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The Optimum pH of the Green Snow Algae, *Chloromonas tughillensis* and *Chloromonas chenangoensis*, from Upstate New York

Ronald W. Hoham†*
*Robert W. Filbin**
*Frank M. Frey**
*Timothy J. Pusack**
*Jeffrey B. Ryba**
*Patrick D. McDermott** *and*
*Ryan A. Fields**

*Department of Biology, Colgate University, Hamilton, New York 13346, U.S.A.
†rhoham@mail.colgate.edu

Abstract

The optimum pH of two species of snow algae from Upstate New York were assessed by studying three axenic strains of *Chloromonas tughillensis* in a pH range of 3.0–7.0 and three non-axenic strains of *Cr. chenangoensis* in a pH range of 3.0–8.0. Growth was examined at 0.5 pH intervals. Cell counts at the termination of the experiments differed among strains and among pH intervals in individual strains for both species, and strains of *Cr. tughillensis* responded differently to changes in pH ($p < 0.001$) while strains of *Cr. chenangoensis* did not ($p = 0.193$). Cell counts and absorbance data for *Cr. tughillensis* indicated an optimum pH of 4.9–6.1 using regression analysis. Strains of *Cr. chenangoensis* exhibited higher but insignificantly different counts between pH 7.0 and 8.0 with maxima at pH 7.5, but pH optima were not determined. When the range was expanded to include pH 8.5–9.0, an optimal pH of 7.0–8.0 was determined for strain CU 722B, and this is the first snow alga reported to have an optimum alkaline pH. The highest absorbance values, however, occurred between pH 3.0–4.5 and pH 7.0–8.0. The pH values recorded in the field were 5.0–5.3 for *Cr. tughillensis* and 6.7–7.6 for *Cr. chenangoensis*.

Introduction

Snow or cryophilic algae occur worldwide (Kol, 1968), primarily in alpine regions and/or regions with consistent snowfalls >200 cm year^{-1} (Duval and Hoham, 2000). Cryophilic algae are well adapted to living in extreme conditions of low temperature, high irradiance levels, low nutrient concentrations, high acidity, and desiccation after snow melt (Hoham and Duval, 2001). Active phases for growth of snow algae occur for approximately one to two weeks per year during snowmelt, often in early April in Upstate New York (NY), which coincides with a peak in snow acidity (Hoham and Mohn, 1985).

The pH of snow meltwater varies from 3.8 to 8.1 with algae present (Hoham, 1975; Ling and Seppelt, 1998). Snow algae have a preference for acidic conditions, but some species are more tolerant of acidity than others (Hoham and Duval, 2001). Those with an acidic optimal pH include *Chloromonas* (*Cr.*) *rosae* v. *psychrophila* (published as *Cr.* sp.) (pH 4.0–5.0) and *Cr. hohamii* (published as *Cr. polyptera*) (pH 4.5–5.0) (Hoham and Mohn, 1985). Kol (1968) reported the field pH for 80 cryophilic algal species and established that most were found between pH 5.0 and 7.0, and none were above pH 7.0.

Some algae are more indifferent to pH. The snow alga *Cr. polyptera* was found in meltwater with pH 6.7–8.1 (Ling and Seppelt, 1998); the green alga *Mougeotia* sp. had an optimum pH for growth in lakes of ~5.2, but in laboratory experiments it was pH 8.0 (Graham et al., 1996); and the green alga *Chlamydomonas applanata* grew at pH 3.4–8.4 with an optimum pH of 7.4 (Visviki and Santikul, 2000).

Acidic pH can negatively affect algal growth and development (Ellis and Machlis, 1968), which may result from changes in nutrient availability and ability to take up nutrients (Ouellet and Benson, 1951; Raven, 1980; Hoham and Mohn, 1985; Umbach, 1985). Cryophilic algae affect environmental pH, either increasing it by removing CO_2 from snow during photosynthesis (Hoham et al., 1989) or decreasing it by excreting organic compounds (Newton, 1982).

