4 Microbial Ecology of Snow and Freshwater Ice with Emphasis on Snow Algae

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4.1 Overview

The physical and chemical properties of snow and ice result in a habitat that can support microbiological activity. Microorganisms such as bacteria, algae, and fungi are commonly found thriving in snow, glacial ice, lake ice, ice shelves, and sea ice (Kol, 1968a; Horner, 1985). Microbes found living in these habitats may encounter extreme conditions of temperature, acidity, irradiation levels, and minimal nutrients, and they are subjected to desiccation when liquid water is no longer available (Hoham, 1992). These environments are suitable habitats for microorganisms only when liquid water is present for at least part of the year, allowing for growth and reproduction of the microbes (Pollock, 1970). Even though viable microbial cells and spores are present, the surface of the vast Antarctic polar plateau and the Greenland ice sheet is virtually lifeless because of the lack of water in the liquid phase (Catranis and Starmar, 1991). In contrast, the bottom surfaces of the Antarctic glaciers are wet because of basal melting and may serve as an important habitat for microbes (Scherer, 1991). The sea ice of the Arctic Ocean often contains blooms of snow algae during the summer when meltwater is present on the ice surface (Melnikov, 1989; Gradinger and Nürnberg, 1996). Microbes are abundant in snow fields and in depressions and ponds found on glaciers (Kol, 1968a; Wharton et al., 1985); those found in snow and ice have special adaptations and mechanisms for living in cold temperatures and tolerating the long period of desiccation during the time of nonactive growth and the high irradiance levels characteristic of summer (Bidigare et al., 1993). Microorganisms play a fundamental role in the biogeochemistry of snow and ice (Jones, 1991) and are closely involved in the primary production, respiration, nutrient cycling, decomposition, metal accumulation, and food webs associated with these habitats (Fjerdingstad et al., 1978; Hoham, 1980; Jones, 1991; Hoham et al., 1993; Hoham and Ling, 2000). Microbes also contribute to melting of snowpacks and formation of cryoconite holes (Kol, 1968a; Wharton et al., 1985).

4.2 Introduction

This chapter focuses on the microbial ecology of snow fields and glaciers with some discussion of lake ice and the relatively freshwater surfaces of ice shelves. It does not include sea ice that is predominantly a marine environment (see reviews on this subject by Horner, 1985; Palmisano and Garrison, 1993; Kirst and Wiencke, 1995; Horner, 1996) or biological ice nucleation (see the comprehensive bibliography on this subject by Warren, 1994).

4.2.1 Historical Perspective

Snow and ice microorganisms have been recorded since the time of Aristotle but were first brought to the attention of the scientific community in 1819 (Bauer, 1819). Most of the work in snow and freshwater ice microbiology has focused on the snow algae (Kol, 1968a; Hoham, 1980) and ice algae and cyanobacteria from dry valley lakes in Antarctica (Parker et al., 1982a, 1982b). However, there is an increasing awareness of algae from permanent glaciers (Ling and Seppelt, 1990, 1993; Yoshimura, Kohshima, and Ohtani, 1997). Much less is known about fungi from snow (Hoham et al., 1993) and glacial ice (Abyzov, 1993), and, until recently, eubacteria from snow (Margesin and Schinner, 1994) and glacial ice have been scarcely studied (Schinner, Margesin, and Pümpel, 1992). The effects of grazers, such as ciliates, rotifers, and collembola, on snow microbe populations have been included in some studies (Pollock, 1970; Aitchison, 1989; Hoham et al., 1993).

Between 1819 and the mid-1960s, research emphasized the systematics, taxonomy, and distribution of snow and ice algae, with most investigations coming from Europe (Wille, 1903; Kol, 1968a), North America (Kol, 1968a), Japan (Fukushima, 1963), and one of significance from the South Orkney Islands (Fritsch, 1912). In the past three decades, however, the ecology and physiological ecology, life histories, taxonomy, distribution, ultrastructure, biochemistry, physiology, and interrelationships with snow chemistry of snow microorganisms have been examined. This chapter emphasizes this more recent research.

4.2.2 Locations of Snow and Ice Microorganisms

Snow and ice algae and other microbes are well known from alpine and high latitude regions of Europe (Kol, 1968a; Schinner et al., 1992). Their distributions in western North America have been reported regionally (Kol, 1942; Stein and Brooke, 1964; Garric, 1965; Stein and Amundsen, 1967; Hoham and Blinn, 1979; Wharton and

Vinyard, 1983) and more recently in the eastern parts of the continent (Handfield et al., 1992, Duval, 1993; Hoham et al., 1993, Duval and Hoham, 2000). Other notable reports include those from Japan (Kobayashi and Fukushima, 1952; Fukushima, 1953, 1963), Australia (Marchant, 1982, 1998), New Zealand (Kol, 1968b; Thomas and Broady, 1997), New Guinea (Kol and Peterson, 1976), the Himalayas (Kohshima, 1984b, 1987a, 1987b; Yoshimura et al., 1997), and some parts of South America (Lagerheim, 1892; Kol, 1968a), the Arctic (Kol, 1942, 1963; Sinclair and Stokes, 1965; Kobayashi, 1967; Kol, 1968a; Kol and Eurola, 1973; Melnikov, 1989; Gradinger and Nürnberg, 1996), and Antarctica and surrounding islands (Fritsch, 1912; Llano, 1962; Hirano, 1965; Fogg, 1967; Kol, 1971; Akiyama, 1979; Ishikawa, Matsuda, and Kawaguchi, 1986; Bidigare et al., 1993; Broady, 1996; Ling, 1996; Mataloni and Tesolin, 1997; Ling and Seppelt, 1998). Little is known about snow and ice microbes from Africa and many alpine areas of Asia and South America, but Duval, Duval, and Hoham (1999) recently discovered a snow alga from the Atlas Mountains of Morocco. Because similar extreme environments may exist or may have existed in the past on the planet Mars, NASA has included these microbes as one of four life systems on Earth as possible analogs for life on early and present day Mars (Wharton et al., 1989a, 1989b; Rothschild, 1990).

4.3 Populations

4.3.1 Population Diversity and Density

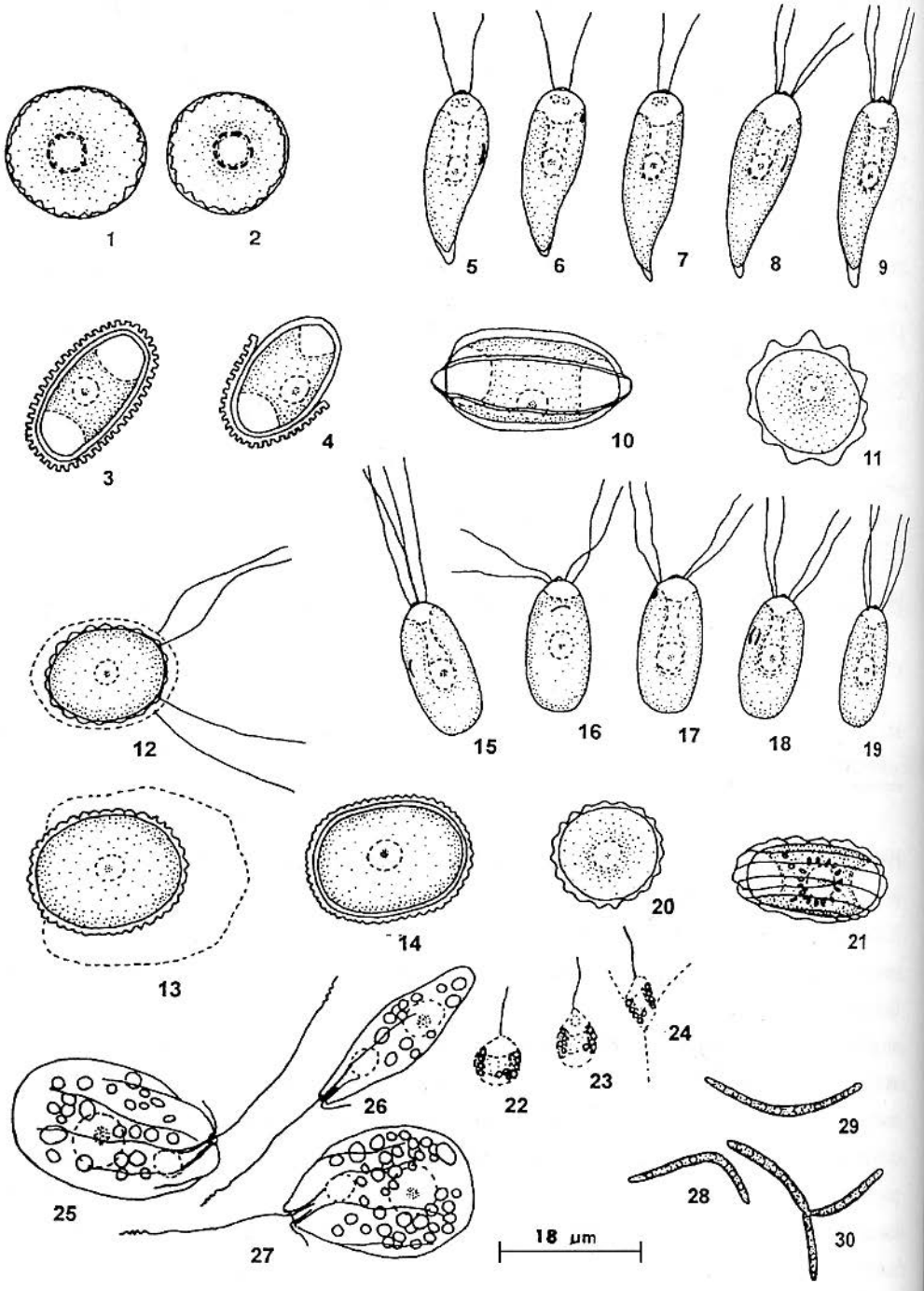
This section emphasizes the bacteria, fungi, algae, protozoa, and some invertebrates that are found in snow and ice. Of these microbes, the algae have received the most attention. Algae found in snow and ice include Chlorophyta (green algae), Euglenophyta (euglenoids), Chrysophyta (Xanthophyceae [yellow-green algae], Chrysophyceae [golden algae], Bacillariophyceae [diatoms]), Pyrrhophyta (dinoflagellates), and Cryptophyta (cryptomonads). The Cyanophyta (blue-green algae) are discussed as cyanobacteria. Specific overviews on these groups in snow and ice have been published for the green algae (Kol, 1942, 1968a; Hoham, 1980; Hoham et al., 1993), euglenoids (Kiener, 1944; Hoham and Blinn, 1979; Hoham et al., 1993), yellow-green algae (Kol, 1968a; Hoham et al., 1993), golden algae (Fukushima, 1963; Stein, 1963; Hoham and Blinn, 1979, Hoham et al., 1993), diatoms (Wharton, Parker, and Simmons, 1983; Wharton and Vinyard, 1983), dinoflagellates (Kol, 1968a; Gerrath and Nicholls, 1974), cryptomonads (Javornický and Hindák, 1970), and cyanobacteria

Table 4.1. *Maximum populations of snow and ice algae recorded for green algae (Division Chlorophyta).*

Species	Snow color	Location	Cells mL^{-1} of snow water equivalent (SWE)	Reference
Chlamydomonas nivalis and Trochiscia americana	Red	California, USA	6.3×10^4	Thomas (1994)
Chlamydomonas nivalis	Red	Oregon, USA	2.3×10^5	Sutton (1972)
Chlamydomonas sp.	Green	Czech Republic	1.4×10^6	Lukavský (1993)
Chloromonas brevispina	Green	Washington, USA	5.0×10^5	Hoham et al. (1979)
Chloromonas pichinchae	Green	Washington, USA	1.0×10^6	Hoham (1989a)
Chloromonas rubroleosa	Red	Antarctica	2×10^5	Ling and Seppelt (1993)
Chloromonas sp.-B	Salmon-orange	Massachusetts, USA	8.6×10^5	Hoham et al. (1993)
Chloromonas sp.-C	Salmon-orange	Massachusetts, USA	3.0×10^5	Hoham et al. (1993)
Mesotaenium berggrenii	Grey-pink	Antarctica	1.0×10^5	Ling and Seppelt (1990)

(Wharton et al., 1981, 1983). Several of these microbes are illustrated in Figures 4.1 to 4.30 (Hoham and Blinn, 1979).

Population sizes of snow and ice algae are best known for the green algae that color snow green, red, and orange in North America, but other reports include those from Europe and Antarctica (Table 4.1) (also see Müller et al., 1998a). In a snow algal study on a Himalayan glacier, algal biomass estimated by total cell volume rapidly decreased as the altitude increased (Yoshimura et al., 1997). Populations of eubacteria in snow and ice have been studied only recently, and relatively little is known about their species composition in this habitat. Moiroud and Gounot (1969) confirmed the presence of psychrophilic bacteria from glacier ice. A relatively low eubacterial recovery was made from the Agassiz ice cap, Ellesmere Island, Canada (Handfield et al., 1992). Of the 17 microorganisms that grew at 1°C and 4°C and were defined as psychrophilic, there were 4 Gram-positive bacteria, 9 algae (after publication they were determined to be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Figures 4.1 to 4.30. Cryophilic organisms found in southwestern United States (bar = 18 μm) (from Phycologia [Hoham and Blinn, 1979], 18, 141). Figures 4.1 and 4.2: *Chlamydomonas nivalis*, a green alga. Resting spores with smooth cell wall, red protoplast, and central pyrenoid surrounded by starch plates. Figures 4.3 and 4.4: *Chloromonas brevispina* (formerly *Cryocystis brevispina*), a green alga. Zygospores with central nucleus surrounded by parietal chloroplast. Note two large lipid bodies, one at each pole of the cell. Note shedding of primary wall with spines and exposure of inner smooth secondary wall (Figure 4.4). Figures 4.5 to 4.11: *Chloromonas nivalis*, a green alga. Figures 4.5 to 4.7: Vegetative cells with two flagella, papilla, two apical contractile vacuoles, centrally located nucleus, and parietal cup-shaped chloroplast. Note median, lens-shaped eyespot (Figure 4.5), anterior oval eyespot (Figure 4.6), and cell without eyespot (Figure 4.7). Figures 4.8 and 4.9: Planozygotes with four flagella otherwise similar to vegetative cells. Note median, lens-shaped eyespot (Figure 4.8) and cell without eyespot (Figure 4.9). Figures 4.10 and 4.11: Zygospores with central nucleus surrounded by parietal chloroplast. Note two large lipid bodies, one at each pole of cell (Figure 4.10). Note wall with flanges extending full length of cell (Figure 4.10) and flanges in cross section (Figure 4.11). Figures 4.12 to 4.14: *Cryocystis granulosa* cell type (the zygote of a *Chloromonas* [unpublished]), a green alga. Figure 4.12: Quadriflagellate cell giving rise to *Cryocystis granulosa*. Note central nucleus surrounded by protoplast, cell with developing wart-like protuberances, and membrane surrounding cell. Note expansion of membrane around cell (Figure 4.13) and shedding of membrane and development of primary and secondary walls (Figure 4.14). Figures 4.15 to 4.21: *Chloromonas polyptera*, a green alga. Figures 4.15 to 4.19. Planozygote (formerly *Carteria nivale*) with four flagella, papilla, centrally located nucleus, and parietal, cup-shaped chloroplast. Note median, lens-shaped eyespot parallel to longitudinal axis of cell (Figure 4.15), anterior, lens-shaped eyespot perpendicular to longitudinal axis of cell (Figure 4.16), anterior oval eyespot (Figure 4.17), two median, lens-shaped eyespots (Figure 4.18), and cell without eyespot (Figure 4.19). Figures 4.20 and 4.21: Zygotes (formerly *Scotiella polyptera*) with central nucleus surrounded by central chloroplast. Note small lipid granules (Sudan IV test) surrounding nucleus, and two large lipid bodies, one at each pole of the cell (Figure 4.21). Note wall with numerous flanges in cross section (Figure 4.20) and extending full length of cell, sometimes branching (Figure 4.21). Figures 4.22 to 4.24: *Chromulina chionophilia*, a golden alga. Cells with a parietal, band chloroplast, one contractile vacuole, a single flagellum, and 8–25 chrysolaminarin bodies. Note three rhizopodia extending from the plasmalemma (Figure 4.24). Figures 4.25 to 4.27: *Notosolenus* sp., a colorless euglenoid. Cells with ridges on pellicle, central to posterior nucleus, numerous paramylon bodies, one contractile vacuole at anterior end of cell, and two flagella, the longer projected anteriorly, the shorter posteriorly. Cells in narrow diameter view (Figure 4.26) and wide diameter view (Figures 4.25 and 4.27). Figures 4.28 to 4.30: *Selenotila nivalis*, a fungus. Cells with radiating arms possessing numerous vacuoles alternating with granular cytoplasm.

yeasts; M. Handfield, personal communication, 1994), 3 yeasts, and 1 uncertain (this was also a bacterium; M. Handfield, personal communication, 1994). Of the 220 microorganisms that were isolated at 25°C, 104 were eubacterial isolates and most were fungi. The eubacteria were categorized as Gram-positive rods (37), Gram-negative rods (30), cocci (20), streptomyces (5), and uncertain forms (12). Of these 104, 25 (24 percent) grew at 4°C and were defined as psychrotrophic. The origin of these microbes was considered terrestrial because they grew poorly in seawater. The low recovery values suggested that environmental conditions on the ice cap permit only minimal microbial activity.

Thomas (1994) reported a direct correlation between eubacterial and algal populations in snow from Tioga Pass, California. The highest populations of eubacteria (3.2×10^5 cells mL^{-1}) were found with large populations of algae (4.9×10^4 cells mL^{-1}) in red-colored snow, and snow not colored by algae contained 11 to 33 percent of the eubacterial populations and 0.1 to 5 percent of the algae found in red snow. Three separate eubacterial species were recognized but not identified from these samples, and the green algae identified were red-colored spores of *Chlamydomonas nivalis* and *Trochiscia americana* (*T. americana* is probably a resting spore of a green algal biflagellate). Baross and Morita (1978) suggested that large populations of psychrophilic bacteria exist in association with snow algae.

