.7 in all cases.

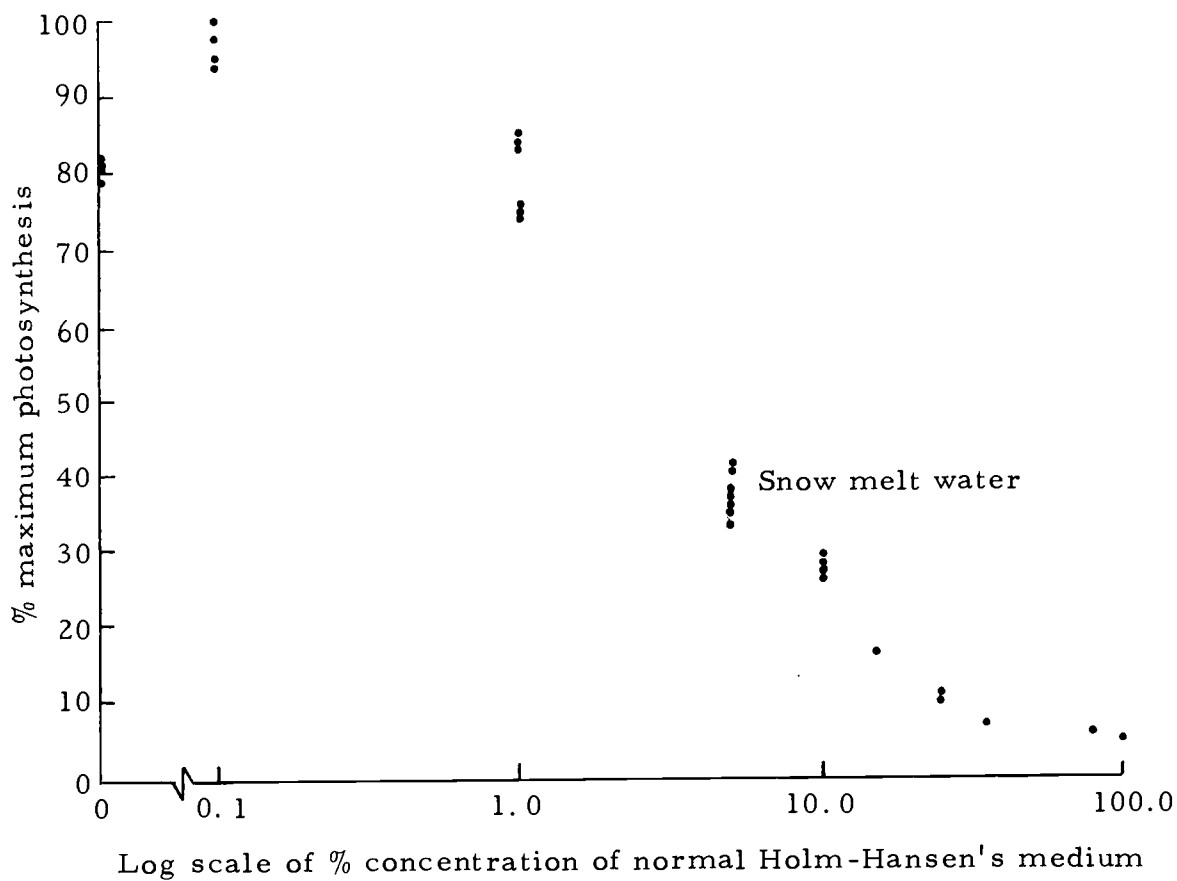


Figure 24. In this experiment a collection of spores from the field were suspended in Holm-Hansen's medium, ranging from 100% of the normal to 0.1 % of the normal strength. The zero point is distilled water. Relative photosynthesis was then measured in each of the solutions.

