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Optimum Growth Temperatures of Three Species of Green *Chloromonas* Snow Algae from Upstate New York and the White Mountains, Arizona

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Abstract

The optimum temperatures of three species of snow algae were studied using four strains of *Chloromonas* (*Cr.*) *rosae* v. *psychrophila*, six strains of *Cr. tughillensis*, and one strain of *Cr. chenangoensis*. These axenic strains were from Upstate New York except for two of *Cr. rosae* v. *psychrophila* from the White Mountains, Arizona. Temperatures tested were from 2.5 to 20°C. The high elevation subalpine *Cr. rosae* v. *psychrophila* from New York and Arizona grew from 4 to 20°C and had the greatest cell counts at 4 to 15°C. In contrast, the subalpine to temperate low elevation strains of *Cr. tughillensis* grew from 2.5 to 10°C and optimally at 2.5 or 5°C, and *Cr. chenangoensis* grew from 2.5 to 7.5°C and optimally at 2.5 and 5°C. *Chloromonas tughillensis* and *Cr. chenangoensis* belong to a genetic subclade with low temperature optima, whereas *Cr. rosae* v. *psychrophila* belongs to a subclade with broad temperature optima. In acclimation experiments, there were no significant differences in cell counts when acclimating two Adirondack, New York, strains of *Cr. rosae* v. *psychrophila* for two weeks prior to experiments vs. using non-acclimated strains that were moved from 4°C directly to 4, 10, 15, or 20°C. For *Cr. tughillensis*, four of six strains had significantly higher cell counts when grown at 2.5°C after acclimation at 7.5°C for five months. These are the first reports of temperature optima of snow algae from eastern North America.

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Introduction

Microbes that thrive in cold-temperature aquatic habitats have received considerable attention in recent decades. Comprehensive reviews include those of marine sea-ice biota (Horner, 1985; Staley and Gosink, 1999) and freshwater snow and ice (Hoham and Duval, 2001). The temperature range for growth of psychrophilic (cold-thriving) microbes has varied by definition with optimum growth near 0°C but a high limit for growth at 10°C (Hoham, 1975a), 15°C (Morita, 1975), or 20°C (Arango, 1981). The term cryophilic, instead of psychrophilic, has been used historically when referring to microbes that live in snow (Kol, 1968; Hoham, 1975a).

In the snow ecosystem, algae have received more attention than other microbes because their pigments color the snow red, orange, and green, which make them readily visible. Snow or cryophilic algae are found in subalpine, alpine, and polar habitats worldwide and are well adapted to living in extreme conditions of low temperature, high acidity, high irradiance levels, low nutrient concentrations, and desiccation after snowmelt (Hoham and Duval, 2001). Their ability to thrive in extreme conditions has led astrobiologists to speculate that snow algae may represent a possible life form for extraterrestrial life (Rothschild, 1990). Their germination and rapid growth requires that air temperatures remain above freezing for several consecutive days, which results in a liquid meltwater near 0°C (Pollock, 1970; Hoham and Duval, 2001). In the past four decades the knowledge of cryophilic algae has greatly expanded through research in physiology, ecology, ultrastructure, and laboratory studies using cultures, molecular probes, and molecular phylogenies.

Studies that have examined the ecology and life history of a single species (autecological) have revealed a better understanding of the optimal conditions under which snow algae live (Hoham et al., 1998, 2000, 2006, 2007). Obligate snow algae have optimal growth at temperatures below 10°C and do not grow above that temperature (Hoham, 1975a, 1980). *Chromulina chionophilia* (Stein, 1963), *Chloromonas* (*Cr.*) *pichinchae* (Hoham, 1975a, 1975b), and *Raphidonema tatrae* (Hindák and Komárek, 1968) did not grow above 10°C making them obligate cryophiles, whereas *Raphidonema nivale* had optimal growth at 5°C but grew at 15°C and was not considered an obligate cryophile because it may persist after snowmelt on soil or in lakes (Hoham, 1975a). Species of snow algae that belong to *Chlamydomonas* and *Chloromonas* probably have been derived from temperate species of these genera and have invaded the snow habitat at least two or three times during their evolutionary history (Hoham et al., 2002).

Snow algae have adaptive features that prevent freezing of cells. *Chlamydomonas nivalis* exhibited increased levels of unsaturated fatty acids in cell membranes, which allowed for increased membrane fluidity at colder temperatures (Morris et al., 1979). Lipids were more common in vegetative cells of *Cr. pichinchae* that had been exposed to subfreezing conditions, which enabled cells to remain viable because lipids occupied space previously filled with liquid water (Hoham, 1975a, 1975b). *Chlainomonas rubra* appeared much more sensitive to freezing; cells often lysed and did not recover when frozen quickly (Hoham, 1975a). In the green alga, *Dunaliella salina*, proline was an important cryprotectant synthesized under cold stress (Helliot and Mortain-Bertrand, 1999).

Even though snow algae live and grow at or near freezing temperatures, what percentage of these cryophiles are truly

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obligate or how well have they adapted to the temperatures in which they live? The objectives of this research were to determine if three species of green *Chloromonas* snow algae from Upstate New York (NY) and the White Mountains (AZ) are obligate cryophiles, have adapted to the temperature in which they live, show temperature differences between strains of the same species, and are similar or different from previously studied snow algae worldwide. The following were investigated: (1) the optimum growth temperature of different strains of *Cr. rosae* v. *psychrophila*, *Cr. chenangoensis*, and *Cr. tughillensis*; and (2) how varied preacclimation temperatures affected these temperature optima for *Cr. rosae* v. *psychrophila* and *Cr. tughillensis*. One hypothesis was that the higher elevation taxon (*Cr. rosae* v. *psychrophila*) would have a colder optimum temperature than the lower elevation taxa (*Cr. chenangoensis* and *Cr. tughillensis*), and another was that preacclimated cultures would have better growth than non-preacclimated cultures at all temperatures tested. This is the first optimum temperature study done on snow algae from eastern North America.