4.3.2 Snow Microbe Associations

Weiss (1983) found encapsulated Gram-negative bacteria in the loose fibrous network surrounding cells of the snow alga, *Chlamydomonas nivalis*, collected from Yellowstone National Park, Wyoming, and the Sierra Nevada Mountains, California. Bacteria were not found in control samples taken from white snow. Weiss concluded that the association between bacteria and snow algae was characteristic of red snow populations and was not a chance association. Paerl (1980) indicated that such interactions can be a mutual benefit in the form of increased nutrients, but Weiss (1983) did not have the experimental evidence to support this possibility. Interestingly, Thomas (1994) located bacteria in controls (white snow samples) from similar study sites in the Sierra Nevada Mountains. Hoham et al. (1993) reported a similar association between bacteria and fungi with *Chloromonas* snow algae from eastern North America. They found bacteria and fungi adhering to the outer gelatinous matrix surrounding resting spores in this *Chloromonas*, and it was not known if a symbiotic association occurred between these microbes.

Kol (1968a) reported that, of 466 species of microorganisms found in snow, 77 were fungi and 35 were bacteria; the remainder were algae. She indicated, however, that

none of the bacteria and only 6 fungal species were cryobionts. Three of the fungi were parasites on *Chlamydomonas* spp. and the desmid *Ancylonema nordenskioldii*. Kol (1968a) illustrated several developmental stages of the remaining 3 fungi, *Chionaster nivalis*, *Chionaster bicornis*, and *Selenotila nivalis*. These fungi are found most commonly with large populations of snow algae (Hoham and Blinn, 1979; Hoham, Mullet, and Roemer, 1983; Hoham et al., 1993) and do not appear to enter into any type of symbiotic association with the algae, but they probably receive their required carbon for growth from them (Hoham et al., 1989). Schinner et al. (1992) reported 430 strains of microbial bacteria and fungi from alpine soils and cryoconite of glaciers from the European Alps. Of these isolates, 77 percent were bacteria, 5 percent were actinomycetes, 20 percent were yeasts, and 3 percent were hyphomycetes. Active microbial communities were found in the slush layers of the winter cover of lakes in the Pyrenean and Tyrolean Alps in spite of the low temperature and seasonal occurrence of the habitat (Felip et al., 1995). The microbes included bacteria, flagellates (algae and heterotrophs), and ciliates. The snow alga *Chlamydomonas nivalis* was restricted to surface pools.

Sinclair and Stokes (1965) reported psychrophilic yeasts that were isolated from polar ice, snow, and soil. It is now apparent that many fungi found in snow are yeasts. Yeasts were reported from snow bacterial studies (Handfield et al., 1992; Thomas, 1994), and Hoham and Clive (unpublished data) found a direct correlation of large yeast populations in snow with large snow algal populations from Killington Ski Area, Vermont, and Whiteface Mountain, New York. Thus, the data collected on populations of snow bacteria and snow fungi indicate a direct correlation between large populations of these microbes with large populations of snow algae.

In some parts of the Pacific Northwest, the snow fungus *Phacidium infestans* grows throughout melting snowpacks (Hoham, 1975b; Hoham and Mullet, 1977; Hoham, Roemer, and Mullet, 1979; Hoham, 1987). Snow algae passively adhere to this fungus, but physical connections between them do not occur as in lichen symbiosis. However, exchange of metabolites may take place between these microbes. Upon complete melting of the snowpacks, *Phacidium* with its adhering algae becomes dried threads or strands draping over soil, rocks, tree branches, shrubs, and forest cover such as mosses and lichens. *Phacidium* also grows during winter under a heavy layer of snow over unfrozen soil when temperatures remain near 0°C at the soil surface and under snow cover where air humidity is sufficient (Vuorinen and Kurkela, 1993). Under these circumstances, the blight may parasitize coniferous seedlings in winter as it does during the time of spring snowmelt. It is not known if *Phacidium* exchanges metabolites with other microbes such as bacteria, algae, and fungi during the winter.

In Antarctic dry valley lakes, the algal mats located under the ice were dominated by the cyanobacteria *Phormidium frigidum* and *Lyngbya martensiana* (Wharton et al., 1983). Heterocystis cyanobacteria (*Anabaena cylindrica* and *Nostoc commune*) were restricted to moat, pond, ice surface, and cryoconite areas (Wharton et al., 1981). Except for moat and anaerobic prostrate mats, pennate diatoms were abundant in all samples, particularly in surface layers of liftoff and aerobic prostrate mats. These diatoms were often seen gliding between filaments of the cyanobacteria and included species of *Navicula*, *Nitzschia*, and *Pinnularia*. Green algae were rare in all below-ice samples. Heterotrophic bacteria and yeasts were observed in the surface layers of mats, but these microbes were not identified. Viruses may regulate heterotrophic and autotrophic protozoan, algal, and bacterial community successions in ice-covered Antarctic lakes (Kepner, Wharton, and Galchenko, 1997).

4.3.3 Food Chains and Food Webs

Primary consumers, protozoa and rotifers, are part of the food web that occurs in snow (Pollock, 1970; Hardy and Curl, 1972; Hoham et al., 1979, 1983; Hoham, 1987; Bidigare et al., 1993; Hoham et al., 1993). Some of these protozoa and rotifers from more temperate latitudes were reported to select and digest green cells over the more brightly colored orange and red cells (Pollock, 1970). It was suggested that the latter may be spores that were hard to digest. Ciliated protozoa from Antarctic snow, however, consumed both red- and green-colored algal cells (Bidigare et al., 1993). A population of ciliated protozoa was reported from Stowe ski area, Vermont, where there were large numbers of the snow alga *Chloromonas* and the snow fungus *Chionaster* (Hoham et al., 1993). However, there were no reports of consumption of the snow fungus by the protozoa as there were for the snow alga. Some protists called chytrids parasitize snow and ice algae (*Chlamydomonas* spp. and *Ancylonema*) (Kol, 1968a).

Larger Oligochaete invertebrates known as ice worms are common to certain Alaskan glaciers (Moore, 1899) and on snow fields at Mt. Rainier, Washington (Welch, 1916; Wailes, 1935). Welch (1916) reported two species of the ice worm *Mesenchytraeus* from Mt. Rainier and that certain snow algae were probably their chief sources of food. Kohshima (1984a, 1984b, 1987a) reported cold-tolerant insects and copepods that lived on a Himalayan glacier by feeding on the algae and bacteria. Aitchison (1983, 1989) reported that subnivean, winter-active collembola in Manitoba prefer feeding on species of fungi from the family Dematiaceae (see Aitchison, Chapter 5). During winter, the snow cover ranged from 30 to 60 cm and the subnivean temperature varied from −3°C to −6°C. Even though the collembola were more active at +5°C, they

continued to feed at temperatures of −2°C and −5°C (Aitchison, 1989). These fungi are subnivean and therefore unlike the fungal species that are found in and on snow at the time of snowmelt. In addition, Antor (1995) discussed the importance of arthropod fallout on alpine snowpatches for the foraging of alpine birds in the Spanish Pyrenees.

Hoham et al. (1993) discussed food webs in snow involving bacteria, algae, fungi, protozoa, rotifers, tardigrades, insects, arachnids, subnivean mammals, and higher plants. Trophic categories included autotrophs, heterotrophs, decomposers, detritivores, and contaminants.

4.4 Cell Structure and Cell Physiology

4.4.1 Cell Structure

Snow- and ice-microbe cell structure was reviewed by Kol (1968a) and Hoham (1980). Having flagella, for example, allows cells to move in the meltwater that surrounds the snow crystals at the time of snowmelt (see Pomeroy and Brun, Chapter 2). The dominant snow algae are flagellates that belong to the order Volvocales of the green algae including *Chlamydomonas* and *Chloromonas* (Kol, 1968a; Hoham, 1980) and occasionally *Smithsonimonas* and *Chlainomonas* (Kol, 1968a; Hoham, 1974a, 1974b). Even though *Carteria* with four flagella has been identified from snow (Fukushima, 1963; Stein and Amundsen, 1967; Kol, 1968a), it is probable that all *Carteria* from snow are planozygotes (resting phases) of other biflagellate green algae (Hoham et al., 1983). Other flagellates in snow include the golden algae (Chrysophyceae) *Chromulina* and *Ochromonas* (Fukushima, 1963; Stein, 1963; Hardy and Curl, 1972; Hoham and Blinn, 1979; Hoham et al., 1993), euglenoids *Euglena* and *Notosolenus* (Kiener, 1944; Hoham and Blinn, 1979; Hoham et al., 1993), cryptomonad *Cryptomonas* (Javornický and Hindák, 1970), and dinoflagellates *Gyrodinium* and *Gymnodinium* (Kol, 1968a; Gerrath and Nicholls, 1974). However, other snow microbes that are not flagellates (algae, fungi, bacteria) are passively moved in the snow meltwater. These diverse microbes have different nutritional requirements that affect the dynamics of snow chemistry in the snowpacks (see Tranter and Jones, Chapter 3).

Cell wall structures reduce desiccation, penetration of ultraviolet (UV) light, and grazing by protists and animals, and they adhere cells to substrates. Although the cell wall of red-colored cells of *Chlamydomonas nivalis* includes both protein and cellulose components (Tazaki et al., 1994b), the chemical nature of coverings surrounding other snow algal cells has not been identified. However, the wall composition may be similar to that described for the different algal groups to which snow algae belong

(Bold and Wynne, 1985; Sze, 1993). The cell wall ultrastructure of some Volvocalean snow algae has been examined by scanning electron microscopy (SEM). *Chlainomonas* has two external envelopes in *Chlainomonas kolii* (Hoham, 1974b, 1980), and the one in *Chlainomonas rubra* (Stein and Brooke, 1964; Hoham, 1974a, 1980), which may be proteinaceous (Hoham, 1980), has not been examined by SEM. The SEM of the zygote wall in species of *Chloromonas* shows an outer primary wall composed of flanges or ridges (*Chloromonas nivalis*) (Hoham and Mullet, 1977, 1978) (*Chloromonas polyptera*) (Hoham et al., 1983) or short protuberances or spines (*Chloromonas brevispina*) (Hoham et al., 1979) and a secondary inner wall that is smooth in these species (Hoham, 1980). Similarly, the zygote wall of *Chlamydomonas nivalis* has both a primary wall of truncate extensions and a smooth inner wall (Kol, 1968a; Kawecka and Drake, 1978). The combination of a secondary and primary wall may reduce desiccation and UV penetrance. In a separate study of *Chlamydomonas nivalis* (probably asexual resting spores), SEM shows an outer smooth wall surface associated with bacteria (Weiss, 1983) and a possible symbiotic association between these microbes was suggested. Transmission electron microscopy (TEM) reveals that this wall has three layers: a fibrous inner layer, a dense central core, and a smooth compact outer layer (probably asexual resting spores). These cells were surrounded by a loose fibrous network in which encapsulated Gram-negative bacteria and unidentified surface debris were seen, thus showing a close physical association between the alga, bacteria, and debris. Using single cell dielectric spectroscopy, the cell wall of *Chlamydomonas nivalis* resting spores was found to have an extremely low permittivity of 3 to 5 (Müller, Schnelle, and Fuhr, 1998b). In *Chloromonas* and *Chlamydomonas*, the flanges, spines, and truncate processes may reduce grazing and help anchor these cells to substrates after snowmelt. SEM also has helped to resolve difficult taxonomic questions associated with these species.

TEM reveals information about internal cell structure that may give insight on how cells are more suited for the environment where they live, and it has been used to examine two species of snow algae, *Chlamydomonas nivalis* and a *Chloromonas* sp. Weiss (1983) found abundant clear granules in the cytoplasm and several thylakoid membrane stacks and occasional starch grains in the chloroplast of *Chlamydomonas nivalis*. Marchant (1982) reported massive accumulations of carotenoid vesicles in the cytoplasm of *Chlamydomonas nivalis* from Australian snow samples visible by TEM, and Weiss (1983) suggested that these pigment vacuoles were the same as the granules (which contained lipids) found in his samples of *Chlamydomonas nivalis* from Wyoming and California. Lipids are more common in mature zygotes and zygospores that are

usually orange or red or in vegetative cells that have been exposed to subfreezing conditions (Hoham, 1975a, 1975b). Large accumulations of red-colored astaxanthin esters that occur in extrachloroplastic lipid globules in *Chlamydomonas nivalis* reduce photoinhibition and photodamage (Bidigare et al., 1993). Andreis and Rodondi (1979) used TEM to infer that the red stage of *Chlamydomonas nivalis* was in a condition of high metabolic activity because of the large number of mitochondria present. They also found intracytoplasmic globules of pigments that joined together and flowed in the vacuoles after local tonoplast dissolution and active cell wall synthesis in asexual reproductive stages. TEM sections of *Chlamydomonas nivalis* from Svalbard revealed ribosomal-rich cytoplasm in green biflagellate zoospores and a domination of oil droplets and starch grains in red asexual cysts (Müller et al., 1998a). Fjerdingstad et al. (1974) examined TEM sections prepared from red snow samples but published micrographs of bacteria labelled as *Chlamydomonas nivalis*.

Food reserves stored in snow algal cells are potential sources of nutrition for grazers. The common food reserves in the snow and ice algae that belong to the Chlorophyta or green algae are carbohydrates and lipids (Kol, 1968a; Hoham, 1980). Starch is found in rapidly dividing cells or in newly formed zygotes that are usually green (Hoham, 1980). Stein and Bisalputra (1969) reported crystalline bodies in the chloroplast of a *Chloromonas* (published as *Chlamydomonas* sp.). These inclusions were hexagonal, 0.2–0.9 μm in diameter, and it was suggested that they were proteinaceous. A structural relationship between these inclusions and the algal chloroplast was not apparent. It was also suggested that these crystalline bodies were involved in storage of photosynthates (carbohydrates). Food reserves in algae other than green algae are typical for their respective groups (Bold and Wynne, 1985; Sze, 1993), but little is known about developmental stages or growth conditions in snow algae associated with these reserves.

Pigments in the snow algae have not been studied critically for most species. However, the pigments in the green alga *Chlamydomonas nivalis* have been identified (Viala, 1966; Czygan, 1970; Bidigare et al., 1993). Viala (1966) first reported that the red pigment was the xanthophyll astaxanthin, and Czygan (1970) indicated that the red pigments were ketocarotenoids and their synthesis paralleled chlorophyll decomposition. (For more information about pigment shifts in snow algae, see the next section on cell physiology and special adaptations.) In the laboratory, Czygan reported a disappearance of red carotenoids that coincided with increasing concentrations of carotenes, other xanthophylls, and chlorophylls a and b, when cells were placed in a nutrient solution for 4 weeks with a light regime of 6,000 lux. However, Mosser,

Mosser, and Brock (1977) recorded a shortwave radiation flux of 86,000 lux in the field for *Chlamydomonas nivalis*, or fifteen times greater than that used by Czygan in the laboratory. Thus, it was not clear if the pigment shift observed by Czygan in the laboratory was due to added nutrients (nitrogen) or to reduced light intensity. Hoham (unpublished data) observed similar pigment shifts in the laboratory with *Chlamydomonas nivalis* cultures where nutrients were not added to the original snow meltwater, but cells were maintained in low level light regimes. In those cultures, however, bacterial populations may have been responsible for the nitrogen levels that caused the pigment shift from carotenoids to chlorophylls in the algae.

4.4.2 Cell Physiology and Special Adaptations

For Antarctic snow algae collected from open exposures, it was hypothesized that species of *Chlamydomonas* remain green when growth is rapid (abundant nutrients and high level of light) and cells maintain a rapid turnover of the Q_b protein that reduces the cells' susceptibility to photoinhibition from damaging wavelengths of light (Bidigare et al., 1993). In the same study, at low growth rates (few nutrients and high level of light), cells synthesize and accumulate red astaxanthin esters to protect against photoinhibition of photosynthesis. Astaxanthin accumulation was linked directly to a decline in nitrogen (Czygan, 1970; Bidigare et al., 1993), and a similar correlation was found for the green algal flagellate *Haematococcus lacustris* (Lee and Soh, 1991). However, depleted P, S, K, or Fe can mimic the effects of N depletion (Czygan, 1968). Ratios of chlorophyll a : astaxanthin esters are highest in green vegetative cells and lowest in red cysts (asexual spores and zygotes) in species of *Chlamydomonas* found in snow in polar regions (Bidigare et al., 1993; Müller et al., 1998a). Red astaxanthin pigments probably accumulate in other genera of green snow algal flagellates – i.e., *Chlainomonas*, *Chloromonas*, and *Smithsonimonas* – through a similar correlation of rapid growth and low nitrogen supply, but this needs verification. However, not all snow algae respond this way. *Chloromonas* collected from snow in Québec and Whiteface Mountain, New York, reduce NH_4^+-N and NO_3^--N nitrogen levels to near zero but display no visual evidence of secondary carotenoid synthesis and remain green (Hoham et al., 1989). These samples, however, were collected from beneath forest canopies where maximum irradiances were not achieved or were minimal (thus few nutrients and low level of light). Perhaps the forest canopy screens out the damaging UV light in this ecosystem, and thus these algal cells do not need Q_b proteins or red astaxanthin esters for protection against photoinhibition.

Orange to yellow-orange colored carotenoids develop in the zygotes of *Chloromonas nivalis* (Hoham and Mullet, 1977, 1978), *Chloromonas brevispina* (Hoham et al., 1979),

and *Chloromonas polyptera* (Hoham et al., 1983). In these species, the brightly colored pigments are located in large lipid bodies of which there is usually one at each pole of the zygote. These orange to yellow-orange zygotes are located in the upper snow layers where the irradiation levels are highest, and zygotes of these three species collected from lower snow layers are usually green. Another snow alga, *Cryocystis granulosa* cell type (a zygote in the life cycle of an unnamed species of *Chloromonas*) (Hoham, unpublished data), accumulates secondary carotenoids progressively from yellow-green to orange to a sometimes red-orange color in small lipid vesicles dispersed throughout the cell (Hoham and Blinn, 1979). This secondary carotenoid development appears to be more related to aging of cells than to irradiation levels (Hoham, 1980). However, it is not known whether the pigment change in this species is correlated with nutrient (nitrogen) depletion as reported for *Chlamydomonas nivalis* by Czygan (1970) and Bidigare et al. (1993).