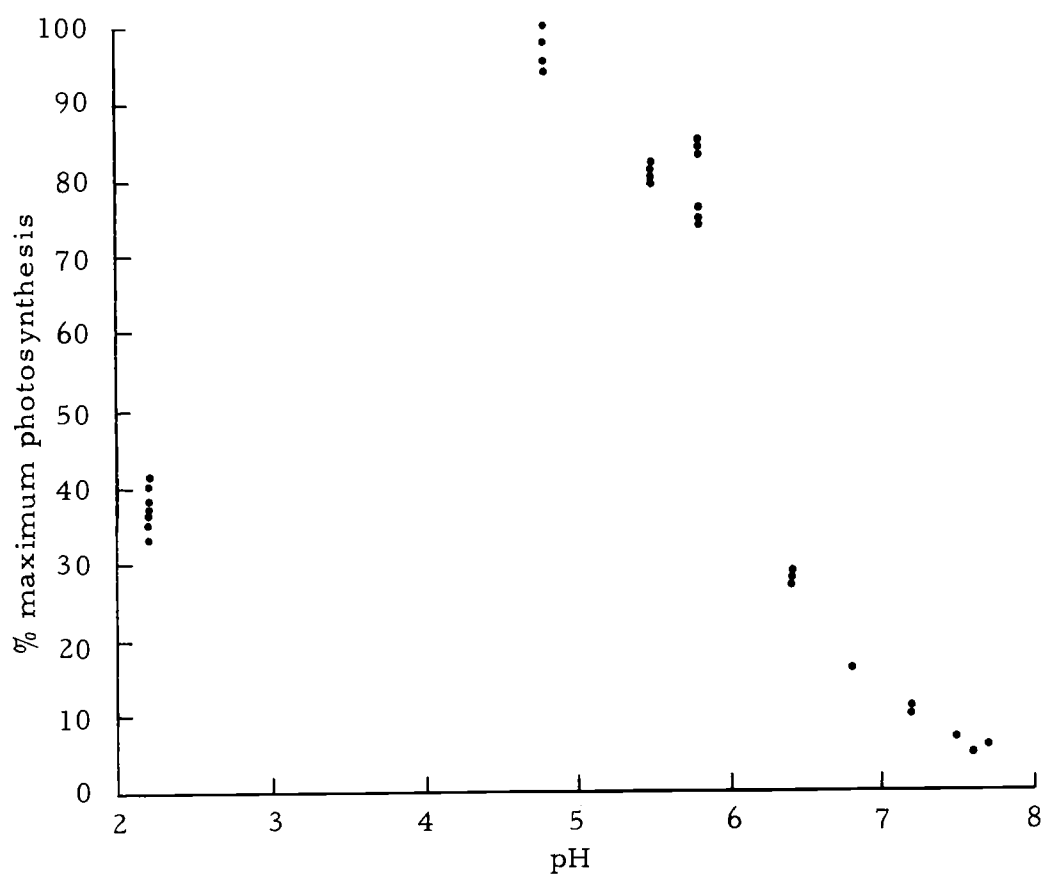


Figure 25. In this experiment a field collection of spores was suspended in various dilutions of Holm-Hansen's medium. Relative photosynthesis was plotted against the pH of several dilutions.

culture this chrysophyte behaves similarly to C. aerophila, C. rosenoffi and C. ferrea (Lund, 1942) in that it is found growing most profusely at the air-water interface. In the snow there is a great deal of surface area which is covered by a film of water which might provide a suitable environment for C. chionophila. This is one of few organisms from the snow for which there is information available from cultured material. Although recently discovered (Stein, 1963) this organism is very amenable to culturing and has had considerable physiological and morphological work performed on it. It is extremely temperature sensitive and lysis occurs on a microscopic slide unless the slide is chilled. Lugol's solution is a good temporary fixative which allows the flagellum to remain intact.

General Description of Cultured Material. Very healthy cultures tend to have cells which are either heart-shaped or spherical and are very motile (Figure 26A). These cells are a rich golden brown except when grown under conditions of high light. Under these conditions they unexpectedly lose a great deal of their carotenoid pigment and the entire culture is a pale green. The eyespot is not always present. There may be one large red eyespot, or, when the cells are grown under high light intensity, near the limit of their tolerance, the pale green cells have two eyespots. The cells exhibit considerable metaboly as they move and there appears to be little rigidity to the cell wall. At elevated temperatures the cells first drop their flagella,

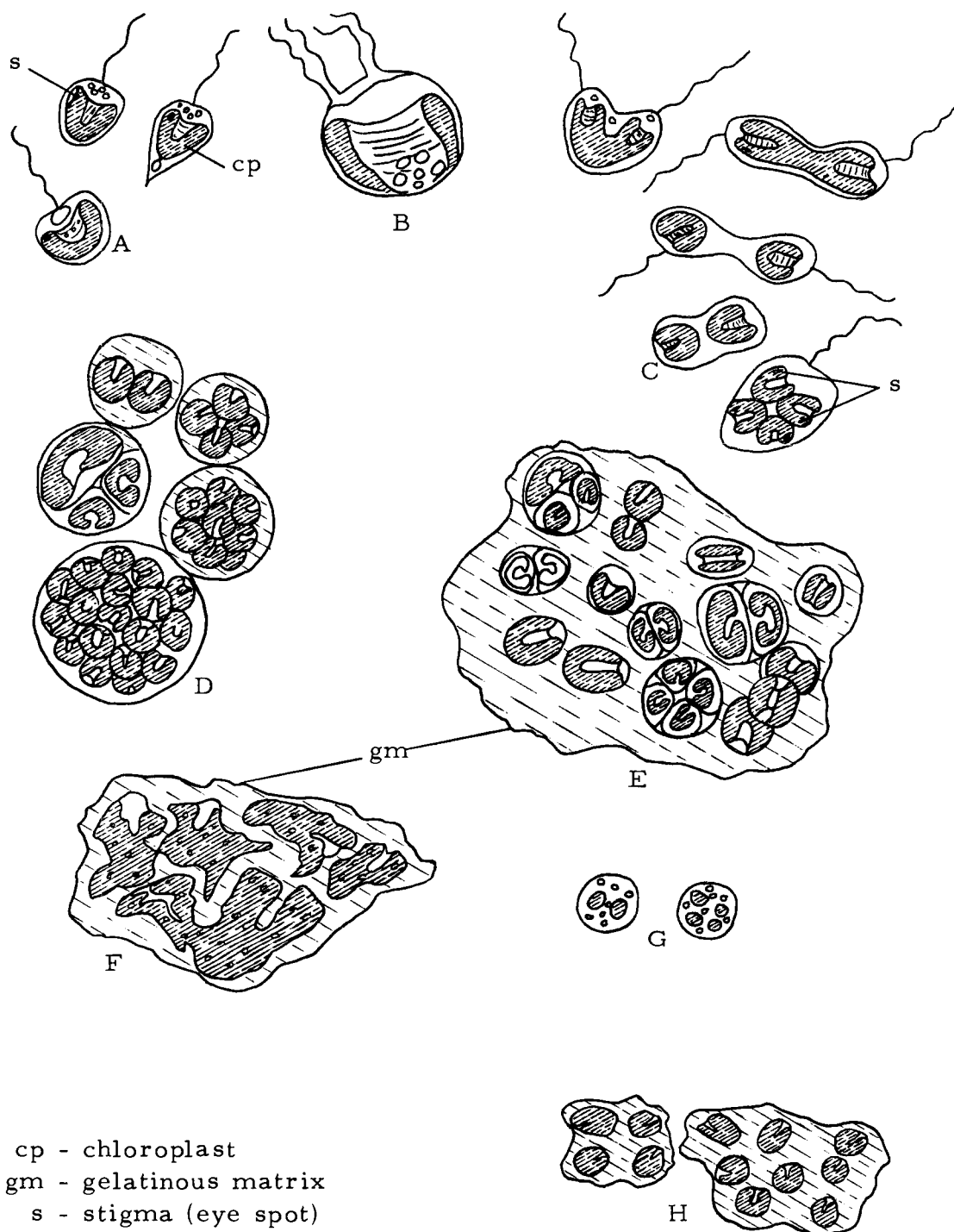


Figure 26. Morphological variation found in Chromulina chionophila Stein grown in laboratory culture.

round into a sphere, and then rupture.