Materials and Methods

COLLECTION SITES, PREACCLIMATION, AND PHOTOPERIOD

Cr. rosae v. *psychrophila* strains CU 204 (UTEX SNO 11) and CU 479A (UTEX SNO 56) were collected from Whiteface Mountain, NY, and CU 381F (UTEX SNO 50) and CU 381G (UTEX SNO 51) from the White Mountains, Arizona. *Cr. tughillensis* strains CU 581A (UTEX SNO 86), CU 581C (UTEX SNO 88), CU 581D (UTEX SNO 89), CU 582A (UTEX SNO 90), CU 582C (UTEX SNO 91), and CU 582D (UTEX SNO 92) were collected from Whetstone Gulf State Park, Tughill Plateau, NY. *Cr. chenangoensis* strain CU 722B (UTEX SNO 147) was collected from the Chenango Valley, Hamilton, NY. The sampling network of these taxa were restricted because *Chloromonas chenangoensis* and *Chloromonas tughillensis* are known from only one site each in NY (Hoham et al., 2006). *Chloromonas rosae* v. *psychrophila* is worldwide in its distribution, but cultures of it exist only from western and eastern North America and two cultures from each region were included in this study. For more information on collection sites, which include dates and elevations, see the UTEX web site at http://www.bio.utexas.edu/research/utex/.

Prior to the experiments, strains were maintained on modified M-1 agar medium (Hoham et al., 2006) for 4 weeks at 4°C. For acclimation to temperatures used in the experiments, see the *EXPERIMENTAL SETUP* section below. Each temperature regime was established in Percival® Model 1–30 BLL Growth Chambers. For *Cr. rosae* v. *psychrophila*, GE Cool White fluorescent lighting was at an irradiance level of 75–140 µmol photons m^{-2} s^{-1} with a 16:8 hour (light:dark) photoperiod, which followed procedures of Hoham and Mohn (1985) and Hoham et al. (1998) for Whiteface Mountain, NY. For *Cr. tughillensis* and *Cr. chenangoensis*, blue light (GE F20T12.B-Blue fluorescent tubes) was used at irradiance levels of ~95 and ~115 µmol photons m^{-2} s^{-1}, respectively, with a 14:10 photoperiod for both species, which followed procedures of Hoham et al. (2006).

GROWTH CHAMBER SETUPS, IRRADIANCE, AND CELL COUNTS

Glassware and media were autoclaved and preacclimated along with sterile pipettes for each experiment. Using sterile technique, cells were washed from agar plates with M-1 medium, and aliquots were placed into Erlenmeyer flasks. Cell concentrations were enumerated using hemacytometers to start experiments at 8×10^3 for *Cr. rosae* v. *psychrophila* and 10×10^3 cells mL^{-1} for *Cr. tughillensis* and *Cr. chenangoensis*, respectively. A 15-mL cell suspension was poured into each test tube, caps were loosely tightened for gas exchange, and replicate test tubes for each strain were arranged along the periphery inside 500 mL beakers. Empty test tubes were placed in the center of the beaker and in equally spaced intervals along the outside to keep experimental tubes vertical and to minimize light variations. Replicates were in triplicate for *Cr. rosae* v. *psychrophila* and quadruplicate for *Cr. tughillensis* and *Cr. chenangoensis*. Beakers were arranged on white blotters for maximum reflectivity of light.

A Licor LI-1935a 3-D "Spherical" sensor attached to a LI-1000 "Datalogger" was used to measure µmol photons m^{-2} s^{-1} irradiance prior to experiments and before and after counts were taken. The sensor was placed equidistant from and in the middle of the beakers to obtain the irradiance level within each chamber. During measurements the growth chamber door was closed until the light reading stabilized, and the shelf was raised or lowered to give the approximate irradiance level needed.

Cells were observed through Zeiss® phase-contrast microscopes and enumerated using hemacytometers weekly for four weeks or the maximum time that populations may exist in field samples. Prior to each enumeration, cells were resuspended five times with a 1-mL sterile pipette to achieve an equal suspension of cells, and this was performed in each chamber with the door open quickly to minimize any changes in temperature or lighting. Four people enumerated separate hemacytometer fields and an average was taken to avoid sampling bias. Absorbance data at 440 nm (not shown) were compiled for all experiments and the results were similar to cell count data.

EXPERIMENTAL SETUP: TEMPERATURE OPTIMA, ACCLIMATION, AND NUTRIENTS

Three replicate cultures of each of the four strains of *Cr. rosae* v. *psychrophila* were grown at 4, 10, 15, and 20°C. Four replicate cultures of each of the six strains of *Cr. tughillensis* were grown at 5, 10, 15, and 20°C. In a separate experiment, four replicate cultures of each of these six strains were grown at 2.5, 5, and 7.5°C. Four replicate cultures of the *Cr. chenangoensis* strain were grown at 2.5, 5, 7.5, and 10°C. All of these cultures were preacclimated for two weeks at each temperature prior to beginning experiments. For all three taxa, 0°C was not tested due to the limits of the growth chambers employed. For each data set, a series of two-way ANOVAs and one-way ANOVAs with post-hoc Tukey tests were used where appropriate to investigate variation in average cell counts at four weeks among strains, growth temperatures, and combinations of strains and growth temperatures.

Three replicate cultures of *Cr. rosae* v. *psychrophila* CU 204 and CU 479A were grown at 4, 10, 15, and 20°C from two different starting conditions. In the first, cells were moved from 4°C to the other temperatures (nonacclimated treatments). In the second, cells were acclimated for two weeks at the other temperatures prior to beginning experiments. Because of significant among-strain variation (see below), these data were analyzed separately for each strain using two-way ANOVAs with growth temperature and acclimation treatment as fixed factors. Four replicate cultures of each of the six strains of *Cr. tughillensis* were grown under four different acclimation-growth temperature treatments. Cells were acclimated at either 2.5 or 7.5°C for five

TABLE 1

Results of two-way ANOVAs for *Chloromonas rosae* v. *psychrophila* (A) and two studies of *Chloromonas tughillensis* (B and C) with cell count as the dependent variable and strain and growth temperature as fixed factors.