The fatty acid (FA) composition of *Chlamydomonas* spp. was determined from Antarctic snow samples collected at Hermit Island (Bidigare et al., 1993) (Table 4.2). Green cells contained more saturated FA (mostly 16:0 and 18:0) and fewer monounsaturated FA, and red cells contained fewer saturated FA and more monounsaturated FA (mostly 16:1 and 18:1). Red cells also contained fewer saturated FA and more monounsaturated FA in their astaxanthin esters, and these fractions were dominated by palmitic acid (16:0) and oleic acid (18:1). In the same study, 91 percent of the astaxanthin present in Antarctic red vegetative cells were diesters, whereas 91 percent of the astaxanthin present in *Chlamydomonas nivalis* red cysts from Wyoming were monoesters. In the red snow samples collected from Hermit Island, approximately 5 percent of the total pool of FAs was associated with the astaxanthin esters. In a separate study with red-colored *Chlamydomonas nivalis* collected near Resolute, Cornwallis Island, Canada, Tazaki et al. (1994a, 1994b) found palmitic, stearic, oleic, and behenic acids in these cells. High concentrations of *n*-alkanes with n-C_{24} was characteristic of their red-colored snow algae, suggesting the presence of hydrocarbons that could be derived from the Arctic cold desert and/or organic debris from wind-transported bacteria.

The shift in FA composition of red-pigmented snow algae may serve as a cryoprotective function (Bidigare et al., 1993). Samples of red and green *Chlamydomonas* were transported from Antarctica to Texas A&M University in 1989 at −70°C (the 1990 samples were transported at 0°C); after thawing, the red cells were intact but the green cells had lysed from the freezing process at −70°C. The green cells were primarily vegetative; however, the red cells were in transition between vegetative cells and cyst formation. Survival of the red cells may be explained partly by the predominance of unsaturated FAs, which increases membrane fluidity at this low temperature

Table 4.2. *Fatty acid composition (relative %) and content of whole cells and the astaxanthin esters of* Chlamydomonas *spp. sampled from Hermit Island, Antarctica.*

	Total fatty acids†		Astaxanthin esters†
Fatty acid (FA)*	Green cells	Red cells	Red cells
14:0	2.3	3.9	4.1
15:0i	0.4	ND*	ND
15:0a	0.8	ND	ND
15:0	1.3	ND	TR†
$16:3\Delta^{7,9,12}$	ND	2.0	ND
$16:2\Delta^{9,12}$	1.8	1.5	ND
$16:1\Delta^{7}$	0.4	0.4	ND
$16:1\Delta^{9}$	4.0	17.9	1.8
16:0	26.5	0.5	26.8
17:0	0.8	0.3	0.3
$18:3\Delta^{9,12,15}$	ND	1.4	ND
$18:2\Delta^{7,10}$	ND	ND	1.6
$18:2\Delta^{9,12}$	2.3	2.4	0.7
$18:1\Delta^{9}$	11.1	59.2	51.5
$18:1\Delta^{11}$	3.1	2.0	ND
18:0	34.5	0.2	5.1
$20:5\Delta^{5,8,11,14,17}$	0.5	0.9	0.7
20:0	3.0	0.7	0.8
$20:6\Delta^{4,7,10,13,16,19}$	0.6	0.3	4.3
$22:2\Delta^{10,13}$	ND	ND	0.7
$22:1\Delta^{13}$	2.0	0.5	0.7
22:0	1.6	5.4	0.4
$24:1\Delta^{15}$	1.6	ND	ND
24:0	1.3	0.4	ND
Total %	100.0	100.0	100.0
% saturated FA	72.5	11.4	37.5
% monounsaturated FA	22.2	80.0	54.0
Total FA (μg mg^{-1} dry weight)	47.9	45.4	2.5

*Superscript numbers stand for references.

†ND; not detectable, TR; trace levels.

Note: From Bidigare et al. (1993).

(Roessler, 1990). In their review of bacteria, Margesin and Schinner (1994) stated that psychrotrophs synthesize neutral lipids and phospholipids containing more unsaturated FAs when grown at low temperatures to a greater extent than do mesophiles. An increase of unsaturated FAs leads to a decrease in the lipid melting point and maintains the lipid in a liquid and mobile state, which is necessary for survival. It was proposed that the growth temperature range of an organism depends on the ability of the organism to regulate its lipid fluidity. The lower growth temperature limits on cold-adapted organisms is fixed by the freezing properties of dilute aqueous solutions inside and outside the cell and not by chemical properties of cellular macromolecules.

The snow alga *Chlamydomonas nivalis* was more tolerant to stress (freezing injury, shrinkage, and rehydration) than other more temperate species of *Chlamydomonas* (i.e., *Chlamydomonas reinhardii*, *Chlamydomonas moewusii*, and *Chlamydomonas eugametos*) (Morris, Coulson, and Clarke, 1979). The mechanism suggested for this resistance was that more unsaturated FAs in *Chlamydomonas nivalis* allows for greater membrane fluidity. They found that the average number of double bonds per fatty acid was higher in *Chlamydomonas nivalis* than in *Chlamydomonas reinhardii*. This was mainly due to the differences in the 16:3 and 18:4 content, which made up more of the total FAs in *Chlamydomonas nivalis* than in *Chlamydomonas reinhardii*. Viable cells of *Chlamydomonas nivalis* were recovered from −196°C following cooling rates between 0.3°C and 8°C min^{-1} with optimal survival at a cooling rate of 2.4°C min^{-1}. *Chlamydomonas nivalis* was also more resistant to the stresses of shrinkage and rehydration; the median lethal concentration of NaCl was 1.2 M for *Chlamydomonas nivalis* and 0.4 M for *Chlamydomonas reinhardii*. A higher unsaturated to saturated FA ratio was reported in *Chlamydomonas nivalis* than in *Chlamydomonas reinhardii* (Morris et al., 1981). A reduction of viable cells in the mesophilic species *Chlamydomonas reinhardii* was found by cooling to −2.5°C and below, and this was correlated with changes in structure and function of the cell membrane (Grout, Morris, and Clarke, 1980). A significant difference in ^{86}Rb uptake was indicated for *Chlamydomonas nivalis* versus *Chlamydomonas reinhardii* in media undercooled to −5°C (Clarke, Leeson, and Morris, 1986). It was suggested that this uptake difference, which was greater in *Chlamydomonas nivalis*, may relate to the more highly unsaturated FAs in the cell membrane of this snow alga and that an evolutionary adjustment of membranes in *Chlamydomonas nivalis* of living at near 0°C involved an increase in the unsaturation of membrane phospholipids. (The taxon, *Chlamydomonas reinhardtii* is the correct spelling, but we have followed the spellings from individual publications that use *C. reinhardtii*, *C. reinhardii*, and *C. reinhardi*.)

At culture temperatures of 10°C and 20°C, the Antarctic green alga, *Chlorella vulgaris* strain SO-26, was compared with the mesophilic *Chlorella sorokiniana* strain C-133 for FA composition (Nagashima et al., 1995). Even though these microbes were not reported from snow or ice, they are representative when cold-tolerant and temperate strains are being compared. When culture temperatures were changed from 20°C to 10°C, the increase of the ratio of unsaturated to total FAs was considerably greater in strain SO-26 than in strain C-133. The results indicated that, in photosynthesis, the properties of the Antarctic *Chlorella* SO-26 were more psychrophilic than those of the mesophilic *Chlorella* C-133; both strains could be acclimated by culture temperature, at least partly because of FA unsaturation. In both strains, the major fatty acids were palmitic (16:0), linoleic (18:2), and linolenic (18:3).

Green cells of *Chlamydomonas nivalis* from the Canadian Arctic rich in Ca were involved in active photosynthesis and red cells low in Ca were in a resting stage (Tazaki et al., 1994b). Protamine, stearic acid, and decanoic acid were found in Ca-rich green cells and carminic acid and nopalcol BR-13 were found in Ca-poor red cells.

The properties of cold-adapted bacteria and fungi were reviewed by Margesin and Schinner (1994). Even though snow and ice are just two of the several cold-adapted habitats in their review, the information is included here because of its applicability to snow and ice habitats. They reported that proteins from cold-adapted species are not prone to cold denaturation and their enzymes have higher catalytic efficiencies than those from warm-adapted species. These efficiencies are associated with formation of loose, more flexible structures that allow catalytic conformational changes to occur with less energy input. Activation free energies of metabolic reactions are proportional to adaptation temperature; thus, these values are lower in cold-adapted homologs of an enzyme than in warm-adapted homologs. Enzymes from cold-adapted microorganisms have a shift of the optimum activity toward lower temperatures, which reflects adaptation to their natural habitat. Greatest enzyme formation occurs at temperatures much lower than the optimum temperature for growth; this may compensate for the slow rate of enzymatic activity and probably ensures a high utilization of substrate in the cold environment (Devos et al., 1998). Optimal enzyme activity in the psychrophilic alga *Chloromonas* ANT1 from Antarctica was compared with the mesophilic *Chlamydomonas reinhardtii*; optimal activity was 20°C lower in ANT1 for the two enzymes studied (Loppes et al., 1996).

Margesin and Schinner (1994) also discussed a rapid protein turnover in psychrotrophic bacteria that could be an energy-saving mechanism that provides amino acids during synthesis of new proteins for adaptation, especially when these organisms

live in nutrient-poor environments. Protein synthesis at low temperatures might be the result of specialized proteins that allow for reinitiation of protein synthesis. The ability of an organism to adapt to low temperatures depends not only on the ability to synthesize protein but also may involve regulation and interaction of a number of cellular components and processes. Five psychrophilic bacteria collected near the Antarctic station Dumont d'Urville (the habitat was not given) secreted exoenzymes maximally in culture at temperatures close to that of their environment (−2°C to 4°C) (Feller et al., 1994). Higher temperatures (17°C to 25°C) induced faster growth rates but reduced cell development and enzyme secretion. The suggested optimal activity temperature for these enzymes was 30°C to 40°C, about 20°C lower than that of mesophiles.

In the same review, Margesin and Schinner (1994) indicated that synthesis of polysaccharides is greater in cold-adapted microorganisms than in mesophiles, and some psychrotrophs show a different attack on metabolizing substrates at different temperatures. These same organisms display greater and more efficient transport of solutes across cell membranes than do mesophiles. Membrane transport at low temperature is facilitated by the high lipid content in the membranes of psychrophiles and psychrotrophs. The increased lipid content could reflect an increased cell size in some psychrophiles at lower temperatures and be part of a mechanism to increase the cell membrane surface area and the ability of the cell to take up nutrients at 0°C.

Using gas liquid chromatography, Roser et al. (1992) found high levels of carbohydrates in the snow alga *Mesotaenium berggrenii* from Antarctica. Sucrose and glucose made up 61 percent of the total carbohydrate, glycerol accounted for 11 percent, and 26 percent was unknown. The other snow algae in their study showed much lower levels of intracellular carbohydrates. These algae, including species of *Chloromonas*, *Chlorosarcina*, and *Chlamydomonas*, caused algal blooms in low nutrient areas away from penguin rookeries. The dominance of inositol in one sample of *Chlorosarcina* was not considered major because of the very low sugar/polyol levels detected on a chlorophyll a basis. The lack of detectable polyols or sugars in most of these snow algae raised questions about how these cells tolerated freezing during the winter months. Using ^{13}C nuclear magnetic resonance analysis, Chapman, Roser, and Seppelt (1994) confirmed the earlier findings of Roser et al. (1992) from Antarctica that *Mesotaenium berggrenii* contained sucrose and glucose, and no significant quantities of sugars, polyols, or amino acids were found in extracts of a *Chloromonas* that caused red snow. In an earlier study, Tearle (1987) detected high levels of polyols

in two species of Antarctic snow algae but did not indicate which compounds were detected.

4.5 Life Cycles, Laboratory Mating Experiments, and Cultures

4.5.1 Life Cycles and Speciation

Life cycles of snow algae have emphasized green algal flagellates (Hoham, 1980), and the phases of these life cycles correlate with physical and chemical factors at the time of snowmelt (Hoham, 1980; Hoham et al., 1989; Jones, 1991; Hoham and Ling, 2000) (see Chapters 2 and 3). Active metabolic phases occur in spring or summer when the snow melts, nutrients and gases are available, and light penetrates through the snowpack (Hoham, 1980). The process begins with germination of resting spores at the snow–soil interface (old snow–new snow interface in persistent snow fields) producing biflagellate zoospores (Hoham, 1980). These cells swim in the liquid meltwater surrounding the snow crystals toward the upper part of the snowpack, and their position in the snowpack is determined by irradiance levels and spectral composition. Visible blooms of snow algae occur a few days after germination. Both asexual and sexual biflagellates develop in some species. The sexual cells (gametes) fuse to form resting zygotes; in other species, asexual resting spores develop directly from asexual biflagellate vegetative cells (Hoham, 1980; Hoham et al., 1993; Ling, 1996). The resting spores eventually adhere to the soil or debris over the soil when the snowpack has melted or remain on old snow in persistent snow fields. From year to year, populations of snow algae stay in approximately the same localities. The resting spores remain dormant during summer; may form daughter cells through cell division after the first freezes in autumn; are covered with new snow in fall, winter, and spring; and do not germinate again until the factors repeat themselves (Hoham, 1980). The entire life cycle process is illustrated in Figure 4.31. It is not known how long resting spores remain viable, but some retained in their original meltwater have remained viable for more than 25 years (R. Hoham, personal observation).

Since snow algal flagellates typically are restricted to the snow environment and are not found in lakes or other bodies of water, the species selected for in snow are those that require minimal nutrients. It is important that the snow alga produces some type of resistant spore before complete snowmelt, and nutrient depletion in snow appears to correlate with the transition from vegetative phase to resistant spore (Czygan, 1970; Hoham et al., 1989; Jones, 1991; Hoham and Ling, 2000) (Table 4.3). The selection

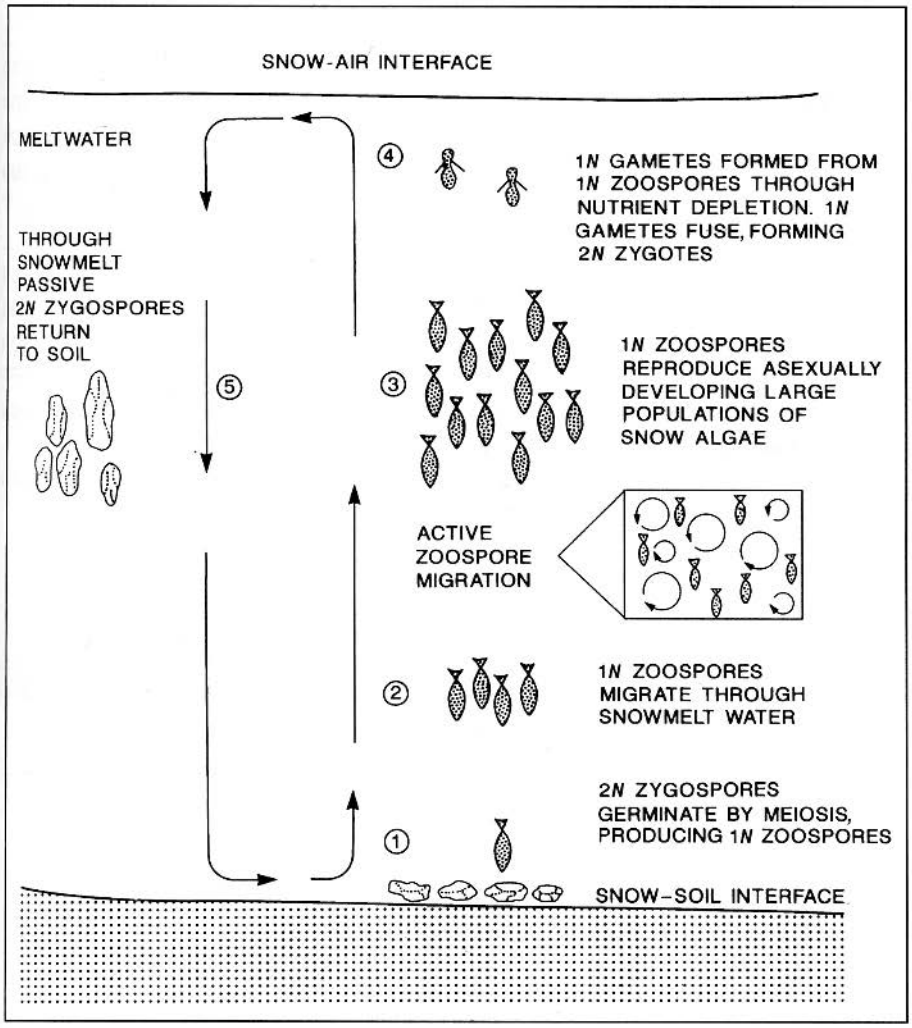


Figure 4.31. Life cycle of snow algal flagellate (*Chloromonas*) with sexual life history (modified from Gamache, 1990; Jones, 1991).

for phototactic species allows populations to migrate for optimal photosynthesis and to locate new nutrient sources.

Extensive studies of snow algal life histories have been done with the green algae (order Volvocales), and these life histories are complex (Fukushima, 1963; Hoham, 1975b; Hoham and Mullet, 1977, 1978; Kawecka and Drake, 1978; Hoham et al., 1979; Hoham, 1980; Kawecka, 1981; Hoham et al., 1983; Kawecka, 1983/1984; Ling, 1996), and one of these life histories is illustrated in Figure 4.32. A number of snow algae that were assigned to the order Chlorococcales and other volvocalean algae are zygotic

Table 4.3. *Snow algal populations (cells ml^{-1}) of* Chloromonas *(Chlorophyta) from Lac Laflamme, Laurentian Mountains, Québec.*

9 May 1988 samples*	Vegetative cells	Resting spores	Total
1 Algae	73,300	0	73,300
1 Control	4,200	0	4,200
2 Algae	123,600	0	123,600
2 Control	2,200	0	2,200
17 May 1988 samples†	**Vegetative cells**	**Resting spores**	**Total**
1 Algae	107,800	800	108,600
2 Algae	193,900	20,000	213,900
3 Algae	39,300	26,500	65,800

*Control samples without visible algae were taken adjacent to samples with visible algae.

†Note shift from vegetative cells to resting spores when compared with 9 May samples; controls were not tabulated for 17 May.