The organism appears to be neustonic in growth habit, often covering the surface of the culture medium a golden brown, and vigorous aeration is needed to get a dense culture. Figure 26B shows an anomalous form which appears from time to time in culture. The larger size and multiple flagella would indicate that karyokinesis has occurred without cytokinesis. This may be an artifact brought about by the culture conditions.

Figure 26C shows a number of division stages of this organism. The pictures are remarkably similar to those which Fukushima (1963) has drawn to represent Ochromonas smithii. Fukushima has chosen to view the series as a conjugation of gametes, whereas, I feel they represent simple cellular division. The motile cell containing four daughter cells remains a mystery but is perhaps the motile form of the multicelled stage shown in Figure 26D.

In Figure 26D there appear five cell types which may represent several reproductive stages. There is a remarkable resemblance between the figures shown here and the ones illustrated by Kol (1967) to represent Ellipsoidion perininun var. cryophila and Chloridella glacialis Kol. These life stages have been found only in rapidly growing cultures, usually within a few days after inoculation. The three-celled configuration shown here has so far been seen only in one clone of C. chionophila which is predominantly non-motile. Figure 26E

shows the cells of this clone which often occur in a gelatinous mass. In this clone of C. chionophila this mode of division is the dominant form over long periods of time, with only a small percentage of the cells being motile. At other times this same clone will exhibit the same type of growth which is found in all other clones in the culture collection, with nearly all cells motile. Cells in the gelatinous matrix are somewhat more resistant to elevated temperatures than are the solitary cells. When compressed under a coverslip these cells closely resemble the Protoderma brownii Fritsch (1912) which was collected in Antarctica. Fogg (1967) noted that his Ochromonas sp. collected in the South Orkney Islands also resembled the organism as described by Fritsch from fixed material.

In Figure 26F there is an illustration of C. chionophila as it appears when grown on 1 percent agar with nutrient salts added. A section of the agar below the colony may be removed and placed, with the colony attached, into liquid medium after which the progeny become motile and are soon found at the surface layer of the medium.

Figure 26G represents another infrequently occurring form in which the plastids are greatly reduced. The internal structure of these cannot be resolved because of their small size.

Figure 26H is an illustration of what most likely is an encysted form of this organism. This is a very hard matrix and can be broken only by applying physical pressure to the matrix. Upon fracturing the

matrix small Chromulina-like, spherical cells are released, but at present none of the cells within one of these matrices has given rise to any progeny nor have any of those which were physically released from the matrix.

This organism is seldom seen in collections and is very sensitive to fixative agents. Chromulina chionophila will form a thick, brown, surface film in culture medium four days after collection, from a sample which appeared free of cells at the time of collection. A bloom of this organism in the field is seldom observed.

Chlamydomonas nivalis (Bauer) Wille. As the organism which is most frequently listed as the primary species in collections of snow algae and one which has been used as the species to which many red spores are assigned it is important that this organism be cultured and the life cycle examined. There is the possibility, as stated by Kol (1942), that once the method of keeping the "species" in pure culture was developed then the path would be open for solution of the problem of classifying the red spore forming Chlamydomonas of the snow. Fukushima (1963) did report on the life cycle of Chlamydomonas nivalis and in particular the zygote formation of the organism. I have had three strains of C. nivalis in culture for over three years.

Sterile cotton was saturated with sterile nutrient medium and found to be an excellent substrate for growth. Cells spread outward from the point of inoculation. As the colored area progresses it is

possible to pick a region within the area where one phase of the life cycle is predominant. Cells on the surface and small tufts of cotton, in particular, become orange while the cells in the center of the cotton are still green, although formed into spores. Hanging drop preparations and batch cultures were made using old spores of laboratory-grown material and were also observed. Figures 27 and 28 are composites of these sources of information.

In Figure 27 the asexual phase of the life cycle is illustrated. This is the most interesting phase of the cycle. The cycle as illustrated begins with a zoospore dropping its flagella and rounding into a thick-walled spore. As the spore develops it may go in either one of two directions. First it may divide and form thick walled autospores within its own cell wall. These are released and they in turn may form more large thick walled spores like the mother cell (Figure 27H, J, F). This type of reproduction is especially prominent in a culture when it is at temperatures near 20°C, or in a medium that has a pH of 7.5-8.5 at 0°C. It appears that this mode of reproduction might be of some survival advantage when the organism is under some physiological stress, as suggested by Prescott (1961) for Arctic Algae in general. This would be of great advantage if freezing and thawing cycles were a problem. This could also account for the variation in spore sizes found in most field collections as could the rounding of zoospores into spherical spores. The C. nivalis spore may also