	Source	df	*F*
(A) *Cr. rosae* v. *psychrophila* 4/10/15/20°C	Strain	3	14.52***
	Temp	3	45.38***
	Strain * Temp	9	2.66*
	Error	32	
(B) *Cr. tughillensis* 5/10/15/20°C	Strain	5	28.65***
	Temp	3	921.45***
	Strain * Temp	15	22.98***
	Error	72	
(C) *Cr. tughillensis* 2.5/5/7.5°C	Strain	5	39.27***
	Temp	2	110.92***
	Strain * Temp	10	18.21***
	Error	54	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

months prior to experiments and then grown at either 2.5 or 7.5°C for four weeks. Nutrients (nitrate, ammonium, and sulfate) were analyzed in these experiments using Dionex Ion Chromatography after five weeks or one week after final cell counts because there was not enough time to analyze both after four weeks. Due to the significant among-strain variation (see below), these data were analyzed using a series of two-way ANOVAs with acclimation and growth temperature as fixed factors, and then a series of one-way ANOVAs with post-hoc Tukey tests were used to investigate differences among the four acclimation-growth temperature combinations separately for each strain.

Results

TEMPERATURE OPTIMA

Separate two-way ANOVAs were performed for *Cr. rosae* v. *psychrophila* and *Cr. tughillensis* with cell count as the dependent variable and strain (*Cr. rosae* v. *psychrophila*, four levels; *Cr. tughillensis*, six levels) and growth temperature (four levels) as fixed factors. Each analysis showed that there was a significant main effect of strain for each species (Table 1); therefore, the data were analyzed for each strain of a particular species separately using a series of separate one-way ANOVAs.

For *Cr. rosae* v. *psychrophila*, average cell counts significantly varied among temperature groups for each strain (CU 204: $F_{3,11} = 20.81$, $p < 0.001$; CU 479A: $F_{3,11} = 29.09$, $p < 0.001$; CU 381F: $F_{3,11} = 28.01$, $p < 0.001$; CU 381G: $F_{3,11} = 6.13$, $p < 0.050$). As suggested by the two-way ANOVA, strains varied with respect to the temperature that yielded the highest average cell counts (Fig. 1A). Post-hoc Tukey tests showed that average cell counts at 20°C were significantly lower than all other temperature groups for all strains, but did not identify a statistically significant temperature optimum for any strain.

In the first experiment with *Cr. tughillensis*, average cell counts significantly varied among temperature groups for each strain (CU 581A: $F_{3,15} = 261.06$, $p < 0.001$; CU 581C: $F_{3,15} = 433.96$, $p < 0.001$; CU 581D: $F_{3,15} = 45.27$, $p < 0.001$; CU 582A: $F_{3,15} = 266.10$, $p < 0.001$; CU 582C: $F_{3,15} = 175.50$, $p < 0.001$; CU 582D: $F_{3,15} = 92.49$, $p < 0.001$). Although strains varied with respect to overall average cell counts (Fig. 1B), post-hoc Tukey tests showed that average cell counts at 5°C were significantly higher than all other temperature groups for each strain. In the second experiment with *Cr. tughillensis*, average cell counts again significantly varied among temperature groups for each strain (CU 581A: $F_{2,11} = 64.44$, $p < 0.001$; CU 581C: $F_{2,11} = 41.43$, $p < 0.001$; CU 581D: $F_{2,11} = 12.28$, $p < 0.010$; CU 582A: $F_{2,11} = 63.49$, $p < 0.001$; CU 582C: $F_{2,11} = 7.61$, $p < 0.05$; CU 582D: $F_{2,11} = 59.85$, $p < 0.001$). Post-hoc Tukey tests showed that different strains had different optimum temperatures (Fig. 1C); the optimum temperature for strains CU 581A and CU 581D was 5°C, whereas the optimum temperature for the other four strains was 2.5°C.

For *Cr. chenangoensis*, average cell counts significantly varied among temperature groups (CU 722B: $F_{3,15} = 526.50$, $p < 0.001$). A post-hoc Tukey test showed that average cell counts at 2.5 and 5°C were not different from each other, but were significantly higher than average cell counts at 7.5 and 10°C (Fig. 1D).

ACCLIMATION

Separate two-way ANOVAs with growth temperature (four levels) and acclimation status (two levels) for *Cr. rosae* v. *psychrophila* strains CU 204 and CU 479A showed that average cell counts significantly varied among temperature groups for both strains (CU 204: $F_{2,12} = 78.13$, $p < 0.001$; CU 479A: $F_{2,12} = 10.71$, $p < 0.010$), but there was no significant main effect of acclimation (CU 204: $F_{1,12} = 0.525$, $p > 0.100$; CU 479A: $F_{1,12} = 2.66$, $p > 0.100$) or interaction between growth and acclimation temperature (CU 204: $F_{2,12} = 0.714$, $p > 0.100$; CU 479A: $F_{2,12} = 0.373$, $p > 0.100$). Within temperature groups, average cell counts between the acclimated and not-acclimated groups were strikingly similar (Figs. 2A and 2B).