Note: From Hoham et al. (1989).

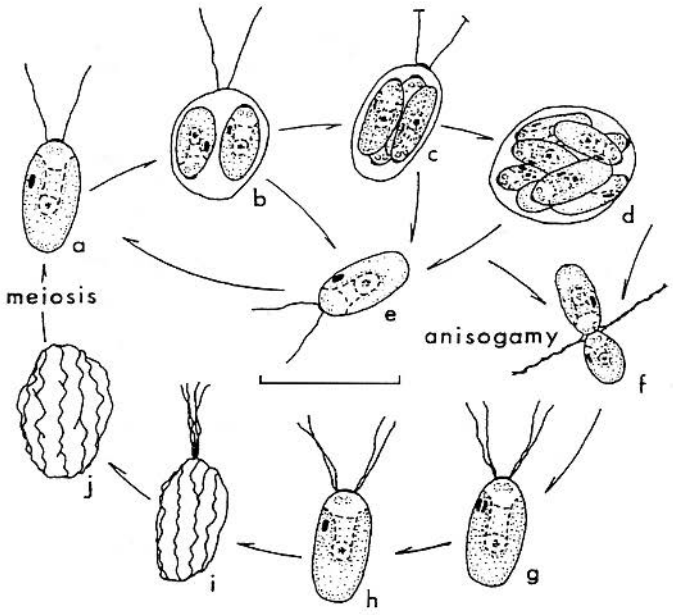


Figure 4.32. Life cycle of *Chloromonas polyptera* (bar = 18 μm). (a) Vegetative cell; (b–d) cleavage of vegetative cells; (e) zoospore; (f) anisogamy; (g) planozygotes (formerly *Carteria nivale*) with two eyespots; (h) planozygote with one eyespot; (i) zygote (formerly *Scotiella polyptera*) with four flagella; (j) zygote without flagella (from Hoham et al., 1983).

stages of the snow alga *Chloromonas*. In this list are *Scotiella* (Hoham, 1975b; Hoham and Mullet, 1977, 1978; probably Kawecka, 1983/1984), *Cryocystis*, *Cryodactylon*, *Oocystis*, and *Trochiscia* (Hoham et al., 1979; Ling, 1996), and *Carteria* (Hoham et al., 1983). In one study (Hoham et al., 1979), the zygotes of *Chloromonas brevispina* were recognized as seven previously described taxa assigned to the Chlorococcales. Ling (1996) reported that a species of *Trochiscia* was the zygote of the green algal flagellate *Desmotetra* in the family Chlorosarcinaceae. *Trochiscia rubra* and *T. cryophila* are stages of the zygotic phase of the snow alga *Chloromonas brevispina* (Hoham et al., 1979).

Zygotes of *Chloromonas* and the closely related *Chlamydomonas* have not been studied in most species of these genera (Huber-Pestalozzi, 1961; Ettl, 1970, 1976; Ettl and Schlösser, 1992). A number of life histories have been suggested for the snow alga *Chlamydomonas nivalis* (Fukushima, 1963; Kol, 1968a; Sutton, 1972; Hoham, 1980), with more specific studies on asexual aplanospore formation (Kawecka, 1981) and sexual reproduction (Kawecka, 1978; Kawecka and Drake, 1978). Bridge formations form between gametes in *Chlamydomonas nivalis* (Kawecka and Drake, 1978) and probably in *Chlainomonas rubra* (Hoham, 1974a). Abnormal sexual mating configurations occur in *Chlamydomonas nivalis* (Kawecka and Drake, 1978), *Chloromonas rostafiński* (Kawecka, 1983/1984), and other species of *Chloromonas* (Hoham, 1992; Hoham et al., 1993, 1997, 1998).

Asexual reproduction would be the fastest route to produce resting spores because more time is needed to complete the sexual phase of the snow algal life cycle (Hoham, 1992). *Chloromonas* sp.-A (Hoham et al., 1993) produces asexual resting spores that resemble *Scotiella cryophila* in field material. Ling (1996) reported that *Scotiella polyptera* from Windmill Islands, Antarctica, appears to be asexually produced from a *Chloromonas* and differs from *Scotiella polyptera* reported in North America, which are the sexual zygotes of *Chloromonas polyptera*. Thus, in localities where the snowpack is inconsistent from year to year, asexual reproduction may be favored by natural selection. However, some of these populations of snow algae that lack in genetic diversity and that live in an inconsistent habitat may be headed for evolutionary extinction.

Species definition within *Chlamydomonas* and other related genera in the order Volvocales (this includes the snow algae *Chloromonas* and *Chlainomonas*) has been difficult. Ettl and Schlösser (1992) stated that is not possible to recognize species of *Chlamydomonas* as populations of interfertile individuals because reproduction is unknown in more than 80 percent of the described species. Relationships within these genera were suggested on the basis of cell wall structure and/or the specificity of cell

wall autolysins. Nucleic acid base sequence data may permit more reliable lineages at the species and generic levels. Using restriction fragment length polymorphism analysis of the region of DNA that codes for the 18S ribosomal RNA subunit (rDNA) and the adjacent internal transcribed spacer (ITS) amplified by polymerase chain reaction, Hoopes (1995, personal communication), however, indicated that three strains of the snow alga *Chloromonas* sp.-A (Hoham et al., 1993) from the same locality (White Mountains, Arizona) have dissimilar nuclear rDNA sequences, suggesting that genetic differences may have evolved in the same species from the same location. This is in contrast to the findings of Judge, Scholin, and Anderson (1993) where strains of an algal dinoflagellate species from a single locality had similar rDNA sequences, suggesting that these sequences could be used to delineate regional populations for morphologically similar species.

Using nuclear 18S rDNA gene sequence data, it was suggested that cold-tolerant species of *Chlamydomonas* and *Chloromonas* have a common ancestry because they align into a single group or clade (Buchheim, Buchheim, and Chapman, 1997). However, when additional species of *Chloromonas* from snow were added to the study (*Chloromonas brevispina*, *Chloromonas pichinchae* and *Chloromonas* sp.-D), they formed a second clade implying that cold tolerant species of *Chloromonas* may have evolved or originated in snow habitats at least twice (Bonome, Leebens-Mack, and Hoham, 2000). These three species of *Chloromonas* have unique cell divisions where parental cells retain their flagella when they form cell packs in *Chloromonas brevispina* (Hoham et al., 1979) and *Chloromonas pichinchae* (Hoham, 1975b) or show cell transformations from oblong to spherical cells in *Chloromonas* sp.-D (Marcarelli et al., 2000). The alignment of the cold tolerant taxa presented by Buchheim et al. (1997) is also supported by chloroplast rbcL gene sequence data (Morita et al., 1999).

4.5.2 Laboratory Mating Experiments

Using modifications of Hoshaw (1961), laboratory mating experiments revealed that strains 593A and 593C were normal + and − mating strains from Québec that produced normal mating pairs (Figure 4.33 [see plate section]) and zygospores (Hoham et al., 1993). These were the first normal mating strains of *Chloromonas* isolated from snow in North America, and zygospores with markings similar to those of *Chloromonas brevispina* developed in the laboratory in these strains (Hoham et al., 1979; Hoham and Clive, unpublished data). Additional strains of *Chloromonas brevispina* from Québec and Vermont have been crossed with strains 593A and 593C, some of which are self-mating and others appear to have no mating potential (Yoder, Knapp,

and Martin, 1995, personal communication). Similar variation in genetic compatibility within a species complex was also reported for the green algal desmid *Micrasterias thomasiana* (Blackburn and Tyler, 1987). In the normal sexual mating *Chloromonas* sp.-D from the Tughill Plateau, New York, three types of mating pairs were observed (oblong–oblong, oblong–sphere, and sphere–sphere). The oblong-oblong mating pairs diminished through the 8-hour time duration of the experiments and the spherical-type mating pairs peaked 6–7 hours after the experiments began (Hoham et al., 1997, 1998; Hoham and Ling, 2000). Another *Chloromonas* from widely separate geographical areas (Adirondack Mountains, New York, White Mountains, Arizona and Jay Peak, Vermont) is asexual and belongs to *Chloromonas* sp.-A (Hoham et al., 1993) and not to *Chloromonas polyptera*, as suggested earlier by Hoham (1992). Even though *Chloromonas* sp.-A produces rare abnormal mating configurations, resting zygotes did not develop. The abnormal mating pairs (Figure 4.34 [see plate section]) observed in the laboratory for *Chloromonas* sp.-A were similar to those observed in the field for *Chlamydomonas nivalis* (Kawecka and Drake, 1978) and *Chloromonas rostafiński* (Kawecka, 1983/1984). In very rare cases, triple fusions were observed (Hoham, 1992), and these were mostly between geographically isolated strains of *Chloromonas* sp.-A. Similar triple fusions were noted in *Chlamydomonas nivalis* from red snow in Poland (Kawecka and Drake, 1978) and in *Chloromonas* sp.-D from the Tughill Plateau, New York (Hoham et al., 1997, 1998). Populations of snow algae that reproduce asexually instead of sexually result in later generations that lack in genetic diversity. Abnormal mating pairs would promote asexual reproduction if nonviable zygotes are the end product. In another volvocalean green alga, *Gonium viridistellatum*, crosses between geographically isolated strains resulted in deterioration of 90 percent of the zygotes (Nozaki, 1989). In a fusion-arrested strain, zym-26-3 of *Chlamydomonas monoica* obtained after UV irradiation, genetic analysis revealed two unlinked mutations, one (cf-1) responsible for the failure to complete cell fusion and the other (ger-8) interfering with late stages of spore maturation and germination (VanWinkle-Swift, Aliaga, and Pommerville, 1987).

4.5.3 Culture Collections and Growth Media

Hoham (1989a, 1992) and Hoham et al. (1993) indicated that a culture collection of about 100 strains of snow algae was maintained at Colgate University, Hamilton, New York. This collection now includes about 200 strains representing approximately three dozen species, many of which are identified and bacteria free. However, there are several other strains not identified with certainty or not bacteria free. These algae are grown on M-1 medium, which was developed for snow algae

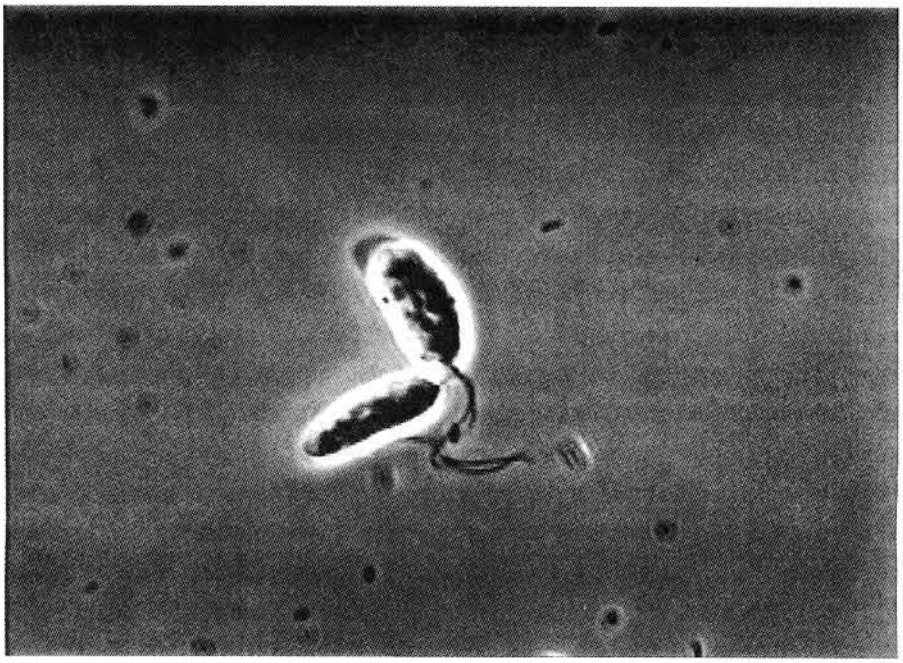


Figure 4.33

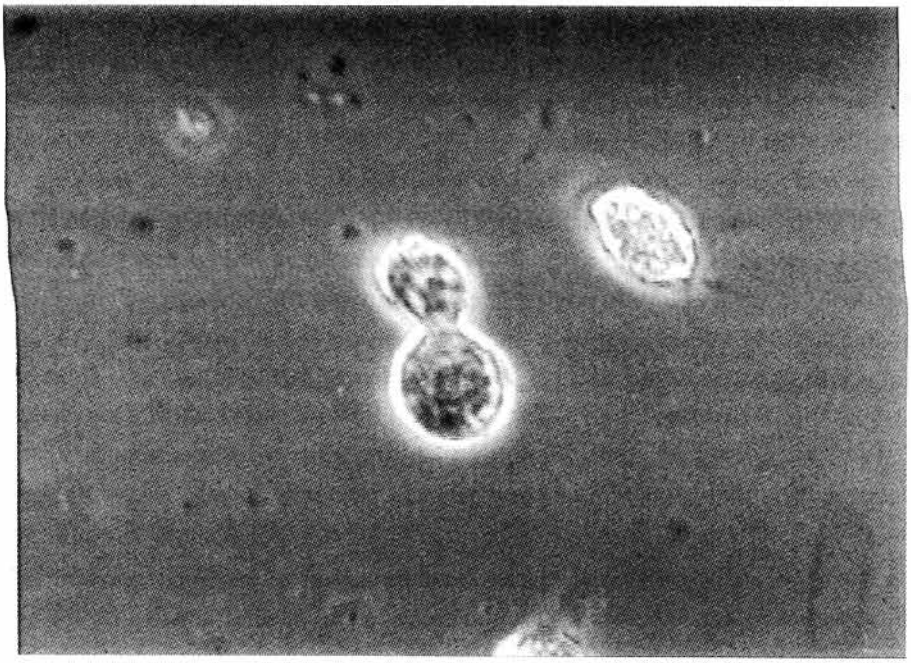


Figure 4.34

(Hoham et al., 1979), and this medium, which has been slightly modified (Hoham, unpublished data), has been most successful for growing a variety of snow algae.

Strains of algae isolated from snow are available from the UTEX collection, Texas (Starr and Zeikus, 1993); the SAG collection, Germany (Schlösser, 1994); and other worldwide collections (Miyachi et al., 1989). Sugawara et al. (1993) published a world directory of cultures of bacteria, fungi, and yeasts that includes snow microorganisms.

Historically, different media have been used to grow snow algae. These include Chu No 10, Benecke's and Bristol's in Japan (Fukushima, 1963), BMT and Tris in Canada (Stein, 1964; Stein and Brooke, 1964), mineral medium supplemented with vitamins in Colorado (Stein and Amundsen, 1967), Holm-Hansen's and Pringsheim's in Oregon (Sutton, 1972), PFW in Washington (modified Provasoli's [1966] enriched sea water medium) (Hoham, 1973), M-1 in New York (Hoham et al., 1979), and Bold's Basal with and without soil extract in Antarctica (Ling and Seppelt, 1993). Handfield et al. (1992) used brain heart infusion (BHI), trypticase soy broth (TSB), plate count agar (PCA), and sea water agar (SWA) media for growing snow and ice bacteria in Canada.

4.6 Evolution and Origins

4.6.1 Snow and Ice Microorganisms

Hypotheses of microbial evolution indicate that thermophiles were the first to evolve (Brock, 1967), followed by mesophiles and psychrophiles. The evolution of psychrophiles and psychrotrophs is probably due to many genetic events (Morita, 1975) because they are distinguished by more than one parameter from mesophiles. There are no reports that indicate how the code or sequence of codons for mesophilic enzymes differs from the code for cold-adapted enzyme homologs (Margesin and Schinner, 1994). Elucidation of the three-dimensional structure of proteins from cold-adapted organisms and a comparison with closely homologous mesophilic proteins may contribute to a better understanding of protein folding and dynamics (Feller, Thiry, and Gerday, 1991).

There are no known fossils of snow algae, but fossils may be difficult to locate because the cells are not typically in lake beds or in rock layers (Hoham, 1980). It was

Figures 4.33 and 4.34. Normal and abnormal mating in *Chloromonas* sp.-D (see Hoham et al., 1993). Figure 4.33: Normal mating pair of gametes showing two pairs of intertwined flagella. Figure 4.34: Abnormal mating pair of gametes without flagella showing a connecting bridge.

suggested that present day populations of snow algae may be remnants of much larger populations that existed through the ice ages (Kol, 1968a). If this is the case, were they derived during recent ice ages during the Quarternary or do they go back to the Carboniferous or before? Snow algae probably were derived from species of soil or aquatic algae by natural selection when glaciers and semipermanent snow fields were more prevalent during the ice ages (Hoham, 1980).

Snow algae maintain populations from year to year in approximately the same microhabitats (Hoham, 1980). Hundreds to thousands of snow algal resting spores adhere to dried fungal strands of *Phacidium infestans*, and these strands could be broken up and distributed by wind or animals to new locations (Hoham, 1987). These potential inoculae would have better chances of producing populations of snow algae simply because of their numbers compared with individual resting spores that might be distributed by wind to unsuitable habitats.

Chloromonas sp.-B, found exclusively on artificial snow, may be distributed from one ski slope to another on skis (Hoham et al., 1993; Dybas, 1998). Resting spores of this species have a gelatinous matrix to which other microbes such as bacteria and fungi adhere. This same material would permit these cells to stick to skis and survive indoors at room temperature in storage until the next season, when they could inoculate a new ski slope. This suggestion is supported by the alga's occurrence at four disjunct ski slopes in three states in New England; it has yet to be found in natural alpine snow areas in New England or elsewhere. Possibilities for the origin of this species include the mutation of an existing species found in snow or a lotic species (from rivers or streams) that adapted to snow when artificial snow was processed from flowing water near ski resorts.