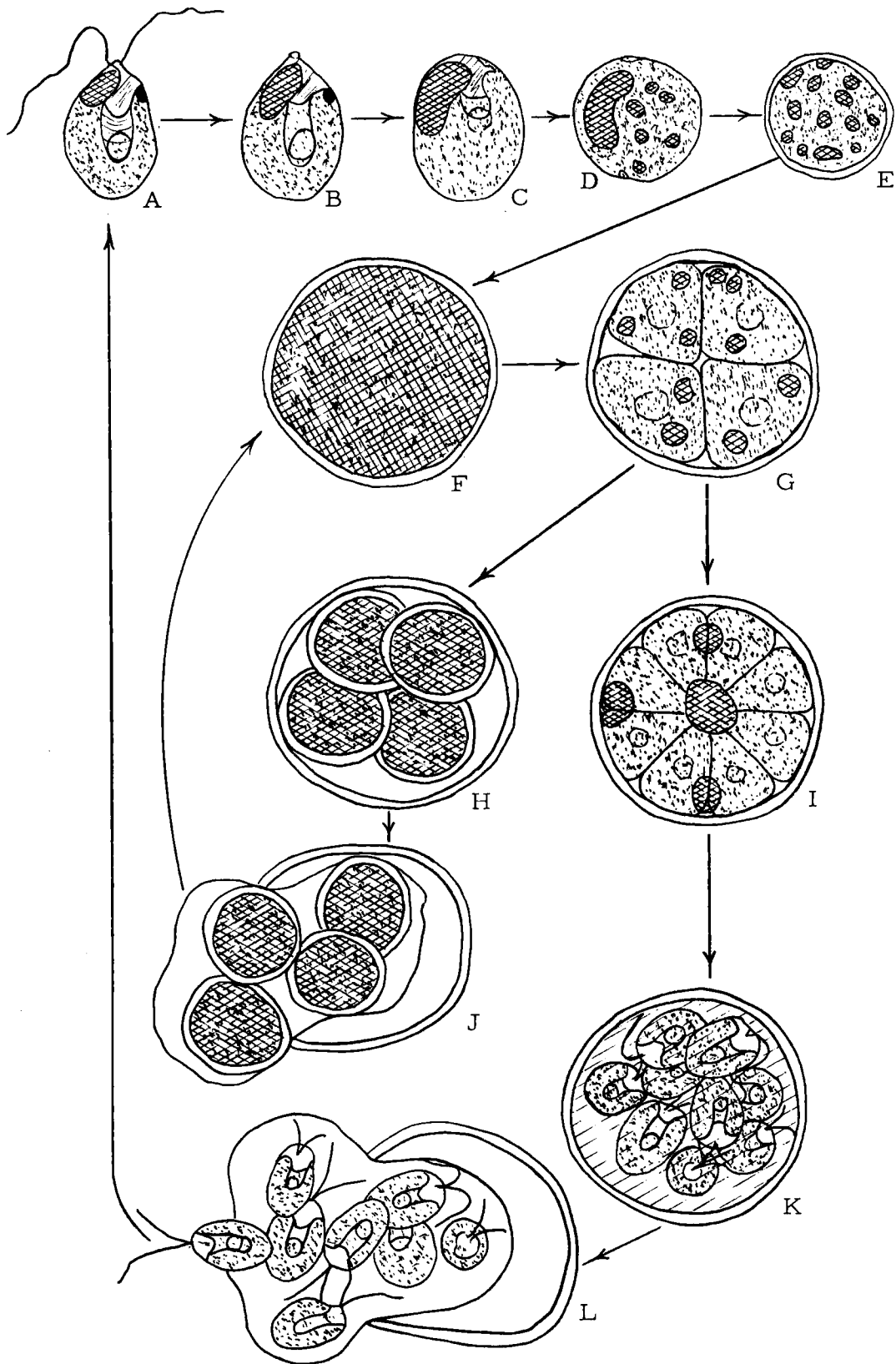


Figure 27. Asexual reproduction in *Chlamydomonas nivalis* (Bauer) Wille.

continue to undergo division of its contents, and the divided cells develop into zoospores which are released (Figure 27I, K, L). The cellular membrane containing the daughter cells is extruded through the cell wall which has been "gelatinized" (Figure 27L). The cells quite often swim within the cell membrane until it ruptures.

The life cycle of the C. nivalis observed in laboratory cultures is shown in Figure 28. The conjugation is somewhat simplified as there are often tens of cells clumping in a given area from which pairs of cells may emerge. The cells may conjugate as shown here or as shown by Fukushima (1963). Under phase contrast microscopy it was seen that the cell membrane of the male gamete was often left behind and pinched off as the wall formed around the zygote. The ornate cell walls associated with the zygote of C. nivalis in the field do not develop in the laboratory nor does the deep red pigment, although a deep orange does occur. The zygote may divide to form zoospores which are released to function as gametes or in the initiation of the asexual cycle described above, or the zygote may divide into four cells which then divide internally to form a large number of zoospores. The zoospore itself may stop swimming, divide and form a large number of daughter zoospores while retaining the typical Chlamydomonas shape and then release these to function as gametes or vegetative cells.