In the experiment with *Cr. tughillensis*, separate two-way ANOVAs for each strain with growth temperature (two levels) and acclimation temperature (two levels) as fixed factors showed significant effects of growth temperature on average cell counts, but mixed results of acclimation temperature on cell counts (Table 2). There was substantial variation among strains with respect to how growth and acclimation temperatures affected cell counts (Fig. 3A). To investigate this further, a coded variable was created for each strain with four levels to represent the combination of growth and acclimation temperature. Then, a series of separate one-way ANOVAs were used for each strain and post-hoc Tukey tests were performed to identify homogeneous subsets. For strains CU 581A, 581C, 582A, and 582C, growth at 2.5°C following acclimation at 7.5°C yielded significantly higher average cell counts than all other treatment combinations. There was no significant difference between average cell counts at 2.5°C following acclimation at either 2.5 or 7.5°C for strain CU 581D. Growth at 7.5°C following acclimation at 2.5°C was significantly lower than all other groups for strain CU 582D, but average cell counts did not differ among the other three treatment combinations.

Differences in nitrate, ammonium, and sulfate concentrations for *Cr. tughillensis* cultures grown at 2.5°C following acclimation at 7.5°C and cultures grown at 7.5°C following acclimation at 2.5°C were assessed using separate two-way ANOVAs for each nutrient with temperature treatment (two levels) and strain (six levels) as fixed effects. There were significant temperature and strain main effects and interaction effects for nitrate (temperature: $F_{1,36} = 1837.61$, $p < 0.001$, strain: $F_{5,36} = 85.31$, $p < 0.001$; interaction: $F_{5,36} = 41.50$, $p < 0.001$), ammonium (temperature: $F_{1,35} = 385.94$, $p < 0.001$; strain: $F_{5,35} = 42.68$, $p < 0.001$; interaction: $F_{5,35} = 27.03$, $p < 0.001$), and sulfate (temperature: $F_{1,36} = 1012.95$, $p < 0.001$, strain: $F_{5,36} = 66.04$, $p < 0.001$,

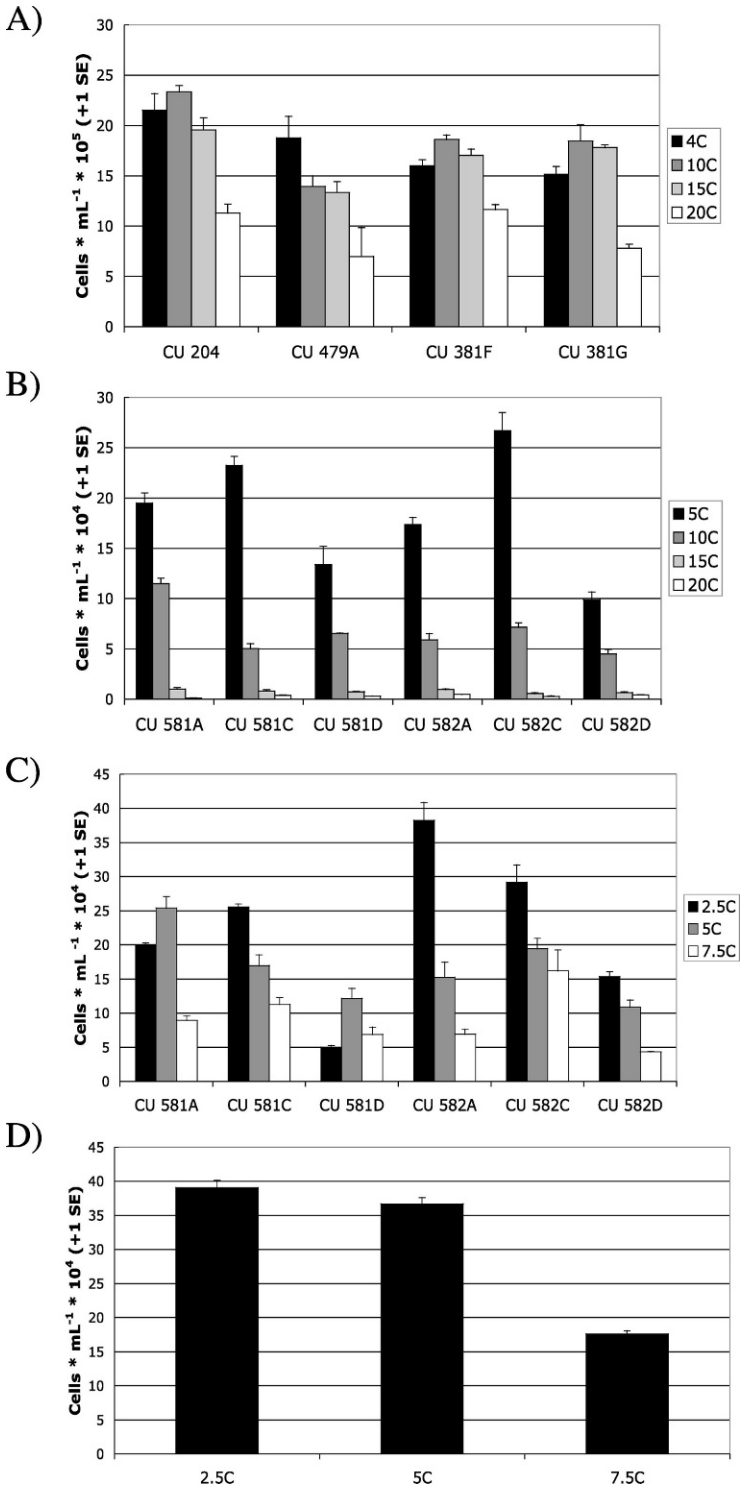

FIGURE 1. Mean cell counts (+1 SE) of growth after four weeks for different strains of *Cr. rosae* v. *psychrophila* (A), *Cr. tughillensis* (B and C), and *Cr. chenangoensis* (D) at different temperatures (°C).

interaction: $F_{5,36} = 45.70$, $p < 0.001$). Separate one-way ANOVAs with post-hoc Tukey tests showed that nitrate concentrations were lower for each strain grown at 2.5°C following acclimation at 7.5°C than at 7.5°C following acclimation at 2.5°C ($p < 0.001$ in all cases; Fig. 3B), and that the magnitude of the difference varied among strains (significant interaction term in two-way ANOVA). These analyses showed similar results for ammonium concentration for all strains ($p < 0.001$; Fig. 3B) except CU 582D, for which there was no difference ($p > 0.100$). Sulfate concentrations were similar to those of nitrate concentrations ($p < 0.001$ in all cases; Fig. 3B).