A stable habitat under extreme conditions was observed in perennially ice-covered Antarctic lakes (Wharton et al., 1989c). This ecosystem harbors a diverse assemblage of microbes including those in Antarctic lake ice and most of the life in the Ross Desert (Wharton et al., 1983, 1993). Cells of the desmid *Mesotaenium* were found in the surface slush of an Antarctic lake (Ling and Seppelt, 1990); however, these cells were washed in from the meltwater of surrounding snow. Barsdate and Alexander (1970) reported ice bubbles containing purple sulfur bacteria and algae. Benthic microbial communities were torn from an Antarctic lake bottom and transiently found in lake ice (Parker et al., 1982a) and may be later removed and dispersed by wind (Simmons, Vestal, and Wharton, 1993). In southern Victoria Land, Antarctica, the macroalga *Prasiola calophylla* was described living on wet ablating glacial ice walls (Vincent, Howard-Williams, and Broady, 1993). This alga remained unattached around small

ice projections and probably is dispersed as wind-borne inoculae. After snowmelt, propagules of unicellular snow algae become air borne in Antarctica when the soil surface dries (Marshall and Chalmers, 1997).

Microscopic observations of shallow ice samples (0–1.2 m) collected from the Agassiz Ice Cap in the Canadian Arctic showed extremely low numbers of bacteria (Handfield et al., 1992). In this study, bacterial distribution was consistent with wind deposition rather than the presence of an autochthonous or self-maintained population. Bacterial concentrations differed with summer and winter layers of snow, similar to pollen deposition patterns. Superficial meltpools that form during mid-summer periods may support the growth of psychrophilic strains (Handfield et al., 1992).

4.7 Interrelationships of Physical Factors with Snow and Ice Microorganisms

4.7.1 Temperature

Are the microbes found living in snow and ice optimally adapted to living at temperatures near 0°C? Temperature studies done in the laboratory for snow microbes were reviewed by Hoham (1975a, 1980), and it was suggested that true snow algae have optimal growth at temperatures below 10°C. The snow alga *Chloromonas pichinchae* was designated as an obligate cryophile (psychrophile) because the motile vegetative cells grew at temperatures between 1°C and 5°C, developed into abnormal clumps at 10°C, and did not survive at temperatures above 10°C (Hoham, 1975a, 1975b). Working primarily with bacteria, Morita (1975) defined a true psychrophile with an optimal temperature for growth below 16°C, an upper limit for growth at 20°C, and a minimal temperature for growth at 0°C or lower. Psychrotrophic bacteria were defined as organisms that can grow at 0°C but grow optimally at 20°C to 25°C (Morita, 1975). These concepts were discussed further by Baross and Morita (1978).

An optimum temperature was reported at 5°C for a *Chloromonas* sp. from snow (Stein and Bisalputra, 1969), 4°C for several strains of the snow alga, *Chlamydomonas nivalis* (Czygan, 1970), and 4°C for *Raphidonema tatrae* with a maximum temperature for growth below 10°C (Hindák and Komárek, 1968). *Cryptomonas frigoris* grew at temperatures between 2°C and 10°C (Javornický and Hindák, 1970) and *Chromulina chionophilia* grew below 10°C (Stein, 1963). Using a laboratory cooling stage, cells of *Chlainomonas rubra* and *Chlainomonas kolii* shed their flagella at temperatures above

4°C, and cells of *C. rubra* were damaged when frozen at −1°C (Hoham, 1975a). All snow algal cultures from the Windmill Islands, Antarctica, grew well at 3°C, *Chloromonas rubroleosa* and *Chloromonas polyptera* died at 10°C, and most *Chlorosarcina antarctica* were dead at 10°C (Ling, 1996). These all appear to be examples of obligate cryophiles. An optimum temperature range of 3.5°C to 10°C was reported for the first psychrophilic obligate methanotrophic bacterium isolated from the tundra soil in the polar Ural Mountains of Russia (Omelchenko, Vasilyeva, and Zavarzin, 1993).

Ling (1996) also reported that *Palmellopsis* sp. and *Desmotetra* sp. 1 grew at 10°C but died at 15°C, and *Desmotetra* sp. 2 and *Chloromonas* sp. 1 were barely living at 15°C. A temperature range for growth at 0°C to 15°C was reported for *Raphidonema nivale* (optimum temperature 5°C), 1°C to 20°C for *Cylindrocystis brébissonii* (optimum temperature 10°C), and 1°C to 15°C for *Chromulina chionophilia* (Hoham, 1975a). These appear to be examples of nonobligate cryophiles where growth stages should be able to live in environments other than snow such as soils or water. However, of these, only *Cylindrocystis brébissonii* was found living outside of snow. The extracellular protease-producing psychrotrophic bacteria from the cryoconite of glaciers and high elevation alpine soils from the Alps of Europe were studied by Schinner et al. (1992). Most bacterial strains isolated had an optimum temperature within the range of 10°C to 25°C, and almost half of the bacterial strains excreted protease into the medium at a cultivation temperature of 10°C. As reported earlier, Handfield et al. (1992) isolated 17 psychrophilic species of bacteria and yeasts that grew at 1°C and 4°C and 25 psychrotrophic species of bacteria and yeasts that grew at 4°C and 25°C from Agassiz Ice Cap, Ellesmere Island, Canada.

The changes in morphology and viability in 20 species of fungi belonging to 5 different groups were examined by Morris, Smith, and Coulson (1988) during freezing in relation to cooling rate and the presence of glycerol. They found that several species survived freezing and thawing in the absence of glycerol. The conventionally used cooling rate of 1°C min^{-1} was found not to be optimal for all the fungal strains studied. The resistance to freezing in the red-colored snow alga *Chlamydomonas nivalis* may be explained by the presence of astaxanthin ester-rich lipid vacuoles that occupy a large volume of the cell (Marchant, 1982; Bidigare et al., 1993), thus reducing the water content that otherwise would increase the potential for lysing during crystalline ice formation (Giese, 1973). The halite chlorine, present in aerosols and soot materials (Cornwallis Island, Canada), may be critical in depressing the freezing point of the liquid films associated with the snow alga *Chlamydomonas nivalis* and may inhibit evaporation in the low-humidity environment (Tazaki et al., 1994a, 1994b). However, they did not indicate the concentrations of NaCl or soot particles present in this film,

which is an important factor to consider when determining the lowering of the freezing point (J. Raymond, personal communication).

4.7.2 Meltwater Flow and Water Content

Meltwater flow, horizontal ice layers, and vertical ice fingers affect the position of the algae within the snowpacks (Hoham, 1975b; Hoham et al., 1979, 1983) (see Pomeroy and Brun, Chapter 2). Dyes were used to illustrate that meltwater travels laterally several meters in shallow snowpacks (Hardy, 1993, personal communication). Food color dyes were applied at different points from upper surface to ground level in snowpacks under 60 cm in depth to measure rate and directional flow of liquid meltwater (Hoham, 1975b), and water flowed either gravitationally or laterally and often followed the horizontal ice layers and vertical ice fingers. The range of water flow varied between 3 and 150 cm hr^{-1}, and maximum flow occurred during midafternoon when air temperatures were highest, there was a direct sun exposure, and snowbanks were connected. Minimum water flow occurred during late evening and early morning hours when air temperatures were lowest, there was no sun exposure, and in isolated snowbanks. This meltwater flow was correlated with developmental stages and potential sources of nutrients for the snow alga *Chloromonas pichinchae* (Hoham, 1975b). Nutrients in the meltwater would be available at the snow–soil interface for algal growth at the time of resting spore germination and throughout the snowpack for growth of vegetative cells (Hoham et al., 1983).

Significant negative correlations were found between albedo and snow water content and between albedo and snow algal cell numbers (Thomas and Duval, 1995). Water melt, however, in general was not affected by the presence of algae because of their patchiness.

Percent water content (mL of H_2O melted from 100-mL core samples of snow) was reviewed by Hoham et al. (1983) from snowbanks in Washington (44 to 69 percent), Arizona (43 to 63 percent), and Montana (52 to 72 percent), and these percentages were correlated with algal life cycle phases in different species of *Chloromonas*. The highest percentages (57 to 63 percent) associated with the snow alga *Chloromonas pichinchae* occurred in early to midafternoon in Washington snow when air temperatures and light intensities were highest (Hoham, 1975b). At this time, the alga was in the swimming asexual vegetative phase. Gamete production, release, and fusions (sexual phase) occurred during lower percentage readings (47 to 54 percent). Water content percentages of 42 to 52 percent and 60 to 96 percent were recorded in association with the snow alga *Mesotaenium berggrenii* and the pink ice alga *Chloromonas rubroleosa* respectively, from Antarctica (Ling and Seppelt, 1990, 1993).

4.7.3 Light, Cryoconite Holes, and Suncups

Snow readily absorbs long-wave radiation from its warmer surroundings as indicated by "tree well" basins that form around tree trunks (Marchand, 1991). Dust and other particulates on snow reflect little light (Clow, 1987; Dozier, 1987), such that pure snow reflects more light than does snow with dust and particulates (Warren, 1982; Thomas and Duval, 1995) (see Pomeroy and Brun, Chapter 2). Physical and biological forces act together to form cryoconite holes, suncups, ice fingers, and channels (Gerdel and Drouet, 1960; McIntyre, 1984). The absorption of solar radiation by particulates and organic material and subsequent warming above 0°C cause melting and formation of cryoconite holes in glacial ice (McIntyre, 1984; Wharton et al., 1985). Wharton et al. (1985) reviewed cryoconite holes on glaciers and reported several life forms including algae, rotifers, pollen, insects, and cyanobacteria. They also discussed how cryoconite holes may increase the rate of glacial ice wastage. Cryoconite holes or depressions yielded seven pennate diatoms and three green algae on Mt. Athabasca's north glacier, Alberta, Canada (Wharton and Vinyard, 1983); two green algal desmids, *Ancylonema nordenskioldii* and *Mesotaenium berggrenii*, from Columbia Glacier, Alaska (Kol, 1942); the desmid *Cylindrocystis brébissonii* from the Thule icefields, Greenland (Gerdel and Drouet, 1960); three green algae, *C. brébissonii*, *M. berggrenii*, and *Trochiscia* sp., that dominated Yala Glacier, Nepal, from lower to higher elevations, respectively (Yoshimura et al., 1997); two cyanobacteria, *Gloeocapsa* and *Nostoc*, from glaciers in Marie Byrd Land, Antarctica (Broady, 1989); and psychrotrophic bacteria from glaciers in the European Alps (Schinner et al., 1992). Prescott (1978) reported surface algal blooms of *Chlamydomonas* and *Ancylonema*, the latter forming black snow or black ice that developed meltpores in ice by light absorption. Drifting Arctic ice was also reported to contain autochthonous microbial populations (Kawecka, 1986). Red-colored snow algae and associated cryophilic microbes darkening snow are often associated with suncups (Hardy and Curl, 1972). From field data collected in the Sierra Nevada Mountains, California (Thomas and Duval, 1995), red-colored snow algae enhanced shortwave radiation absorption in snow on the order of 7 to 12 percent. However, red snow caused by algal blooms did not decrease mean albedos in representative snow fields because of algal patchiness, and much of the albedo was reduced by dirt and debris over the snow. Snow algae on a Himalayan glacier accelerated glacial melting, affected the mass balance of the glacier by forming dark-colored areas on the glacier, and reduced surface albedo (Kohshima, Seko, and Yoshimura, 1993).

The amount of light that penetrates snow is a function of wavelength, snow density, and depth (Pomeroy and Brun, Chapter 2; Richardson and Salisbury, 1977; Warren,

1982). Of importance to subnivean phototrophs is the amount of photosynthetically active radiation (PAR) received at a particular depth. This region of the light spectrum includes visible or white light (400–700 nm) with blue light penetrating the deepest (Sze, 1993). Curl, Hardy, and Ellermeier (1972) reported that the major portion of spectral energy in snow is between 450 and 600 nm, with a peak at 475 nm. Also, blue and green light at 455 and 526 nm (Hoham et al., 1983) and at 454 and 530 nm, respectively (Hoham, 1975b) penetrated the deepest in snow for most time periods sampled. Although most light is attenuated by snow, 1 percent of incident PAR was measured at a depth of 1 m in wet snow, and this promoted photosynthesis and algal germination (Curl et al., 1972). Light was measured penetrating through 2 m of wet snow, suggesting that photoactive responses can occur under minimal light conditions (Richardson and Salisbury, 1977). In laboratory mating experiments with *Chloromonas* sp.-D isolated from Tughill Plateau snow, New York State, it was found that maximum mating occurred at a photon irradiance of 95 μmol m^{-2} s^{-1} (PAR 400–700 nm) under both wide-spectrum and cool-white regimes, and blue light regimes produced more matings than green or red light regimes (Hoham et al., 1997, 1998).

In polar regions (Antarctica), red snow is characteristic of low nutrient areas, whereas green snow is most often associated with higher nutrients near seabird rookeries (Bidigare et al., 1993; Broady, 1996). In temperate regions, however, the distribution of red, green, and orange snow, colored by populations of algae (Figure 4.35a–f [see plate section]), is related to the amount of sunlight received by the populations (Kol, 1968a; Pollock, 1970; Hoham, 1971). It was noted by Fukushima (1963) that green snow algae occur under shaded tree canopies with light regimes of <50 percent sunlight, whereas red snow generated by the alga *Chlamydomonas nivalis* generally is found in areas of higher light intensity. Photosynthesis in *Chlamydomonas nivalis* is not inhibited at high light intensity ($\leq$86,000 lux) in exposed alpine snow and is well adapted to a high light regime (Mosser et al., 1977). Snow algae are often found concentrated in horizontal ice layers several centimeters below the snowpack surface (Hoham, 1975b, 1980, Hoham et al., 1983). The algae adjust their vertical photoposition in the snowpack and support populations of up to 1×10^6 cells mL^{-1} in these horizontal layers (Hoham, 1987). Snow algal populations are often associated with vertical ice fingers as well (Hoham et al., 1979). Although not phototrophic, concentrated bands of bacteria were noted to occur approximately 20 cm above the soil in Rocky Mountain spring snowpacks (Brooks et al., 1993).

The effects of solar UV-B irradiation on photomovement, motility, and velocity in the snow alga *Chlamydomonas nivalis* and in the colorless euglenoid *Astasia* were studied by Häder and Häder (1989). They found that motility and velocity were

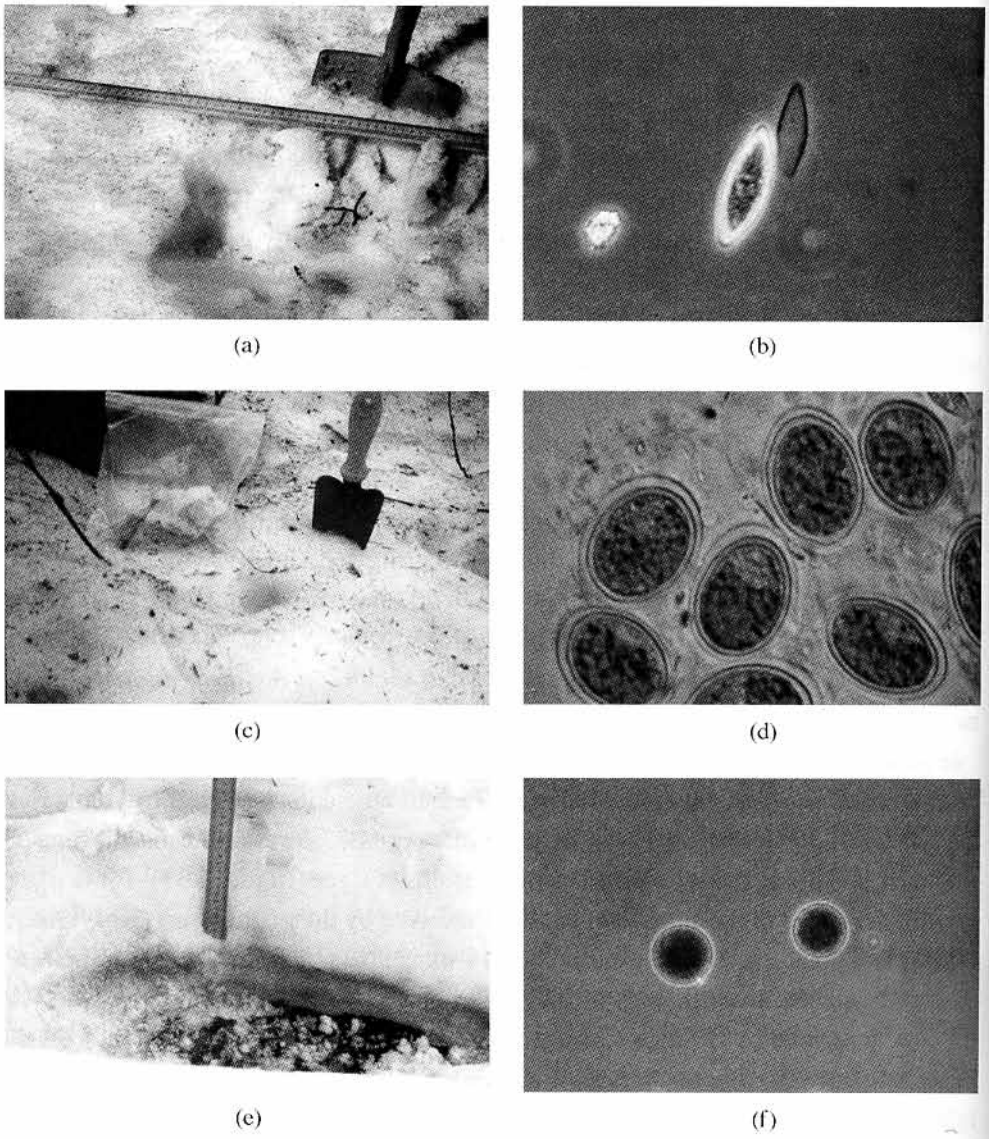


Figure 4.35. (a) Green and orange snow caused by *Chloromonas* sp.-D, Whetstone Gulf State Park, Tughill Plateau, New York [380 M]. (b) Dormant asexual green resting spore of *Cloromonas* sp.-A, Whiteface Mountain, New York [1,340 M]. The resting spore was formerly known as *Scotiella cryophila* (Hoham, unpublished data). (c) Orange snow caused by *Chloromonas* sp.-B, Killington Ski Area, Vermont [1,070 M]. This species is known only from manmade ski slopes. (d) Dormant orange resting spores of *Cloromonas* sp.-B, Wachusett Mountain Ski Area, Massachusetts [525 M]. (e) Red-burgundy-colored snow caused by *Chlamydomonas nivalis*, near Ingalls Pass, Stuart Range, Washington [1,890 M]. (f) Dormant red-burgundy asexual resting spores known as cysts of *Chlamydomonas nivalis*, Raspberry Springs, Coconino National Forest, Arizona [2,957 M].

impaired in the snow alga within 90 min of exposure compared with 3 hours before impairment occurred in *Astasia*. They also found that neither alga demonstrated an obvious phototaxis, but they stated that the test organisms used in their laboratory studies were not adapted to natural conditions. This is interesting because the temperature used in the laboratory was 23°C for *Chlamydomonas nivalis* instead of near 0°C, which is the natural temperature for metabolizing, and personal observations of this alga in North American snow indicate that it is phototactic. The origin of the culture of *Chlamydomonas nivalis* used in their study was not given. Using field experiments in Norwegian snow, the vertical distribution of *Chlamydomonas nivalis* associated with the water surface surrounding the snow crystals and its appearance in the snow layers was related to the melting process instead of to phototaxis (Grinde, 1983). It also appears that Grinde used resting spores stages of *Chlamydomonas nivalis* in the experiments, and these cells would not reform flagella even if wetted. Thus, it is difficult to conclude that *Chlamydomonas nivalis* is not phototactic from this study. Kessler, Hill, and Häder (1992) suggested that biflagellate green cells of *Chlamydomonas nivalis* were strongly oriented by gravity, but direction of gravitaxis was degraded by collisions between cells. For the effects of UV light on photosynthesis, see the next section on primary productivity and respiration.