With these options available to C. nivalis it is small wonder that

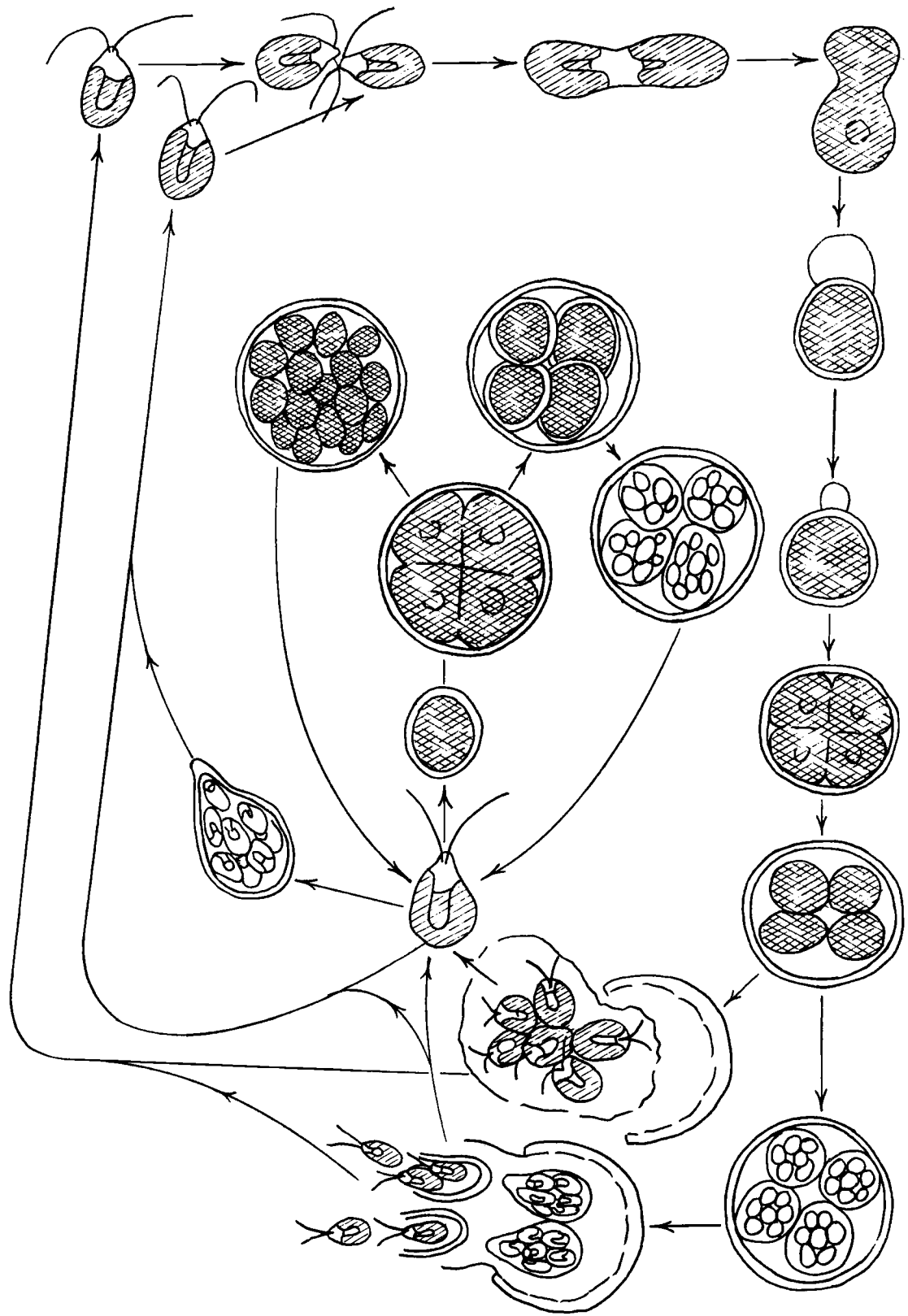


Figure 28. The life cycle of *Chlamydomonas nivalis* (Bauer) Wille in culture.

it can survive in the harsh environment of snow and ice. During the various stages of the life cycle as discussed above, the red pigment content of an individual cell changes. Zoospores themselves contain varying amounts of the oil soluble carotenoid. This probably depends upon the amount of pigment that was allocated to the cell at the time of division of the mother cell. Often the orange (red) pigment will be in a large oil droplet in the center of the sphere around which are formed the daughter cells before they round into zoospores. This pigment is lost during release of the zoospore. If the conditions are right for formation of the red pigment, the amount of pigment increases as the cell begins to round up. Pigment formation occurs in culture in OHH medium when some nutrient becomes limiting to growth, when the cells are exposed to high light intensity, and when the cells are exposed to air by being splashed upon the sides of a culture vessel during aeration. Even in direct sunlight these cells do not form the deep red pigment, at least not the extent that is found in nature.

I have found that if spores are kept in nutrient depleted medium for an extended period such as six months to a year they germinate very quickly when supplied with fresh medium. In one hanging drop experiment I noted that germination took place in less than 90 minutes, and furthermore, although the spores appear to be at identical stages under visual inspection, their growth has actually been halted at

different stages of development. This may be true in nature also. Recently I was able to obtain germination of C. nivalis spores which had been kept in the dark at 5°C for over five years. Cells used in the inoculum had retained their red pigment. All strains of C. nivalis in our collection appear to be the same "species" and very little variation has been noted among them.

Summary and Collation

Not all organisms found in collections from snow are cryobionts. Along with the spores of the various snow algae there is a Microspora sp. which will not grow at reduced temperatures, various mosses which form protonema upon incubation in nutrient medium at 5°C, diatoms which do not grow at 2°C or below, blue-green algae, fungi, pollen, and perhaps many more organisms which are adventitious members of the snow "algae" assemblage. It is an extremely difficult task to separate the cryobionts from the adventitious forms in this case. Only one of the organisms examined here behaves as a true cryophilic species. Chromulina chionophila appears to be restricted to an existence below 15°C. While C. chionophila grows and reproduces at 0°C, it is unable to do so at 15°C and above. This organism has other obstacles to overcome in order to exist in this environment. It must survive freezing and thawing, but as yet a cyst or resistant spore such as seen in Chlamydomonas nivalis has not been positively

identified. Another problem is desiccation and heat during summer. The areas where Chromulina has been collected by the author are not permanently snow-covered and during summer become barren areas of lava rock and sand.

When comparing C. chionophila with C. nivalis one is struck by the lack of similarities between the two. The latter has a thick, tough cell wall which protects it from the rigors of freezing and thawing and summer drying. Chlamydomonas nivalis is not limited to temperatures below 15° or even 25° C whereas C. chionophila appears to be limited to below 15° C. It appears that the common factor between the cryophil and the crybiont is the lack of competition which they face in the snow and ice.

Stichococcus bacillaris and Raphidonema nivale are both reported frequently in collections but seldom if ever are they the primary organism of the collection. Neither of these has a thick-walled vegetative cell but each survives in the snow and ice environment.