Discussion

TEMPERATURE OPTIMA

Phylogenetic studies using 18S rDNA and *rbc*L gene sequence analysis revealed that snow algal species of *Chlamydomonas* (*Cd.*) and *Chloromonas* probably invaded the snow habitat two or three times (Hoham et al., 2002). These snow species were likely derived evolutionarily from temperate species and align with other cold tolerant species of these genera that live in alpine soil, peat, and lakes. Little is known, however, about the origins and evolutionary history of other algal genera found in snow.

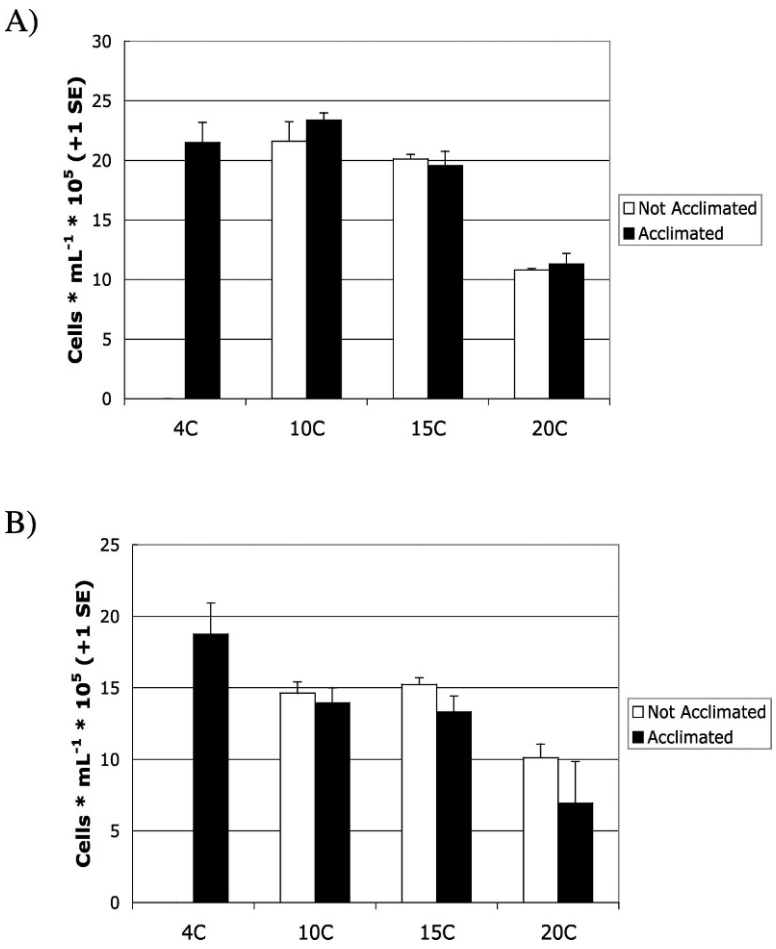

FIGURE 2. Mean cell counts (+1 SE) of growth after four weeks for *Cr. rosae* v. *psychrophila* strain CU 204 (A) and strain CU 479A (B) at different temperatures (°C). Closed bars denote cultures that were acclimated at each temperature; open bars denote cultures that were not acclimated.

Temperature optima studies of snow algae have been conducted qualitatively and quantitatively (Table 3). *Chlainomonas rubra* and *Chlainomonas kolii* shed their flagella at temperatures above 4°C when observed on a laboratory cooling stage and their protoplasts were damaged when cells were maintained at just below 0°C for a few hours (Hoham, 1975a). Lab growth experiments indicate that temperature optima of snow algae vary from 1 to 10°C with several species not being able to survive at temperatures above 10°C, which are termed true or obligate cryophiles.

Red cells of *Chlamydomonas* were more freeze tolerant than green cells because of their higher monounsaturated fat contents (Bidigare et al., 1993). In red cells 80% of fatty acids were monounsaturated, which allowed them to stay intact during a −70°C freezing period, whereas green cells, which had 72% saturated fatty acids, suffered extensive damage. Near-freezing temperature optima in snow algae are reliant upon their ability to maintain lipid fluidity. The snow alga, *Cd. nivalis*, was more tolerant to cold stress (freezing injury, shrinkage, and rehydration) than were three temperate species of *Chlamydomonas* (Morris et al., 1979), and the suggested mechanism was the greater number of unsaturated fatty acids in cells of *Cd. nivalis* that allowed for greater membrane fluidity (Morris et al., 1979, 1981).

In this study, strains of *Cr. rosae* v. *psychrophila* were grown from 4 to 20°C with best growth at 4, 10, and 15°C with no significant differences in growth at these temperatures (Fig. 1A), and at 20°C strains grew significantly less than at the other temperatures. Strains of *Raphidonema nivale* grew from 1 to 15°C and optimally at 5°C and of *Cylindrocystis brébissonii* from 1 to 20°C and optimally at 10°C (Hoham, 1975a). However, none of

TABLE 2

Results of two-way ANOVAs for *Chloromonas tughillensis* strains with cell count as the dependent variable and growth temperature and acclimation temperature as fixed factors.