4.8 Productivity and Biogeochemical Cycles in Snow and Ice

4.8.1 Primary Productivity and Respiration

Snow microbes are often subjected to overnight freezes or repeated freeze-thaw events, and photosynthesis was reported from frozen samples of snow algae that were later thawed (Hoham, 1975a, 1975b; Mosser et al., 1977). Optimum temperatures for photosynthesis in certain strains of snow algae were reported at −3°C to 4°C (Hoham, 1975a; Mosser et al., 1977). Studies of Antarctic lichens also showed near-freezing photosynthetic optima at low PAR levels (Kappen, 1993).

Chlorophyll a concentrations that were very heterogeneous in snow were attributed to populations of snow algae (Thomas, 1972). Using ^{14}C ($H^{14}CO_3^-$ and $^{14}CO_2$), Mosser et al. (1977) reported that *Chlamydomonas nivalis* photosynthesized optimally in the field at 10°C or 20°C but retained substantial activity at temperatures as low as 0°C or −3°C. Using ^{14}C, similar quantities of μg C fixed mm^{-3} cell volume hr^{-1} were recorded for *Chlamydomonas nivalis* (0.05–0.97) (Mosser et al., 1977), mixed populations of snow algae including *Chlamydomonas* sp. (0.04–1.85) (Komárek,

Hindák, and Javornický, 1973), and *Chlamydomonas nivalis* (0.002–0.86) (Fogg, 1967). Larger amounts of fixed carbon (5.7–34.2) were reported for *Chlamydomonas nivalis* (Thomas, 1972), but he questioned these values because of a high assay of CO_2 concentration in the snow meltwater. The carbon concentrating mechanism (CCM) is an inducible mechanism that concentrates CO_2 at the fixation site, which allows for acclimation to a wide range of CO_2 concentrations (Kaplan and Reinhold, 1999). CCM was reported for first time in algae without pyrenoids (*Chloromonas*) (Morita et al., 1998). In cold tolerant species of *Chloromonas*, a biological relationship exists between the absence of pyrenoids and the inability to form a large pool of inorganic carbon in the CCM (Morita et al., 1999).

Carbon production in algal photosynthesis ranged from 1.2×10^{-2} to $12.3 \times 10^{-2} \mu g$ of C (ml snow)$^{-1}$ hr^{-1} in red snow containing *Chlamydomonas nivalis* and *Trochiscia americana* (Thomas, 1994). In adjacent white snow samples, photosynthesis values ranged from 0.00 to $0.16 \times 10^{-2} \mu g$ of C (ML snow)$^{-1}$ hr^{-1}. Even though algal photosynthesis occurred in white snow, ratios of photosynthesis in red snow to white snow ranged from 27 to 79. Thomas (1994) also converted the algal photosynthesis values to units used by Mosser et al. (1977) for red snow comparisons and found that the values reported from both studies fell into the same range. A bacterial production of 2 to $9 \times 10^{-4} \mu g$ of C (ml snow)$^{-1}$ hr^{-1} was recorded in red snow, but only 0.35 to $4.47 \times 10^{-5} \mu g$ of C (ml snow)$^{-1}$ hr^{-1} was recorded in white snow, and ratios of red snow to white snow ranged from 1.2 to 56.4 (Thomas, 1994). In red snow at one site, algal production was 141 to 180 times higher than bacterial production, and it was suggested that the bacteria were utilizing organic matter produced photosynthetically by the algae (Thomas, 1994). These same snow algal and bacterial production interrelationships were discussed further by Thomas and Duval (1995).

The snow alga *Chromulina chionophilia* may possess a photoreactivation enzyme that repairs damage done by UV irradiation to the chlorophyll and other photosynthetic pigments (Hardy and Curl, 1972). The effects of total UV on photosynthetic uptake of radioactive carbon in green and red snow from the Sierra Nevada Mountains, California, were studied by Thomas and Duval (1995). They found UV inhibited uptake by 85 percent in green snow containing *Chloromonas* but inhibited uptake by only 25 percent in red snow containing spores of *Chlamydomonas nivalis* (1994 field data). They concluded that red snow found in open, sunlit areas was better adapted to UV than green snow found in forested, shaded locations. Thomas (1995, personal communication) found that *Chlamydomonas nivalis* photosynthesis was not inhibited by UV as was the case in 1994 (Thomas and Duval, 1995). Thomas attributed these differences in UV inhibition to a snowpack four times greater in 1995 than in 1994 that allowed the

cells in 1995 to become better adapted to UV because of the longer growth season. As mentioned earlier, Häder and Häder (1989) found that UV-B impaired motion in the snow alga *Chlamydomonas nivalis* greater than it did in the colorless euglenoid *Astasia*. However, nothing was mentioned in their study about impairment of photosynthesis.

4.8.2 Dissolved Gases and pH

The dissolved gases CO_2 and O_2 are important criteria concerning microbial populations in the snowpack. The concentration of dissolved CO_2 in snow ranged from 2.5 to 5.0 mg L^{-1}, and it was 9 to 13 mg L^{-1} for dissolved O_2 in snow containing populations of snow algae (Hoham, 1975b; Hoham and Mullet, 1977). Brooks et al. (1993) reported a CO_2 flux at the snow–air interface of 320–360 mg of C m^{-2} day^{-1} and suggested a minor source within the snowpack. They also indicated that most snow is probably oxygenated enough to support metabolic activity for aerobic microbes. In their review of psychrophilic and psychrotrophic bacteria, Margesin and Schinner (1994) indicated that, at low temperatures, solubility and availability of oxygen are increased. Therefore, these organisms are more favorably affected by aeration than are mesophiles.

Snow algae affect pH values in snow (Hoham et al., 1989; Hoham and Ling, 2000). Green snow samples with algae from Whiteface Mountain, New York, had higher pH values than snow without algae (5.87 versus 5.63 at 1341 m and 5.17 versus 4.98 at 1265 m), reputedly the result of CO_2 consumption during photosynthesis (Hoham et al., 1989). Similar increases in pH values were reported in green snow from Svalbard where biflagellate cells were photosynthetically active (Müller et al., 1998a). Newton (1982), however, reported a lower pH (6.2) in regions of Svalbard snow colonized by *Chlamydomonas nivalis* compared with areas that were not colonized (pH 7.0–7.6), suggesting that the alga excreted organic materials (acids and polysaccharides) into the snow, lowering the pH. However, Newton's study was conducted when the asexual spore (cyst) stage was present, a phase that would have reduced metabolic activity. Interestingly, Müller et al. (1998a) found a similar situation in Svalbard where snow with red-orange resting spores of algae were found in lower pH (up to 0.4–0.7 pH unit lower) than in control samples without algae. It appears that the relationship between pH and algae in snow depends on the metabolic state and phase of the snow algal life cycle (Hoham et al., 1989, 1993).

Snow microbes are subjected to high acidity. Observations of pH in snow ranged from 4.0 to 6.3 in western North America (Hoham et al., 1983) to as low as 3.4 for meltwater in the Adirondacks, New York (Schofield and Trojnar, 1980). The pH in snow ranged from 3.5 to 5.4 in south central Ontario, with the lowest pH during the

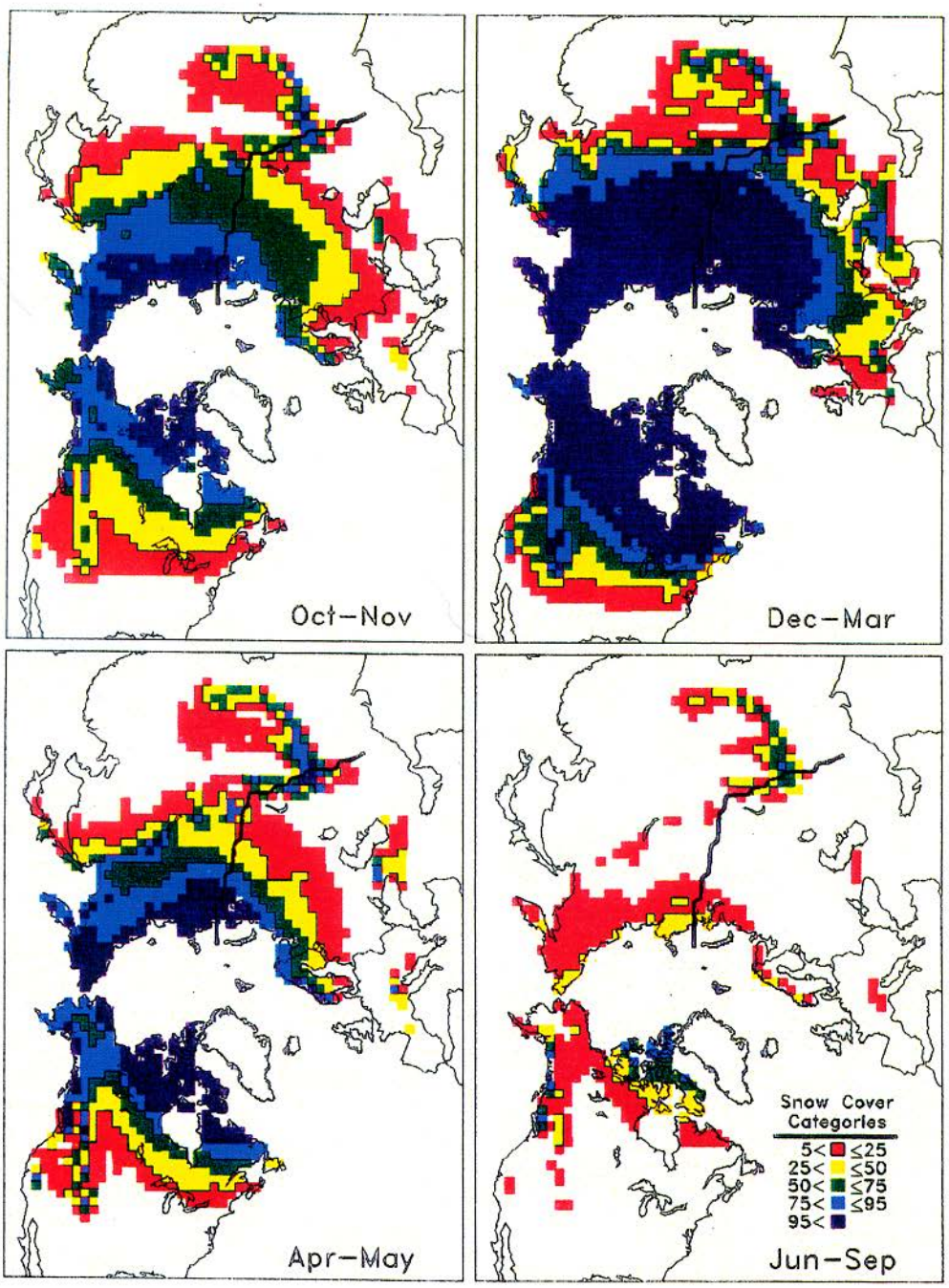


Figure 1.3. Northern Hemisphere seasonal snow cover distribution. White is the least frequent snow cover; blue is the mostfrequent. Snow cover categories assigned to each grid cell are based on the fraction of time with snow cover over the whole period (1973–1992). Category 0, <0.05 of the time with snow cover; 1, 0.05–0.25; 2, 0.25–0.50; 3, 0.50–0.75; 4, 0.75–0.95; 5, >0.95. See Groisman et al. (1994a) for full details.

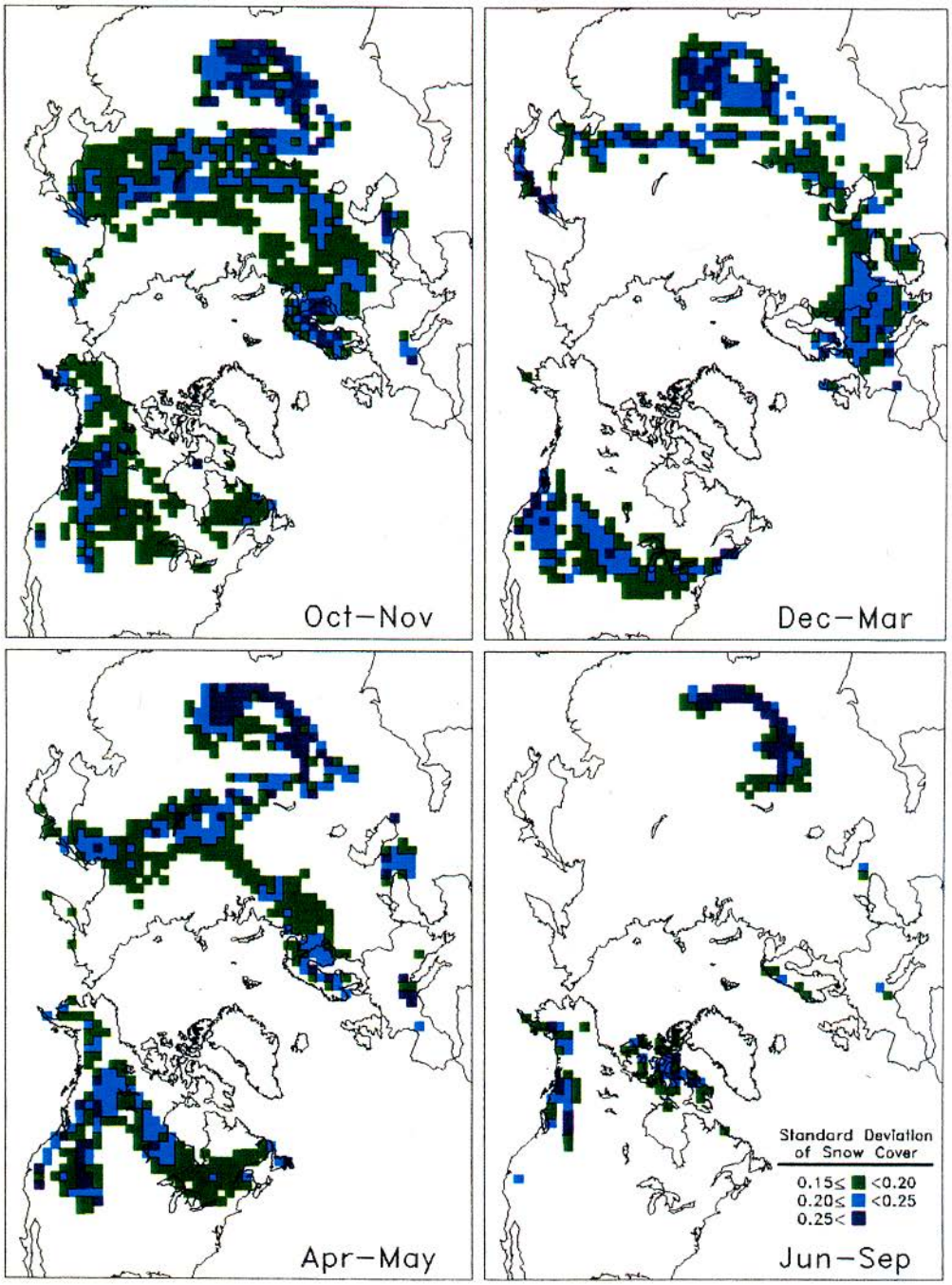


Figure 1.4. Interannual standard deviations of the probabilityof the presence of snow cover for each season. Regions marked in colours are considered "snow transient regions," representing areas where mostvariability has occurred in the past 20 years (Groisman et al., 1994a).