It is possible that there is a cellular "antifreeze" or that the freezing point of the microenvironment surrounding the cell is depressed by the release of organic substances from the cell. Both of these organisms are capable of a high ratio of carbon fixation per unit mass of cellular carbon (Figure 29A, B, C, D, E) at 0° C. Chlamydomonas yellowstonensis is assumed to have the adaptations of

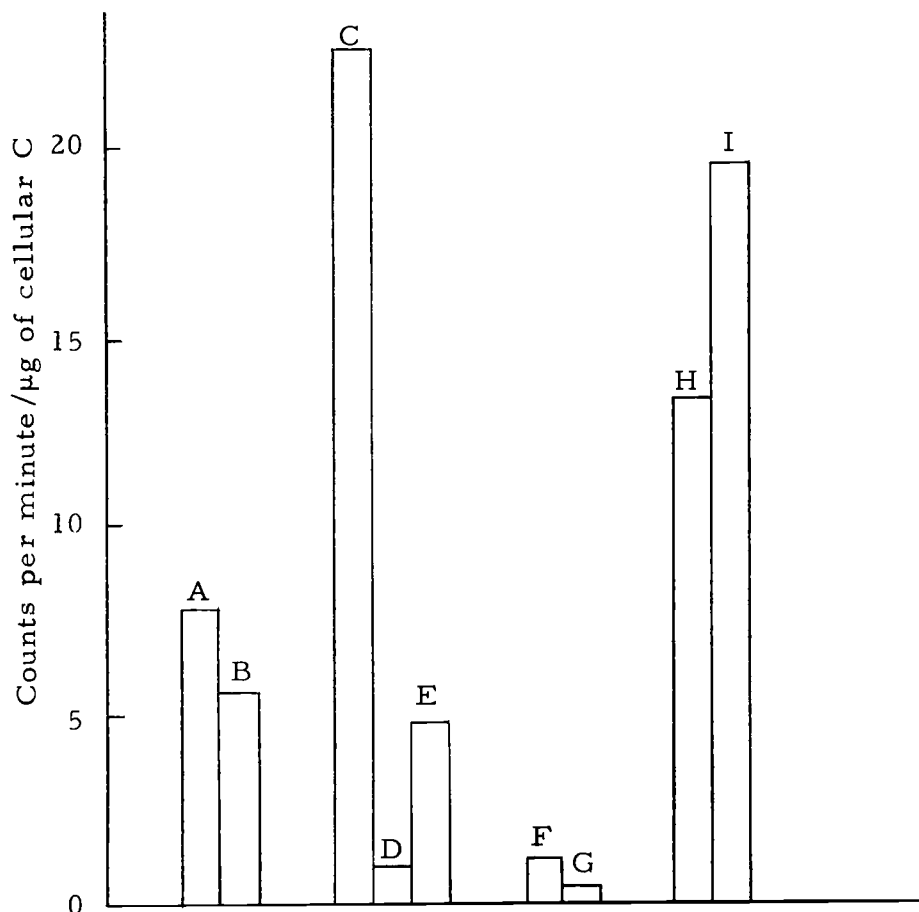


Figure 29. A comparison of the amount of carbon fixed per μg of cellular carbon present.

- A - Stichococcus bacillaris Nag., which has been held in the dark for ten days previous to the experiment.
- B - S. bacillaris, which had 48 hours of dark pretreatment.
- C - Raphidonema nivale Lagerh., which were grown in Holm-Hansen's medium and tested in Pringsheim's medium.
- D - R. nivale, which was grown and tested in Holm-Hansen's medium. The stock culture was 24 hours older than in C.
- E - R. nivale. The cells were treated as in C but the stock culture was six days older.
- F - Collection of spores from the field.
- G - Collection of spores from the field.
- H - Chromulina chionophila Stein. These cells were held in the dark at 0°C for 48 hours previous to the experiment.
- I - C. chionophila. These cells were held in the dark for 16 hours.

C. nivalis, with the exception of the red carotenoid pigment.

In conclusion, it may be said that the evidence gathered thus far indicates that the organisms which have been investigated are not restricted to the ice and snow, but rather are the organisms which have adaptations which allow them to successfully fill an ecological niche where the pressure of competition is lessened by the harshness of the environment.

Literature Cited

- Allen, M. M. (1968)
Simple Conditions for Growth of Blue Green Algae on Plates
Journal of Phycology 4:1-4
- Bauer, F. (1819)
Microscopical Observations on the Red Snow
The Quarterly Review of Literature, Science, and Arts
7:222-229
- Bourelly, P. (1951)
"Catalogues des Collections Vivantes, Herbiers et Documents.
I. L'Algotheque."
Museum Nationale D'Histoire Naturelle Laboratoires de Cryptogamie.
Paris
- Bristol, B. M. (1919a)
On a Malay form of Chlorococcum sinicola (Nag.) Rabenh.
Journal of the Linnean Society of Botany 44:473-482
- _____ (1919b)
On the Retention of Vitality by Algae from Old Stored Soils
New Phytologist 18:92-107
- _____ (1920)
On the Algal Flora of Some Desiccated English Soils: An Important
Factor in Soil Biology
Annals of Botany 34:35-80
- _____ (1923)
Alga in "The Microorganisms of the Soil"
E. J. Russel editor
- Clark, F. C. (1875)
Red Snow
The American Naturalist 9:129-135
- Droop, M. R. (1955)
Some Factors Governing Encystment in Haematococcus pluvialis
Arkiv fur Microbiologie 21:267-272

- Ettl, H. (1965)
Uber Systematische Problems der Gattung Chlamydomonas
Phycologia 5(1):61-70
- Fogg, G. E. (1967)
Observations on the Snow Algae of the South Orkney Islands
Philosophical Transactions of the Royal Society of London
Series B 252:279-287
- Fritsch, F. E. (1912)
Freshwater Algae Collected in the South Orkneys by
Mr. R. N. Rudmose Brown, B. Sc. of Scottish National Antarctic
Expedition, 1902-04
Journal of Linnean Society of Botany 40:293-338
- Fukushima, H. (1963)
Studies on the Cryophytes in Japan
Journal of Yokohama Municipal University (Series C)
43:(144):1-146
- Gessner, F. (1955)
Hydrobotanik. Band I. Deutscher Verlag der Wissenschaften, Berlin
- Hardy, J. T. (1966)
Identification, Culture and Physiological Ecology of Cryophilic Algae
M. S. Thesis, Oregon State University, Corvallis, Ore.
- Hindák, F. (1963)
Systematik der Gattungen Koliella gen. nov. und
Raphidonema Lagerh.
- Hindák, F. and J. Komarek (1968)
Cultivation of the Cryosestonic Alga Koliella tatrae (Kol) Hind.
Biologia Plantarum 10(2):95-97
- Hitchcock, R. (1885)
The Red Snow
American Monthly Microscopical Journal 6:221-224
- Holm-Hansen, O. (1964)
Isolation and Culture of Terrestrial and Freshwater Algae of
Antarctica
Phycologia 4(1):43-51