The alga *Cr. chenangoensis* has been observed in snow only in April 2001, which suggested that this species may have been a soil alga behaving as a snow alga under unusual conditions, or it was possibly blown into the snow by wind. However, an *rbc*L gene sequence analysis of *Cr. chenangoensis* showed that it was part of a subclade containing only snow algal species, which included *Cr. brevispina*, *Cr. nivalis*, *Cr. pichinchae*, and *Cr. tughillensis* (Hoham et al., 2006).

The purpose of this study was to (1) determine the pH optima of three axenic strains of *Cr. tughillensis* and three non-axenic strains of *Cr. chenangoensis*, (2) consider laboratory optima relevance to pH ranges found in the field when the samples were collected (pH 5.0–5.3 and 6.7–7.6, respectively), and (3) establish whether or not snow algae adapt to the pH of M-1 growth medium (pH 5.0–5.1) under laboratory conditions.

Materials and Methods

SOURCE OF MATERIALS AND GROWTH CONDITIONS

Procedures for setting up laboratory experiments followed Hoham and Mohn (1985). Strains CU 582D, CU 581A, and CU 582C of *Cr. tughillensis* were collected in April 1988 from Whetstone Gulf State Park, Tughill Plateau, NY (Fig. 1), from snow with a pH range of 5.0–5.3, and subsequently isolated into axenic culture. Strains CU 721A, CU 722B, and CU 725B of *Cr. chenangoensis* were collected in April 2001 in Hamilton, NY (Fig. 1), from snow with a pH range of 6.7–7.6 and were non-axenic cultures (axenic cultures were not available). All cultures were maintained on modified M-1 medium (Hoham et al., 2006) in a Percival model CTR-66 Growth Chamber at 4 ± 1°C under GE FL40 PL/AQ wide spectrum fluorescent tubes using a 16:8 hour (h), light:dark (L:D) photoperiod. M-1 medium approximates

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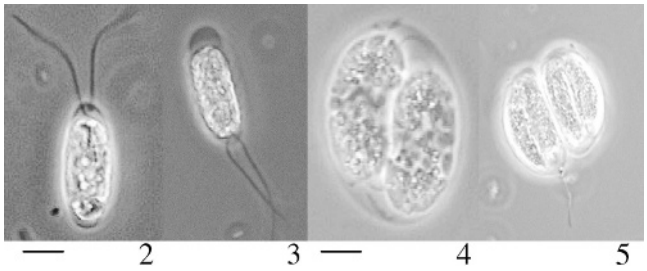
FIGURE 1. Collections sites of *Chloromonas tughillensis* (▲) and *Chloromonas chenangoensis* (■).

nutrients available to algae in snow (Hoham et al., 1979), and 4 ± 1°C was the closest temperature that could be maintained in the growth chamber to the temperature in the field (~0°C).

EQUALIZATION OF CELLS AND GROWTH CHAMBER SETUPS

Materials and growth media were sterilized and stored at 4 ± 1°C, and cells were washed from agar plates using modified M-1 medium. For each strain, 125-mL Erlenmeyer flasks were filled with 50 mL of modified M-1 medium, capped with 50-mL beakers, and inoculated with 1×10^4 cells mL^{-1}. Four replicates were used for each strain of *Cr. tughillensis* for pH 3.0–7.0 and *Cr. chenangoensis* for pH 3.0–8.0 at 0.5 pH increments and the controls where pH levels were not adjusted. The pH experiments were conducted using a 14.5:9.5 h, L:D photoperiod for *Cr. tughillensis* and a 14:10 h, L:D photoperiod for *Cr. chenangoensis* that simulated photoperiods in the field when they were collected. Experimental trays contained eight flasks (two pH points) and were lined with white paper on the inside to standardize reflectivity. All trays for each strain were positioned side-by-side on one shelf, situated on white paper, and a control tray with beaker-capped flasks filled with 50 mL of demineralized water was placed on each end of the experimental trays. All experiments for each species were performed concurrently.