Strain	Source	df	*F*
CU 581A	Growth	1	131.81***
	Acclim	1	3.67
	Growth * Acclim	1	43.78***
	Error	12	
CU 581C	Growth	1	11.55**
	Acclim	1	6.00*
	Growth * Acclim	1	5.94*
	Error	12	
CU 581D	Growth	1	338.83***
	Acclim	1	0.78
	Growth * Acclim	1	19.37***
	Error	12	
CU 582A	Growth	1	179.79***
	Acclim	1	36.78***
	Growth * Acclim	1	159.44***
	Error	12	
CU 582C	Growth	1	50.29***
	Acclim	1	0.52
	Growth * Acclim	1	223.81***
	Error	12	
CU 582D	Growth	1	22.63***
	Acclim	1	5.66*
	Growth * Acclim	1	40.08***
	Error	12	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

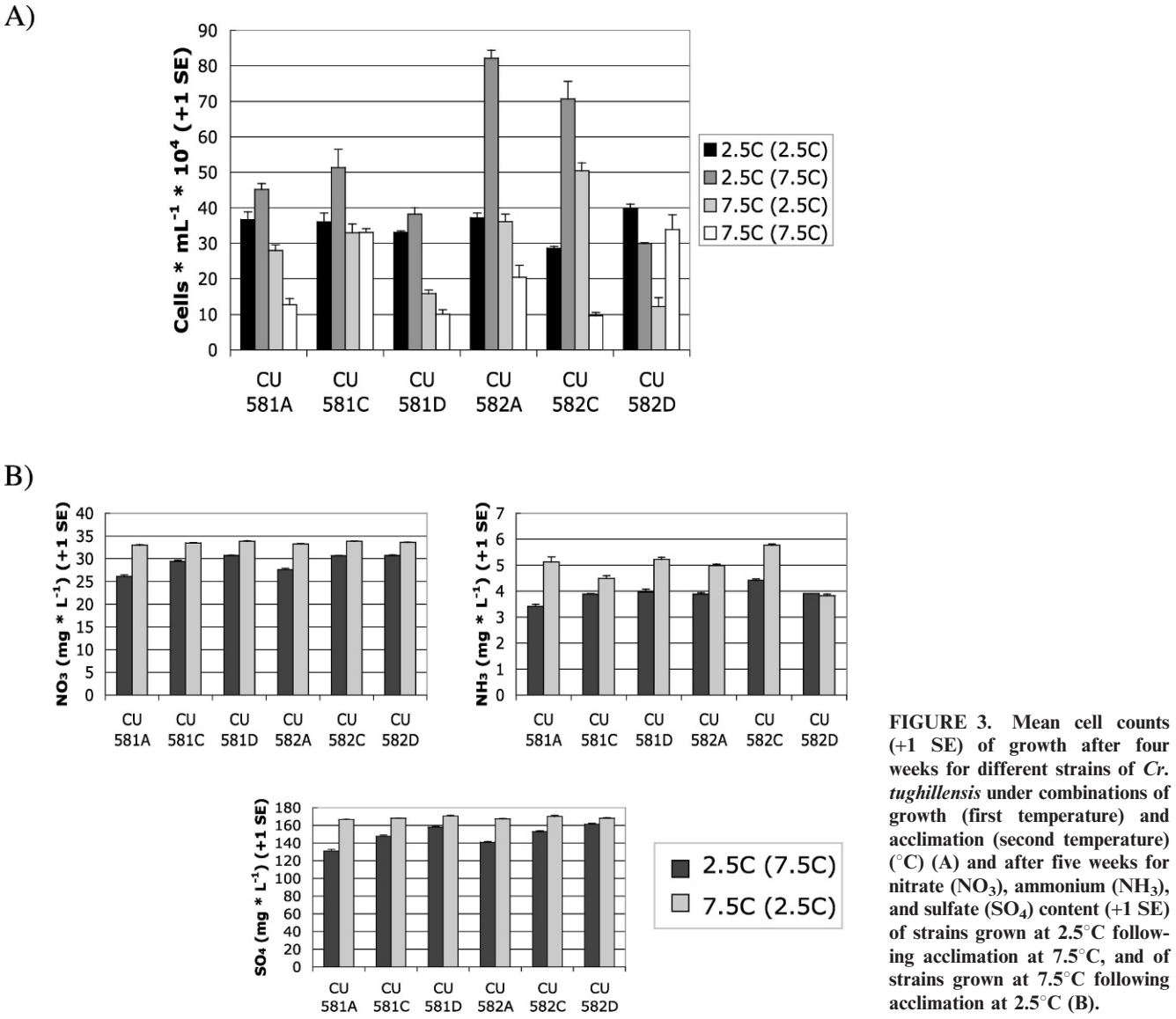

FIGURE 3. Mean cell counts (+1 SE) of growth after four weeks for different strains of *Cr. tughillensis* under combinations of growth (first temperature) and acclimation (second temperature) (°C) (A) and after five weeks for nitrate (NO_3), ammonium (NH_3), and sulfate (SO_4) content (+1 SE) of strains grown at 2.5°C following acclimation at 7.5°C, and of strains grown at 7.5°C following acclimation at 2.5°C (B).

these taxa were considered obligate snow algae or cryophiles because of their ability to grow at temperatures above 10°C, which might allow them to grow on wet soil, peat, or in lakes after snowmelt.

In the first experiment with *Cr. tughillensis* (Fig. 1B), all strains had significantly higher cell counts at 5°C vs. 10, 15, or 20°C, and cultures did not survive at the two higher temperatures after a few weeks. In the second experiment with *Cr. tughillensis* (Fig. 1C), all strains except CU 581D had cell counts that were significantly higher for growth at 2.5 vs. 7.5°C. However, strains CU 581C, 582A, 582C, and 582D showed significantly higher cell concentrations at 2.5 vs. 5.0°C where strains CU 581A and CU 581D were significantly higher at 5.0°C vs. 2.5 and 7.5°C. These results indicate subtle differences in temperature optima among strains of *Cr. tughillensis* with optimum growth at either 2.5 or 5.0°C. For *Cr. chenangoensis* strain CU722B, cell counts were not significantly different at 2.5 and 5.0°C but were significantly higher than at 7.5 or 10°C (Fig. 1D), and cultures died after a few weeks at 10°C (not shown in Fig. 1D).