- Kol, E. (1941)
Green Snow of Yellowstone National Park
American Journal of Botany 28(3):185-191
- _____ (1942)
The Snow and Ice Algae of Alaska
Smithsonian Miscellaneous Collections 101(16):1-36
- Kol, E. and E. A. Flint (1967)
Algae in Green Ice from the Balleny Islands, Antarctica
New Zealand Journal of Botany 6:249-261
- Lund, J. W. G. (1942)
Contributions to Our Knowledge of British Chrysophyceae
New Phytologist 43:274-292
- Nageli, C. (1849)
"Gattungen einzelliger Algen"
Zurich p. 79
- Prescott, G. W. (1961)
Ecology of Freshwater Algae in the Arctic
Recent Advances in Botany 9th International Botanical
- Pringsheim, E. G. (1966)
Nutrition Requirements of Haematococcus pluvialis and
Related Species
Journal of Phycology 11:1-7
- Schroder, Briaitte (1954)
Untersuchungen uber den Einflu Verscheidener
Neutralsalze ave Wachsteneen Zellform und Fadenbildung von
Strichococcus bacillaris und Einigen Anderen Grunalgen.
Arkiv fur Mikrobiologie 20:63-78
- Stein, J. (1963)
A Chromulina (Chrysophyceae) From Snow
Canadian Journal of Botany 41:1367-1370

MISCELLANEOUS OBSERVATIONS FROM FIELD
AND LABORATORY

The Development of Blooms in the Field

Fogg (1967), working in the South Orkney Islands, attributed the increased density of snow algae cells to the mechanical accumulation of cells due to the ablation of the snow. But in the area in which I made my collections the snow fields are, for the most part, of a temporary nature; each year the buildup of the snow algae must begin anew. The red snow is produced with amazing rapidity as the snow begins to thaw and it is doubtful that a mechanical accumulation can account for this. During the summer of 1969, after the late spring bloom had disappeared from the main collection site on Mt. Bachelor, an unseasonal period of cold weather caused continued freezing at this altitude. On the day of my weekly sampling during the cold spell there was no evidence of colored snow nor was any detected in the course of collecting snow for chemical analysis. One week later the snow at the collecting site was red over an area hectares in extent and the surrounding snow fields were red also. The red layer extended to as much as 7 or 8 cm below the surface of the snow or existed as only a red crust in some places. There appeared to be no appreciable ablation of the snow.

In the summer of 1970, when the red snow was first in evidence,

vertical sampling of a sun cup revealed that there was a cell density of 2.3×10^5 cells ml^{-1} in the first 5 cm, diminishing to 3.5×10^4 cells ml^{-1} at 20 cm. These cells were most certainly the product of very recent growth. A week later the area surrounding the few early patches was covered with a rich red color indicating a rapid growth of the algae.

Species Composition of the Blooms

The species composition of these blooms varies from near unialgal to those in which a dozen or more species may be present. The vertical sampling of a sun cup revealed a change in species composition with depth. The forms having little or no red pigmentation formed an increasing proportion of the total cell number with greater depth. There was also a lessening of the red pigment in the normally red species with increased depth.

Summering and Wintering

While dealing with the problems of these organisms in the laboratory it must be remembered that the organisms exist in a far different environment than we provide them. They must survive the cold of winter and the heat of summer. Fukushima (1963) has evidence that the algae spend the summer in the soil. At the collection site of this laboratory the soil is a mixture of lava dust and lava

fragments which, during summer, may be 35°C and very dry. Algae from this soil have been cultured in our laboratory.

The spores or perennating stages are covered by the fall and winter snows to a depth of five or more meters. The cells are in relative darkness for six to eight months. In spring the snow melts and light penetrates to the resting stages. It is possible that the cells germinate and move upwards in the thin layer of water covering the snow or ice crystals toward the optimum light level. In the laboratory, spores inoculated at the bottom of a tube filled with sterile cotton nearly saturated with nutrient media will germinate and appear on the surface in two weeks. Intervening frozen layers in snow would prevent the cells from reaching the surface. The surface layer gradually ablates and the algal bloom becomes visually observable. As snow on the lower slopes disappears the organisms form cysts that are carried into the soil by melt water (Fukushima, 1963). Some of the soil with accompanying algal cells may be blown up higher on the slopes where new blooms begin. These sequences are frequently observed. It is also possible that cells spend their winter at the old-snow/new-snow interface and move toward the surface as conditions are favorable. Slopes where there is permanent snow may have several remnant blooms imposed on one another. This is conjecture in the main, but seems to fit the observations made in the region of our collections. There are also other dispersal means which probably

play a role in the appearance of snow algal blooms, since some of the species observed are not motile. A great deal of dust may collect on the abundant conifers and drip down onto the snow in spring; blooms are frequently found beneath such trees.

Trachelomonas kolii. This organism is regularly seen in late spring in the area from which it was originally described (Hardy and Curl, 1968). As the summer progresses fewer of the cells possessing collars are reported. The sequence of events (Figure 30) has been drawn from observations in the field and the laboratory. When T. kolii loses its lorica and a Chlamydomonas nivalis zygospore loses its cell wall ornamentation, they both have an appearance identical with the hypnospore of C. nivalis. There is no way to visually distinguish these cells. Dr. T. W. Goodwin (pers. comm.) has suggested that an analysis of the carotenoid pigments would reveal the presence or absence of T. kolii.