Growth chamber irradiance levels between 69 and 101 μmol photons m^{-2} s^{-1} for *Cr. tughillensis* and between 69 and 127 for *Cr. chenangoensis* were measured weekly using a LI COR LI-1935A 3-D "Spherical" sensor attached to a LI COR LI-1000 "Datalogger." Trays were shifted two positions three times per week to equalize light intensity amongst trays. Growth chamber lighting approximated irradiance levels received by snow algae in the Adirondacks at 12–20 cm below the snow surface (Hoham et al., 1983). Three times weekly Hach Sension 2 Model 51910 pH electrodes were alcohol sterilized and acclimated to modified M-1 medium, calibrated using pH 4.0 and 7.0 buffers, and then used to measure pH. Flasks were swirled and shaken before obtaining readings and maintained within 0.05 pH using HCl or NaOH, and electrodes were rinsed with sterilized, demineralized water between readings. Cultures were kept on ice when pH readings were taken to maintain a temperature near 4°C and avoid temperature shock.



FIGURES 2–5. Individual cells of *Cr. chenangoensis* (Fig. 2) and *Cr. tughillensis* (Fig. 3). Cell packs of *Cr. chenangoensis* (Fig. 4) and *Cr. tughillensis* (Fig. 5). Scale bar in Figure 2 = 5 μm (for Figs. 2, 3, 5). Scale bar in Figure 4 = 3 μm.

CELL RETRIEVAL AND COUNTING PROCEDURES

Experiments were terminated for *Cr. tughillensis* strains CU 582D, CU 581A, and CU 582C after 20, 24, and 28 days and for *Cr. chenangoensis* strains CU 721A, CU 725B, and CU 722B after 21, 24, and 28 days, respectively, when cell concentrations (5–15 × 10^4 cells mL^{-1}) were similar to those found in field samples (5–20 × 10^4 cells mL^{-1}). Terminating experiments simultaneously was physically impossible due to the time required to end each experiment. Cells were suspended prior to counting them on hemacytometers, and six people enumerated to avoid statistical bias. Tabulation of total cells included individual cells (Figs. 2, 3) and cells in cell packs (zoosporangia/gametangia) (Figs. 4, 5), and the final volume for each flask was recorded. Absorbance and percent transmittance values were taken at 440 nm using a HACH DR/3000 Spectrophotometer to give a second evaluation of population density. Growth was not monitored during the pH experiments because any solution removed for cell counts would have resulted in small differences in growth rates between flasks, and performing absorbance readings would have increased the probability of contamination.

STATISTICAL ANALYSES

Cell counts were averaged for each flask (n = 12 for *Cr. tughillensis*, and n = 10 for *Cr. chenangoensis*) to obtain an overall mean, which were treated as a single replicate. Thus, the total sample size per strain for the experiments with *Cr. tughillensis* and *Cr. chenangoensis* started at 40 and 48, respectively. Three clear outliers were removed from the data sets of *Cr. tughillensis* and *Cr. chenangoensis* (5+ standard deviations away from the mean), which reduced the total sample size for each species to 117 and 141, respectively. The data were analyzed separately for each species in two ways. (1) To determine if variation in mean cell count was associated with strain or pH level, the data for cell counts were log-transformed to improve normality and a two-way ANOVA was performed with strain and pH level as fixed factors. (2) The cell count data were also standardized for each strain relative to the appropriate control so that cell counts for each flask were measured in units of standard deviations away from the control for that strain. This was done to more accurately assess how changes in pH affected cell replication. Specifically, for each strain the average cell count of the control flasks was subtracted from each experimental flask's cell count, and then divided by the standard deviation of the cell counts from the control (e.g., [CU $581A_{\text{pH 3.0, replicate flask 1}}$ − mean CU $581A_{\text{control flasks}}$]/[standard deviation CU $581A_{\text{control flasks}}$]). Another two-way ANOVA was performed on these data for each species with strain and pH as fixed factors. In addition, a separate quadratic regression was used

TABLE 1

Results of two-way ANOVAs on mean cell count (A and B) and absorbance (C and D) data for *Chloromonas tughillensis* with strain and pH as main effects.