Previous studies of snow algae showed an optimum temperature of 5°C for *Chromulina chionophilia* (Stein, 1963); 4°C for *Raphidonema tatrae* (Hindák and Komárek, 1968); 3°C for *Cr. polyptera*, *Cr. rubroleosa*, and *Chlorosarcina antarctica* (Ling, 1996); 2 to 10°C for *Cryptomonas frigoris* (Javornický and Hindák, 1970), *Desmotetra antarctica* and *Desmotetra aureospora* (Ling, 2001); 1 to 4°C for *Chlainomonas rubra* and *Chlainomonas kolii* (Hoham, 1975a); and 1°C for *Cr. pichinchae* (Hoham, 1975a) (Table 3). Cells of *Cr. pichinchae* that survived at 10°C were abnormal and in large non-motile clumps (Hoham, 1975a, 1975b). All of these taxa are obligate cryophiles because they have optimal growth at temperatures below 10°C and do not grow above that temperature (Hoham, 1975a, 1980). Other taxa listed in Table 3 may be obligate cryophiles such as *Cd. nivalis* where cells were cultivated at 4°C, but other temperatures for growth were not tested (Czygan, 1970).

In temperature studies of non-cryophilic algae showing psychrophilic character, two strains of an Antarctic *Chlamydomonas* isolated from floating ice, *Cd.* sp. ICE-L and *Cd.* sp. ICE-W, grew between 0 and 10°C, propagated in the ice, and did not survive at ambient temperatures above 15°C (Liu et al., 2006), which is similar to non-obligate snow algae. A similar temperature range for growth occurred in *Cd. raudensis* isolated from beneath ice in Lake Bonney, Antarctica (Pocock et al., 2004). The marine euglenoid, *Tetreutreptia pomquetensis* isolated from the Gulf of St. Lawrence, grew between 0 and 7°C, but did not survive at 10°C (McLachlan et al., 1999), which is similar to obligate snow algae.

TABLE 3

Temperature ranges and optima for snow algal taxa.

Species	Location	temperature range (°C) for growth	temperature optimum (°C)	Reference
Chlainomonas kolii	Olympic National Park, WA	0–4	0–4	Hoham (1975a)
Chlainomonas rubra	near Mt Stuart, WA	0–4	0–4	Hoham (1975a)
Cd. nivalis[1]	Austrian Alps		4?	Czygan (1970)
Cd. nivalis	Beartooth Mtns, MT-WY	−3 to 30	−3 to 20	Mosser et al. (1977)
Cd. yellowstonensis	Pugh Mtn, WA	0–10–?		Hoham (1975a)
Cr. chenangoensis[2]	Chenango Valley, NY	0–7.5	2.5–5.0	This study
Cr. pichinchae	Washington State	0–10	1	Hoham (1975a, 1980)
Cr. polyptera	Windmill Is., Antarctica	0–10	3	Ling (1996)
Cr. rosae v. *psychrophila*	Whiteface Mtn, NY	0–20	4–15	This study
Cr. rosae v. *psychrophila*	White Mtns, AZ	0–20	4–15	This study
Cr. rubroleosa	Windmill Is., Antarctica	0–10	3	Ling (1996)
Cr. tughillensis	Tughill Plateau, NY	0–10	2.5–5.0	This study
Chlorosarcina antarctica	Windmill Is., Antarctica	0–10	3	Ling (1996)
Chromulina chionophilia	Mt Seymour, BC, Canada	0–10	5	Stein (1963)
Chromulina chionophilia	Pugh Mtn, WA	0–15–?		Hoham (1975a)
Cryptomonas frigoris	High Tatra Mtns, Slovakia	2–10	5?	Javornický and Hindák (1970)
Cylindrocystis brébissonii	Pugh Mtn, WA	0–20	10	Hoham (1975a)
Desmotetra antarctica and *D. aureospora*	Windmill Is., Antarctica	0–15	2–10	Ling (2001)
Raphidonema nivale	near Mt Stuart, WA	0–15	5	Hoham (1975a)
Raphidonema tatrae	Belánske Tatry Mtns, Slovakia	0–10	4	Hindák and Komárek (1968)

[1] *Cd.* = *Chlamydomonas*.
[2] *Cr.* = *Chloromonas*.

Several unsaturated fatty acids were identified from this alga, some of which were unusual, and their high content supported that the degree of unsaturation was indicative of low temperature and membrane integrity as reported for snow algae. Growth of two marine diatoms, *Thalassiosira antarctica* and *Nitzschia frigida*, at temperatures below 0°C was enhanced by increased salinity levels (Aletsee and Jahnke, 1992).

ACCLIMATION

For *Cr. rosae* v. *psychrophila*, there were no significant differences acclimating cultures of Adirondack, NY, strains CU 204 and CU 479A for two weeks prior to experiments vs. using non-acclimated strains that were moved from the 4°C growth chamber directly to the temperatures tested (4, 10, 15, and 20°C) (Figs. 2A, 2B). Consequently strains CU 381A and CU 381B of *Cr. rosae* v. *psychrophila* from AZ were not tested for acclimation. When comparing strains of *Cr. tughillensis* in acclimation experiments (Fig. 3A), four strains (CU 581A, 581C, 582A, and 582C) had significantly higher cell counts at 2.5 (7.5)°C when compared to 2.5 (2.5), 7.5 (2.5), and 7.5 (7.5)°C. When the strains were switched to the lower temperature, their growth rates greatly increased, which supports that optimal growth of *Cr. tughillensis* is closer to 2.5°C than to 7.5°C; however, population sizes varied among strains. After four weeks at 2.5 (7.5)°C, strain CU 582A reached a population size of 8.2×10^5 cells mL^{-1} while CU 582D was at 3×10^5 cells mL^{-1}. For strain CU 581D, there was no significant difference between average cell counts at 2.5°C following acclimation at either 2.5 or 7.5°C, which had significantly higher cells counts than the other two treatments. For strain CU 582D, cell counts at 7.5°C following acclimation at 2.5°C were significantly lower than the other three treatments, which did not differ from one another. In a different study, populations of *Cd. nivalis* showed optimal uptake of $^{14}HCO_3^-$ at −3, 0, 10, or 20°C, which was attributed to responses of different temperature strains (Mosser et al., 1977).