Ecology of Snow Fields

The snow algae are the primary producers of the snow and ice habitats. They are the main suppliers of fixed carbon to the higher trophic levels. In some regions, such as Oregon's Cascade Mountains, the contribution of the pollen of Pinus contorta cannot be overlooked. Fukushima (1963) reports finding ciliates in the snow samples in Japan. I have found a number of unidentified ciliates feeding on the

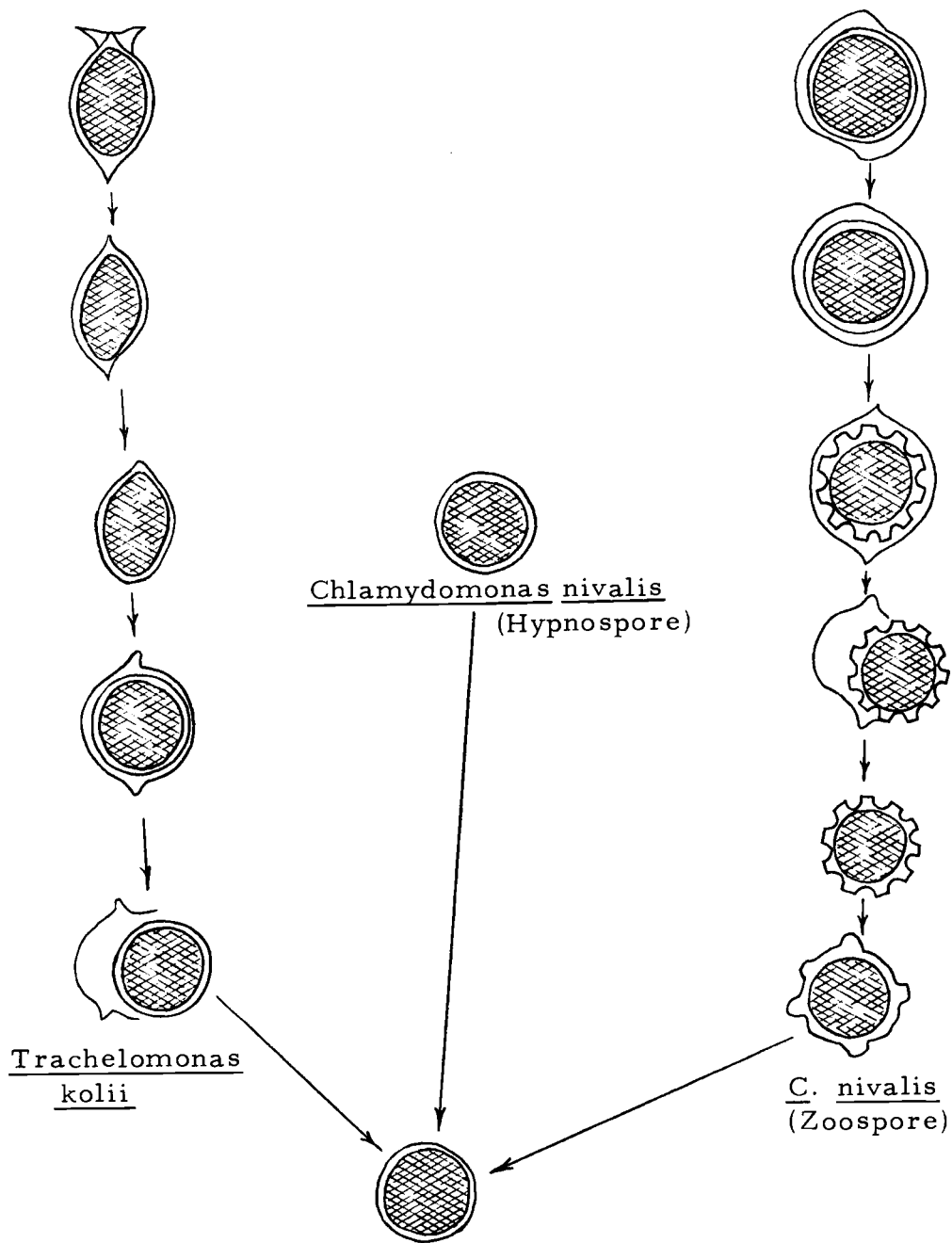


Figure 30. Spore form frequently found in the field and assigned to C. nivalis, although the identification is uncertain.

snow algae. At least one of these is temperature sensitive. In samples from both Antarctica and Oregon there are rotifers living at the expense of the algae; two cultures of algae and rotifers have been maintained in the laboratory for over one year.

The above-mentioned herbivores are of microscopic dimensions. There is one macroscopic herbivore which occurs in the Cascades in relative abundance; the ice worm (Mesenchytraeus solifungus). Recently the gut contents of three of these oligochaetes were examined. The gut of one worm contained the recognizable remains of C. nivalis, Scotiella nivalis, and Raphidonema nivale, with some yeast and other fungi. The gut of the other two worms yielded primarily fungal hyphae, pine pollen, C. nivalis, Scotiella cryophila, Scotiella nivalis, Protococcus spp. and other unrecognized forms. There was also some organic and inorganic detrital material. Often small collembola appear in snow samples associated with snow algal blooms. The feeding habits of these insects are not known. Tardigrades have been observed in samples from both the Oregon Cascades and Antarctica. These are the largest herbivores thus far reported in the snow.

Literature Cited

Fogg, G. E. (1967)
Observations on the Snow Algae of the South Orkney Islands
Philosophical Transactions of the Royal Society of London
B 252-284

Fukushima, H. (1963)
Studies on Cryophytes in Japan
Journal of Yokohama Municipal University
(Series C) 43 (144):1-146

Hardy, J. T. and H. C. Curl, Jr. (1968)
New Species of Trachelomonas
Journal of Phycology 4:9-12