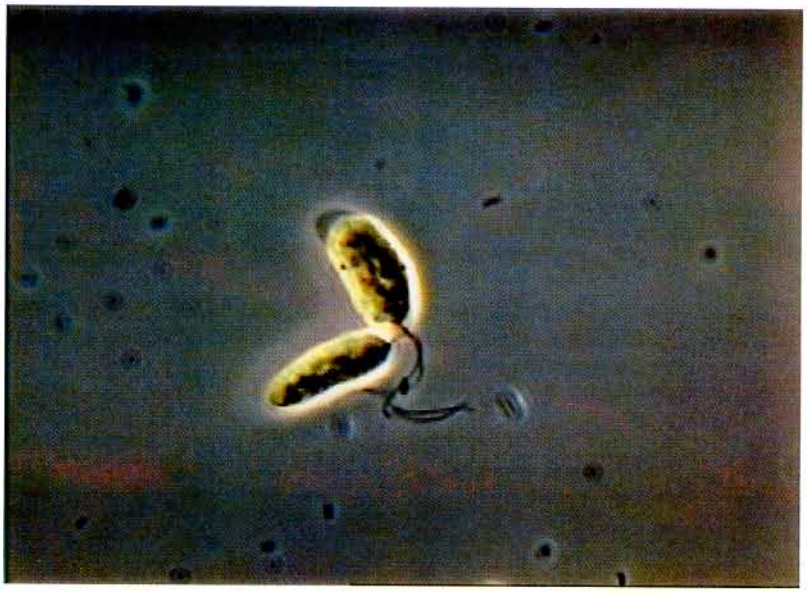


Figure 4.33

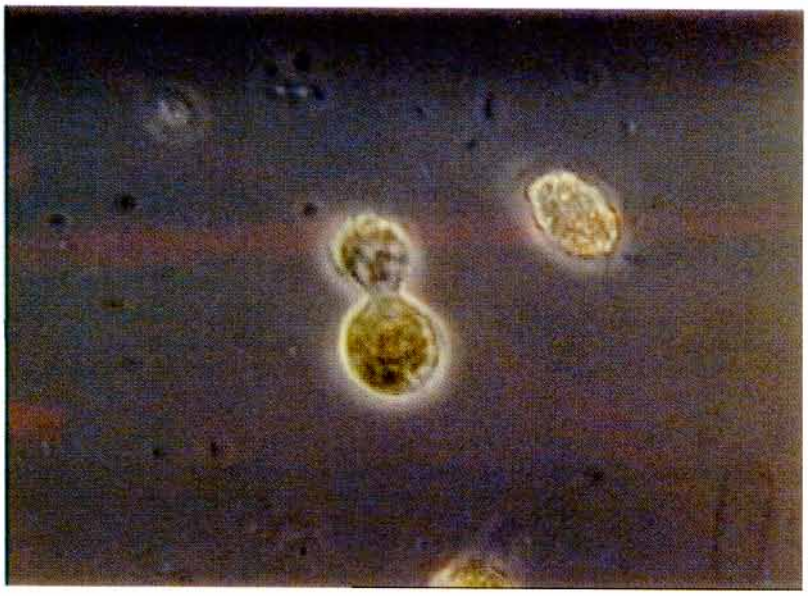


Figure 4.34

Figures 4.33 and 4.34. Normal and abnormal mating in *Chloromonas* sp.-D (see Hoham et al., 1993). Figure 4.33: Normal mating pair of gametes showing two pairs of intertwined flagella. Figure 4.34: Abnormal mating pair of gametes without flagella showing a connecting bridge.

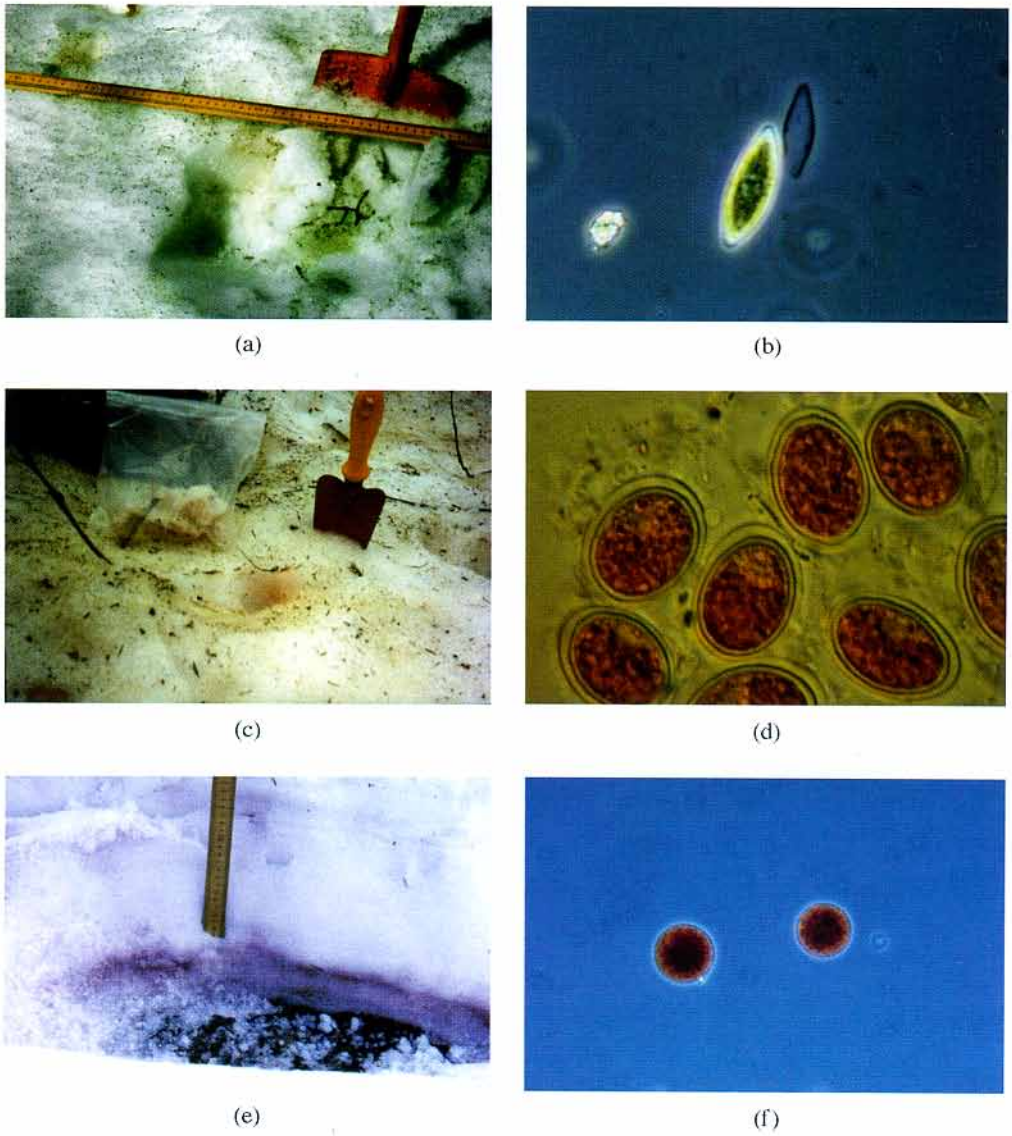


Figure 4.35. (a) Green and orange snow caused by *Chloromonas* sp.-D, Whetstone Gulf State Park, Tughill Plateau, New York [380M]. (b) Dormant asexual green resting spore of *Cloromonas* sp.-A, Whiteface Mountain, New York [1,340 M]. The resting spore was formerly known as *Scotiella cryophila* (Hoham, unpublished data). (c) Orange snow caused by *Chloromonas* sp.-B, Killington Ski Area, Vermont [1,070 M]. This species is known only from manmade ski slopes. (d) Dormant orange resting spores of *Cloromonas* sp.-B, Wachusett Mountain Ski Area, Massachusetts [525M]. (e) Red-burgundy-colored snow caused by *Chlamydomonas nivalis*, near Ingalls Pass, Stuart Range, Washington [1,890 M]. (f) Dormant red-burgundy asexual resting spores known as cysts of *Chlamydomonas nivalis*, Raspberry Springs, Coconino National Forest, Arizona [2,957 M].

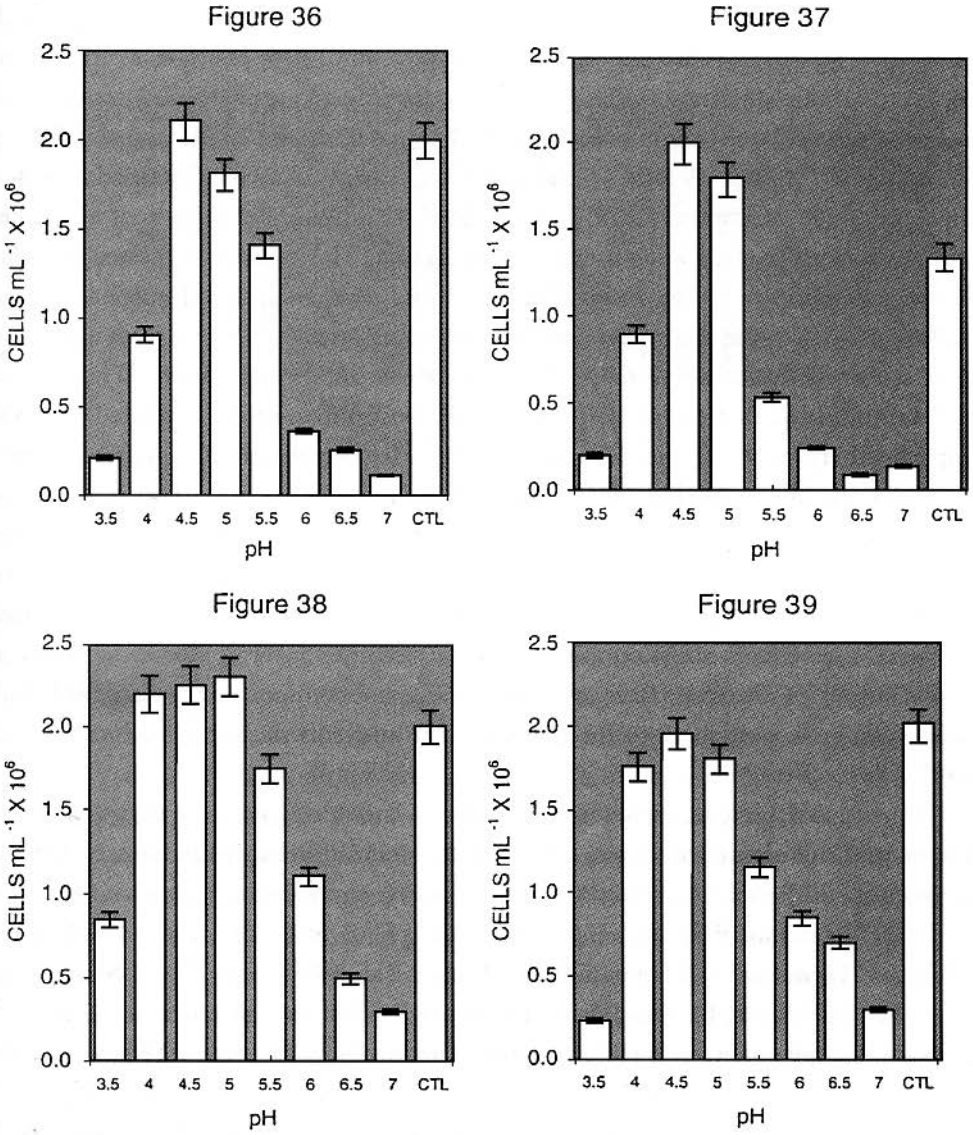
initial snowmelt (Goodison, Louie, and Metcalf, 1986). In Antarctic snow populated by the green algal desmid *Mesotaenium*, the pH was between 4.5 and 5.7 (Ling and Seppelt, 1990); for the green alga *Chloromonas rubroleosa*, the pH was between 4.6 and 6.2 (Ling and Seppelt, 1993). Lukavský (1993) reported a pH range of 4.6–4.9 from snow fields in the High Tatra Mountains, Europe, and a higher pH in old snow from the Bohemian Forest Mountains (no values given), and both habitats were associated with snow algae. A pH range of 4.4–6.2 was reported from Svalbard snow algal samples (Müller et al., 1998a).

The effects of acidic snow may result in the natural selection of snow microbes with greater tolerance to acidity. Hoham and Mohn (1985) reported that strains of the snow alga *Chloromonas* (currently thought to belong to *Chloromonas* sp.-A) from the Adirondack Mountains, New York, had growth optima between pH 4.0 and 5.0 compared with other strains isolated from the White Mountains, Arizona, with pH optima of 4.5–5.0 (Figures 4.36 to 4.39). The significant difference in growth between these geographically isolated strains at pH 4.0 ($P < 0.05$) suggests that snow algae were adapting to the more acidic precipitation found in eastern North America.

4.8.3 Nutrients, Nutrient Cycling, and Conductivity

Snow is an environment that is limiting in nutrients (Hoham et al., 1989; Jones, 1991; Hoham and Ling, 2000), and nutrient loads in the snowpack are spatially distributed (Tranter et al., 1987; Davies et al., 1989). This spatial variability may correlate with the spatial distributions of microbes such as the snow algae (Hoham, 1980; Tranter, 1993, personal communication). Nutrient depletion (particularly NO_3^-) coincided with shifts in phases of the life cycle of snow algae such as *Chloromonas* (Hoham et al., 1989). The importance of NO_3^- on the forest floor, such as in the Adirondacks, New York (Rascher, Driscoll, and Peters, 1987), may play an important role in the life cycles of some snow algal species at the time of their germination. The snowmelt waters concentrated in sulfuric acid and nitrogenous anions probably affect the snow microbiota (Hoham and Mohn, 1985; Bartuma et al., 1990; Williams, 1993), and microbial processes in surface soils beneath the snow may also contribute to this acidity (Arthur and Fahey, 1993). Many of the processes and features occurring in snow such as sublimation, melt, and meltwater channels determine the concentration of nutrients that affect the snow community (Goodison et al., 1986) (see Chapters 2 and 3). Snow ecosystem models have neglected the microbial processes that remove CO_2 and nutrients that help buffer acidic conditions in snow (Hornbeck, 1986).

Even though snow is considered oligotrophic, high nutrient loads of nitrogen ($\leq$4.76 mg L^{-1} N-NH_4^+, $\leq$3.0 mg L^{-1} N-NO_3^-) and phosphorus ($\leq$0.93 mg L^{-1})



Figures 4.36 to 4.39. Final growth of *Chloromonas* sp.-A (see Hoham et al., 1993) at stationary phase in M-1 medium at pH 3.5–7.0. Ninety-five percent confidence intervals at top of each bar ($N = 3$ with an average of 12 counts each). Figures 4.36 and 4.37: White Mountain, Arizona, strains C381F (Figure 4.36) and C381G (Figure 4.37). Figures 4.38 and 4.39: Adirondack Mountain, New York, strains C204 (Figure 4.38) and C479A (Figure 4.39) (modified from Hoham and Mohn, 1985).

were reported in eastern European snow comparable to eutrophic waters (Komárek et al., 1973). Nutrients are deposited on snow by wind, precipitation, weathering of rock, and animals (Jones, 1991). Cell surfaces of the snow alga *Chlamydomonas nivalis* showed prolific accumulation of aerosol debris both locally and globally derived (Tazaki et al., 1994a, 1994b). In samples from Cornwallis Island, Canada, aerosol dust contained clay minerals of P, S, K, Si, Ca and Mg, sea salts, soot and other combustion products, and complex organic debris. The authors suggested that this complex mixture provided a thin film of aerosol soil for the algal nutrient supply. Yellow snow over the European Alps and the Subarctic was derived from a Saharan dust storm in Africa in March, 1991 (Franzén et al., 1994a, 1994b). The authors (1994a) indicated that pollen that fell over Fennoscandian snow originated from the Alps, northern parts of central Europe, and the Mediterranean. Weathering of parent rock may also add to the nutrient composition of snow (Kawecka, 1986). The desmid *Mesotaenium* was observed in snowmelt downslope from moraine and rock aggregations, and this observation was probably a result of enhanced melting or mineral leachate from the upgradient rock (Ling and Seppelt, 1990). Most snow algae from Antarctica were from snow samples located at seabird rookeries where there was an ample supply of nitrogen (Bidigare et al., 1993), and common vegetation, birds, and small mammals contribute to the patchiness of nutrients described in snow (Jones, 1991).

Snow algae deplete nutrients for their growth and development in their life cycles. Concentrations of nutrients (μeq L^{-1}) were lower in Whiteface Mountain, New York, snow containing algae compared with surrounding control samples without algae for $N\text{-}NO_3^-$ (7.7 versus 9.5), $N\text{-}NH_4^+$ (5.3 versus 6.5), $S\text{-}SO_4^{2-}$ (9.4 versus 11.4), Ca^{2+} (7 versus 11), and K^+ (31.3 versus 58.2) (Hoham et al., 1989; Jones, 1991). Similar data from Lac Laflamme, Québec, showed lower concentrations of nutrients (μeq L^{-1}) in snow samples containing algae versus surrounding samples without algae for $N\text{-}NO_3^-$ (2.3 versus 13.4), $N\text{-}NH_4^+$ (1.5 versus 6), $S\text{-}SO_4^{2-}$ (5.9 versus 10.8), Ca^{2+} (1.9 versus 3.5), and Mg^{2+} (1.5 versus 1.6) (Gamache, 1991; Germain, 1991; Jones, 1991) (Figure 4.40). Lower levels of SO_4^{2-}, NO_3^- and NH_4^+ were also found in snow samples with algae in the Himalayas compared with control samples lacking algae (Yoshimura et al., 1997). Lower levels of N (2.6 μeq L^{-1}) and higher levels of Ca (1000 μeq L^{-1}) and P (0.4 μeq L^{-1}) were found in snowpacks where the dinoflagellate *Gymnodinium pascheri* caused snow coloration in Ontario, Canada, compared with other snowpacks in the region lacking the alga (Gerrath and Nichols, 1974). Their nutrient data, however, suggested that surface water may have contaminated their snowpacks containing the dinoflagellate (Jones, 1991).

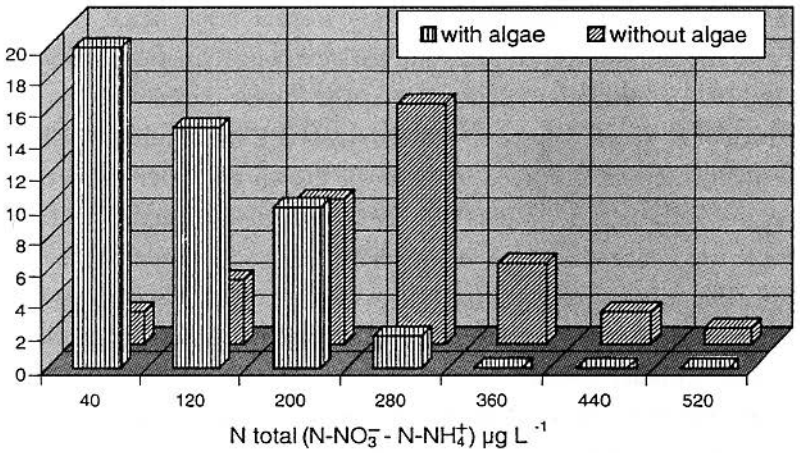


Figure 4.40. Distribution frequency of total N ($N\text{-}NO_3^-$ and $N\text{-}NH_4^+$) concentrations ($\mu g\ L^{-1}$) in a boreal forest snowpack during the spring melt. Snow classed as algal snow is snow with populations of >4,000 cells mL^{-1} (modified from Gamache, 1990; Jones, 1991).