In the nitrate, ammonium, and sulfate experiments, there were significantly lower cell counts among strains of *Cr. tughillensis* grown in the acclimation experiments at 2.5 (7.5)°C vs. 7.5 (2.5)°C except for strain CU 582D in the ammonium experiments, which reflects the higher cell densities at 2.5 (7.5)°C (Fig. 3B). Snow algae deplete nutrients as their cell concentrations increase, and nutrient depletion is a limiting factor for growth. Even though concentrations of the nutrients differed in the two temperature regimes, they were not limiting at either temperature after four weeks. *Chloromonas pichinchae* grew optimally in snow meltwater containing coniferous leaves, bark, and pollen, which contributed nitrogen and phosphorus (Hoham, 1976, 1980). Populations and strains of *Cd. nivalis* and *Cr. pichinchae* probably have different nutrient requirements because concentrations of the same elements differed at three snow collection sites where they were found (Hoham and Duval, 2001), which supports the varying concentrations of nitrate, ammonium, and sulfate among strains of *Cr. tughillensis* observed here.

Conclusions

From field observations and laboratory experiments, strains of the high elevation subalpine *Cr. rosae* v. *psychrophila* from New York and Arizona grew from 0 to 20°C with significantly more growth between 4 and 15°C vs. 20°C; whereas, the low elevation subalpine to temperate strains of *Cr. tughillensis* grew from 0 to 10°C and optimally at 2.5 or 5°C and of *Cr. chenangoensis* grew from 0 to 7.5°C and optimally at 2.5 and 5°C. These results were the opposite of what was expected with the low elevation species having lower optimal temperatures for growth than the high elevation species. However, when examining the placement of these taxa in molecular phylogenies, the two low elevation species,

TABLE 4

Some optimal laboratory conditions for the snow algae, *Cr. rosae* v. *psychrophila*, *Cr. tughillensis*, and *Cr. chenangoensis*.

Parameter	*Cr. rosae* v. *psychrophila*[1]	*Cr. tughillensis*	*Cr. chenangoensis*	References
Spectral composition (nm)	unknown	430–460 (blue light)	430–460 (blue light)	Hoham et al. (1998, 2006)
Irradiance level (μmol photons m^{-2} s^{-1})	unknown	95	115	Hoham et al. (1998, 2006)
Photoperiod (hours, light:dark)	unknown	>20:4	>20:4	Hoham et al. (2000, unpubl.)
Temperature (°C)	4–15	2.5–5.0	2.5–5.0	This study
Life cycle	Asexual only (no mating types)	Two mating types (heterothallic)	Self-mating strains (homothallic)	Hoham et al. (2002, 2006)
pH	4.0–5.0	4.9–6.1	7.0–8.0	Hoham and Mohn (1985), Hoham et al. (2007)

[1] Growth in two Adirondack strains is stimulated by balsam fir extracts (Hoham et al., 2008).

Cr. tughillensis and *Cr. chenangoensis*, align with the high elevation subalpine *Cr. pichinchae* (Hoham et al., 2002, 2006), which has an optimal temperature of 1°C (Hoham, 1975a). From preliminary data (Phil Novis, personal communication, 2007), *Chlainomonas rubra* (subalpine, Pacific Northwest of North America) and *Chlainomonas kolii* (alpine, New Zealand), appear to be closely related members of the same lineage and both species of *Chlainomonas* have temperature optima between 0 and 4°C. All the taxa in this genetic subclade have been studied except *Cr. brevispina* and *Cr. nivalis* and thus far have temperature optima near 0°C regardless of habitat. Also these taxa grow at 0°C in snow meltwater, but this temperature was not tested in this study because it could not be achieved in the growth chambers employed. However, 0°C is not likely the optimum temperature for any of the taxa reported here because results from this study indicate an expanded range of temperature optima rather than a specific temperature. If 0°C were tested it is likely that the optimum temperature range may be broader than reported here.

Interestingly, the high elevation subalpine *Cr. rosae* v. *psychrophila* has a wide range of optimal growth between 4 and 15°C, which is similar to that reported for the alpine *Cd. nivalis* of −3 to 20°C when using $^{14}HCO_3$ uptake (Mosser et al., 1977). It is likely that cells used in their study belonged to *Cd. augustae* and not *Cd. nivalis* because all strains deposited as *Cd. nivalis* in culture collections were later identified as *Cd. augustae*; thus the status of *Cd. nivalis* is not clear (Hoham et al., 2002). *Chloromonas rosae* v. *psychrophila* and *Cd. augustae* belong to the same genetic subclade (Hoham et al., 2002), which is a different subclade from the one above with the colder temperature taxa. Thus temperature optima may reflect a phylogenetic relationship rather than adaptation to elevation or habitat (alpine, subalpine, lower elevation, or polar); however, this cannot be established with certainty until more taxa are studied in these two subclades for their optimal temperature. Preacclimation to the temperatures tested seems to have little affected temperature optima, which suggests that temperature optima are phylogenetically based.

Snow communities include a variety of organisms which make up food chains and food webs at the micro- and macrobiotic level (Hoham and Duval, 2001; Hoham et al., 2007), and algae are the primary producers that support other life forms. Several parameters have been studied in the laboratory for the three species of snow algae used in this study (Table 4). These include light (spectral composition, irradiance level, and photoperiod), temperature, life cycle type, and pH, and each species has it own unique set of criteria that is optimal. Interestingly, the data presented here suggests that temperature optima relate to their phylogenetic history, unlike pH optima (Hoham et al., 2007), which appear to be dictated more by the pH of the habitat in which snow algae live.

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