Coniferous litter, dust, and debris are important sources of nutrients for microbes living in snow. Hoham (1976) investigated the effects that extracts from coniferous litter and different snow meltwaters had on growth of the snow algae *Raphidonema nivale* and *Chloromonas pichinchae*. The conifers used in the leaf litter and bark experiments in the laboratory were the same as those that grew adjacent to snowbanks containing these algae in the Stuart Range of western Washington. The pine pollen used, however, was from a species not found in the Stuart Range. The results of these experiments indicated increased growth for *Chloromonas pichinchae* in all extract concentrations (five species of conifers were used) from the leaf litter and bark except for the highest concentrations where some growth inhibition occurred. A similar result of enhanced growth for *Chloromonas pichinchae* took place in the pine pollen experiment. Results of growth experiments of *Chloromonas pichinchae* in filtered snow meltwater collected from three different sites in the Stuart Range correlated with the coniferous leachate experiments. The best growth occurred in snow meltwater collected from beneath conifers compared with the two samples collected from open exposures. When the two open exposure experiments were compared, *Chloromonas pichinchae* grew best in the sample containing the most atmospheric dust. The tree canopy meltwaters contained more $P\text{-}PO_4$ (18 $\mu g\ L^{-1}$) than snow from open areas (2.2 $\mu g\ L^{-1}$ from the site with more dust and 1.6 $\mu g\ L^{-1}$ from the second site). Increases in $P\text{-}PO_4$ were reported in snow meltwater containing wetted forest litter (Moloney, Stratton, and Klein, 1983; Jones, 1987).

The second snow alga used in this study, *Raphidonema nivale*, responded very differently to the coniferous leaf litter and bark extracts than did *Chloromonas pichinchae* (Hoham, 1976). Several extracts, even at the lowest concentrations used, inhibited the growth of *R. nivale* compared with controls (PFW medium). Even some morphological malformations occurred in *R. nivale* grown in higher extract concentrations. The results of these experiments correlated with field observations that *Chloromonas pichinchae* was more abundant in the snow fields surrounded by conifers than was *R. nivale*. Phenolic compounds have been identified from coniferous spruce leachates at high altitudes in snow that are potentially responsible for allelopathic interferences (Gallet and Pellissier, 1997).

Hoham et al. (unpublished data) conducted additional coniferous leaf litter experiments in the laboratory with the snow alga *Chloromonas* sp.-A (Hoham et al., 1993) from the Adirondack Mountains, New York. This snow alga showed some enhanced growth in balsam fir leaf litter extracts, the same conifer that grows adjacent to the snowbanks containing this alga.

Vitamins such as B_1 and B_{12} are needed by certain microbes that live in snowpacks beneath coniferous tree canopies (Hoham et al., 1989). These vitamins are needed for growth by the golden alga *Chromulina chionophilia* and the colorless euglenoid *Notosolenus*. It is not known, however, if the vitamins are derived directly from the canopy or from other microbes in the snowpack such as bacteria, fungi, or lichen pieces. In laboratory experiments with axenic cultures, the filamentous green snow alga *Raphidonema nivale* required vitamin B_1 but later lost that requirement presumably because of mutation (Hoham, 1971). However, the green snow algae *Chloromonas pichinchae* and *Cylindrocystis brébissonii* demonstrated no vitamin requirements. In the Colgate University Culture Collection, all strains of *Chloromonas* grow without vitamins (Hoham, personal observation). Species of the green algal flagellate group (Volvocales) to which *Chloromonas* and *Chlamydomonas* belong are often found in enriched eutrophic water (Sze, 1993). However, in the case of the snow environment, it appears that volvocalean species have been selected for that require minimal nutrients and do not require specialized molecules such as vitamins for growth.

Tazaki et al. (1994a) found the major presence of Si, P, S, and organics in red and green cells of *Chlamydomonas nivalis* from Cornwallis Island, Canada, and high Ca content in the green cells only. They suggested that both P and S were of vital importance to the algae under conditions of such extreme low temperature. In Svalbard snow, high levels of Fe, Ca, Mg, K, P, and Al were found in algal cells despite the very low concentration of these ions in the extracellular meltwater (Müller et al., 1998a).

The interaction between snow algae and snow chemistry affect conductivity readings in snow (Hoham et al., 1989; Hoham and Ling, 2000). From Whiteface Mountain, New York, conductivity values were lower in snow samples containing algae than in snow samples without algae (13.1 versus 19.5 μS cm^{-1} at 1341 m and 9.6 versus 16.4 μS cm^{-1} at 1265 m; there were not enough samples for statistics). These differences were due to nutrient uptake and algal metabolism. However, conductivity in Svalbard snow was higher in regions of algal colonization (12 μS cm^{-1}) than in regions without algae (4–7 μS cm^{-1}) (Newton, 1982), and it was suggested that algal activity results in an increase of ionic concentrations as well as preferring regions that receive more windblown materials (the latter probably raised the conductivity values here). Other conductivity values reported from snow involving snow algal studies include 8–15 (Arizona; Hoham et al., 1983), 4–6 and 18–20 (Montana; Hoham et al., 1983), 6–33 (Antarctica; Ling and Seppelt, 1990), 25–85 (Antarctica; Ling and Seppelt, 1993), and 0.3–17 (Svalbard; Müller et al., 1998a) μS cm^{-1}. It was suggested that the snow alga *Chloromonas rubroleosa* required a slightly higher nutrient status inhabiting Antarctic snow with a conductivity of 25–85 μS cm^{-1} (Ling and Seppelt, 1993). The relationship between conductivity values and snow algae may depend on the species, the metabolic state and phase of the snow algal life cycle, the metabolic state of other microbes such as bacteria and fungi, and the degree to which the snow has been leached by meltwater (see Tranter and Jones, Chapter 3).

4.8.4 Bioaccumulation of Heavy Metals

Metals enter the nival food chain and accumulate in algal cells thousands of times their concentration in surrounding snow (Fjerdingstad, 1973; Fjerdingstad et al., 1974; Hoham et al., 1977; Fjerdingstad et al., 1978; Hoham, 1980). High levels of trace metals were recorded in red snow from Kulusuk, Greenland (Fjerdingstad, 1973; Fjerdingstad et al., 1974), and lesser amounts of the same trace metals were reported from red snow in eastern Greenland and Spitzbergen (Fjerdingstad et al., 1978). The concentrations of these metals accumulated hundreds to thousands of times higher in the cells of the snow alga *Chlamydomonas nivalis* compared with the values from the surrounding snow meltwater. Hoham et al. (1977) reported that concentrations of trace metals varied in green snow caused by the alga *Chloromonas pichinchae* from Washington, USA, and within the red snow samples caused by the alga *Chlamydomonas nivalis*, from eastern Greenland and Spitzbergen (Table 4.4). This study raised questions concerning whether different species and strains within species have different nutritional requirements. Other studies on heavy metals in snow from Greenland

Table 4.4. *Maximum concentration of elements in snow meltwater expressed in mg L^{-1}.*

Element	Washington, USA*	East Greenland†	Spitzbergen‡
As			0.006
Ba		0.13	
Br	0.002	0.03	0.025
Ca	2.25	16.50	0.73
Cl	0.72	2.13	9.31
Cr		0.005	0.007
Cu	0.01	0.01	0.02
Fe	0.04	0.80	2.83
K	0.21	0.72	2.42
Mn	0.05	0.03	0.04
Mo			0.52
Nb		0.002	0.003
Ni		0.58	0.004
P	0.29	0.25	0.27
Pb		0.003	0.05
Rb	0.002	0.015	0.014
S	0.83	0.13	1.00
Si		2.09	10.2
Sr	0.002	0.06	0.01
Ti		0.25	0.24
Zn	0.71	0.05	0.04
Zr		0.003	0.005

*Green snow, collected June 1976, near Mt. Stuart, elev. 1,387 m.
†Red snow, collected summer 1976, on westbank of Fiord Loch Fyne, Hudson Land, elev. near sea level.
‡Red snow, collected summer 1976, near Sveagruva Coal Mine, elev. 210 m.
From Hoham et al. (1977).

include those of Boutron et al. (1993), Boutron, Candelone, and Hong (1994) and Savarino, Boutron, and Jaffrezo (1994).

4.9 Human Aspects, Interests, and Considerations

4.9.1 Biotechnology

The properties of cold-adapted microorganisms (primarily bacteria) and their potential role in biotechnology were reviewed by Margesin and Schinner (1994)

and Feller et al. (1996). The response to high temperatures by these psychrophiles and psychrotrophs was disruptive in protein synthesis by the inability of RNA formation, alterations of the structure of nucleic acids, inactivation of thermolabile enzymes, activation of lytic enzymes, alteration of the cell morphology, inhibition of cell division, and induction of heat shock proteins. At low temperatures these organisms have slower metabolic rates and higher catalytic efficiencies than do mesophiles. The genetic basis of cold adaptation is not clear. Cold-adapted microorganisms have considerable potential in biotechnological applications of waste treatment at ambient temperatures, enzymology, the food industry, medicine, detergents, environmental bioremediations, biotransformations, and applications in molecular biology.

4.9.2 Human Food

Perhaps a more novel use of snow algae is as a source of human food. Microbio Resources, Inc. (1989), used the microalga *Dunaliella* to produce the 100 percent natural food, Provatene, a natural beta-carotene concentrate. Their 1989 brochure emphasized beta-carotene as an antioxidant. In the 1980s, Paul Bubrick, Microbio Resources, Inc., asked the senior author of this chapter how to grow the snow alga *Chlamydomonas nivalis* in very large volumes to mass produce beta-carotene. However, their "production farms" were located in the hot dry desert east of San Diego, California, an environment not suitable for growing this snow alga. Other difficulties with this project were perceived; for example, the beta-carotene was produced in the resting stages of *Chlamydomonas nivalis*, the phase of the life cycle not readily manipulated in laboratory culture. Even though this project was not undertaken, there may be a potential for using extracts from snow algae such as *Chlamydomonas nivalis* as a food supplement. Microbio Resources, Inc., in business between 1985 and 1995, is no longer in operation.

Another question frequently asked is whether eating snow algae directly may be harmful to humans. A study was undertaken to see if direct consumption of red snow by alpine mountain hikers may cause diarrhea (Fiore, McKee, and Janiga, 1997). Seven healthy volunteers aged 24–56 were given 500 g of red snow containing *Chlamydomonas nivalis*, and none of the volunteers developed diarrhea as suggested in previous unpublished communications from alpine mountain hikers. However, the Denver Medical Hospital reported an acute case of diarrhea and dehydration in September 1997 from a patient who consumed large quantities of red snow while hiking in the Colorado Rocky Mountains (Hoham; personal communication).

4.9.3 Exobiology

Snow and ice microbes were suggested as one of four Earth analogs for life on early and present-day Mars (Rothschild, 1990). The other three analogs were endoevaporites, endoliths, and chemoautotrophs. The search on Mars for signs of microbial life has recommended the polar cap regions as one possible site (McKay and Stoker, 1989; Wharton et al., 1989a, 1989b). The residual ice cap (cap left at the height of summer) at the south pole is composed of frozen carbon dioxide, but on the residual north polar cap the seasonal covering of carbon dioxide frost sublimes by the beginning of summer and exposes an underlying deposit of water ice (Haberle, 1995; Zent, 1996). Bacterial cells frozen in water ice are the only organisms to have survived for a geologically significant time on Earth, and it was suggested that bacterial-like cells may have left traces or remains in a Martian permafrost (Gilichinsky, 1993). Recently, it was hypothesized that 3.6-billion-year-old, bacterial-like fossils from a past Martian biota were found in Martian meteorite ALH84001 collected from Antarctica (McKay et al., 1996). Thus the question of whether Earth microbial life forms such as bacteria, fungi, algae, etc. exist elsewhere in our solar system has not been resolved. Future exploration for life on Mars and the Jovian satellite Europa may resolve this question.

4.10 Other Future Research

4.10.1 Genetics, Molecular Biology, and Ultrastructure

Margesin and Schinner (1994) discussed the transfer of genetic material from mesophilic bacteria to psychrotrophic strains and the expression of three lipase genes from an Antarctic psychrotrophic bacterium into a mesophilic one. In one study using an Antarctic psychrotrophic bacterium (Apigny, Feller, and Gerday, 1993), the molar ratio of stabilizing basic residues Arg/(Arg + Lys) was considerably lower than values obtained from mesophiles and thermophiles, suggesting that this ratio contributes to a more flexible tertiary structure at cold temperatures. Gene transfer and isolation studies have not been done with other snow and ice microbes, and this should be a targeted area for future research.

Speciation of snow microbes is complex (Hoham, 1980). Life cycles of some snow algae have revealed up to seven morphologies or forms in a single species of *Chloromonas* (Hoham et al., 1979). In this study, it was suggested that the different forms of the zygote were related to nutrient depletion. Laboratory mating experiments indicate that complex genetic patterns are emerging in species of *Chloromonas* as well (Hoham et al., 1997, 1998). Using restriction fragment length polymorphism analysis and

polymerase chain reaction amplification, dissimilar rDNA sequences have been found in the same population of a *Chloromonas* from snow in Arizona (Hoopes et al., 1995, personal communication). The use of nuclear 18S rDNA gene sequence data in *Chlamydomonas* and *Chloromonas* has restructured our understanding of these genera and relationships between their cold tolerant taxa (Buchheim et al., 1990; Buchheim et al., 1997; Bonome et al., 2000). Chloroplast rbcL gene sequence data further supports these relationships (Morita et al., 1999). Future research using a combination of laboratory mating experiments, nuclear sequence data, and possibly ultrastructural studies (TEM) will be needed to give a more clear picture of speciation within the snow algae. Snow fungi such as *Chionaster* and *Selenotila* are poorly understood snow microbes. These organisms and other related snow fungi need to be cultured and reassessed. Nuclear sequence data for the snow fungi and snow bacteria would be most revealing.

4.10.2 Ecology and Physiology

More productivity studies are needed to better elucidate community interactions. The recent productivity inquiries on snow bacteria and algae (Thomas, 1994; Thomas and Duval, 1995) are a good beginning. Analysis of glacial ecology is in pioneer stages (Kohshima, 1994; Yoshimura et al., 1997). Productivity studies on snow fungi have not been undertaken. Symbiotic associations between snow and ice microbes need further research. It is not always clear whether symbioses actually occur between microbial populations or whether microbes live passively together.

Snow and freshwater ice ecosystems may serve as models to measure continuous changes in concentrations of UV irradiation on biological systems. Changes in UV concentrations in alpine and polar environments correlated with ozone depletion over the past two decades were discussed by Thomas and Duval (1995). There have not been enough studies with snow microbes to give us a clear picture of their sensitivity to UV wavelengths; however, Thomas and Duval (1995) reported that green-colored snow algae are much more sensitive to UV wavelengths than the red-colored cells. The effect of carotene composition on resistance to UV-C radiation in the green alga *Chlamydomonas reinhardtii* was studied by Ladygin and Shirshikova (1993). UV-C (200–280 nm) light has lethal effects and produces nuclear mutations. These injuries can be repaired by photoreactivating enzymes (photolyases), which have their greatest activity in the region 350–520 nm, or the main maxima of carotenoid absorption. The role that carotenoids may play in minimizing damage to snow algal cells from UV-C damage is not known. An increase in carotenoids was reported in aplanospores of *Chlamydomonas nivalis* collected from the Sierra Nevada Mountains, California, after short-term exposure (2 days) to UV-A (365 nm) (Duval, Shetty,

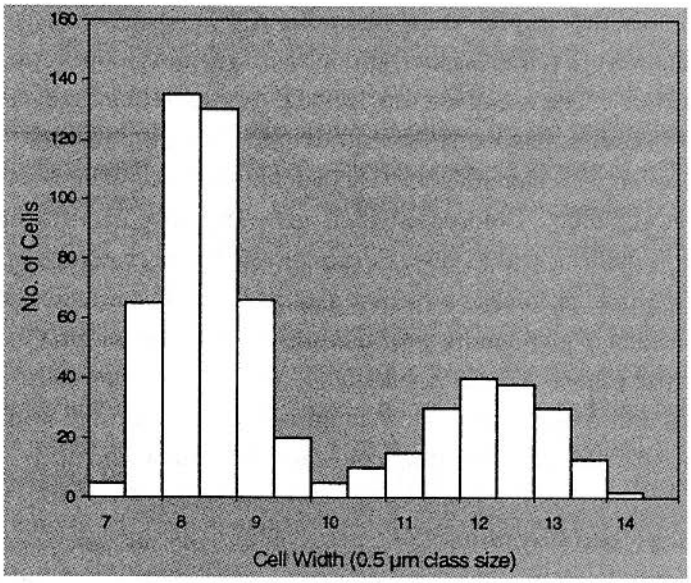


Figure 4.41. Frequency distribution of cell width classes for *Mesotaenium berggrenii* collected near Casey Station (from Ling and Seppelt, 1990).

and Thomas, 1999). In the same study, UV-C exposure (254 nm for 7 days) resulted in greater phenolic-antioxidant production (12–24 percent) when compared to 5–12 percent production under UV-A exposure for 5 days, and UV-C promoted an increase in free proline that did not occur with UV-A. UV-absorbing mycosporine-like compounds were not detected in resting spores of *Chlamydomonas nivalis* from the European Alps (Sommaruga and Garcia-Pichel, 1999).

Snow algal populations of *Mesotaenium* from Antarctica appear to fall into two distinct groups according to cell size (Ling and Seppelt, 1990) (Figure 4.41). Hoham and Blinn (1979) also reported cell size differences within snow algal species from different geographical locations in alpine areas of the American Southwest. The basis of these differences in cell sizes between populations needs to be investigated. Are these differences genetic or might they be caused by an environmental factor? Hoham and Mohn (1985) also suggested that snow algae are adapting to the more acidic precipitation recorded for eastern North America during this century. The potential of snow algae as ecological bioindicators needs further consideration.

It is not known to what degree expansive blooms of snow algae may contribute to the melting of snowpacks. By following the development of these blooms, their impact on alpine watersheds may be more clear. Experiments designed to alter environmental

snowpack regimes, such as the one at Niwot Ridge, Colorado, may give insights into how microbial processes might respond to the disturbance of snow ecosystems (Walker et al., 1993) (see Walker, Billings, and de Molenaar, Chapter 6).

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