		Source	df	*F*
Mean cell count	(A) Unstandardized	strain	2	171.527***
		pH	9	31.699***
		strain*pH	18	7.053***
		error	87	
	(B) Standardized	strain	2	54.565***
		pH	8	23.233***
		strain*pH	16	9.594***
		error	78	
Absorbance	(C) Unstandardized	strain	2	12.325***
		pH	9	40.787***
		strain*pH	18	5.355***
		error	90	
	(D) Standardized	strain	2	8.450***
		pH	8	38.544***
		strain*pH	16	5.947***
		error	81	

For *F*, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

for each strain with standardized cell count as the dependent variable and pH as the independent variable. By taking the derivative of the resulting regression equation, and setting it equal to zero, the optimal pH for each strain was more precisely estimated. An additional experiment using cell counts ($n = 8$) was conducted using strain CU 722B for *Cr. chenangoensis* where the pH range was expanded to include points 8.5 and 9.0 in an attempt to determine optimal pH.

Absorbance data were analyzed in the same way. The total sample size for *Cr. tughillensis* was 120 replicates and 143 for *Cr. chenangoensis*. A two-way ANOVA was used with strain and pH level as fixed factors for each species separately to determine if variation in absorbance at 440 nm differed among strains and pH levels. The data were also standardized as described above and separate two-way ANOVAs were performed for each species with strain and pH level as fixed factors. Finally, equations generated from quadratic regressions were used to determine the optimal pH for each strain as discussed above.

Results

CHLOROMONAS TUGHILLENSIS

Average cell count per flask significantly varied among strains, pH levels, and strain-pH combinations when the non-standardized (Table 1A, Fig. 6A) and the standardized data (Table 1B, Fig. 6B) were used. Optimum growth occurred around pH 5.0 (Figs. 6A, 6B). For all strains, a quadratic regression was a good fit to the data (CU 581A: $R^2 = 0.689$, $p < 0.001$; CU 582C: $R^2 = 0.622$, $p < 0.001$; CU 582D: $R^2 = 0.471$, $p < 0.001$), and point estimates of the optimal pH for each strain were obtained (CU 581A = 5.84; CU 582C = 4.92; CU 582D = 4.97).

Average absorbance per flask varied significantly among strains, pH levels, and strain-pH combinations when the non-standardized (Table 1C, Fig. 6C) and the standardized (Table 1D, Fig. 6D) data were used. Maximum absorbance also occurred around pH 5.0 (Figs. 6C, 6D). For all strains, a quadratic regression was a good fit to the data (CU 581A: $R^2 = 0.740$, $p < 0.001$; CU 582C: $R^2 = 0.617$, $p < 0.001$; CU 582D: $R^2 = 0.754$, $p < 0.001$), and the optimal pH estimates were similar to those obtained from the cell counts (CU 581A = 6.10; CU 582C = 5.06; CU 582D = 4.99).

CHLOROMONAS CHENANGOENSIS

Average cell count per flask varied significantly among strains and pH levels, but not strain-pH combinations when the non-standardized (Table 2A, Fig. 7A) and the standardized (Table 2B, Fig. 7B) data were used. In contrast to the trend observed for *Cr. tughillensis*, optimum growth occurred at pH values around 3.5 or 7.5 (Figs. 7A, 7B). For all strains, a quadratic regression was a decent fit to the data (CU 721A: $R^2 = 0.310$, $p < 0.001$; CU 722B: $R^2 = 0.611$, $p < 0.001$; CU 725B: $R^2 = 0.412$, $p < 0.001$). Point estimates of optimal pH for CU 721A and 725B were not obtained because the best-fit curves were concave-up, and the regression model suggested that the optimal pH for CU 722B was 9.02. However, when the pH range was expanded to include points 8.5 and 9.0 (Figs. 8A, 8B), the optimal pH was 7.0–8.0.

Average absorbance per flask varied significantly among strains, pH levels, and strain-pH combinations when the non-standardized (Table 2C, Fig. 7C) and standardized (Table 2D, Fig. 7D) data were used. Maximum absorbance occurred at pH values around 3.5 or 7.5 (Figs. 7C, 7D). For strain CU 721A, a quadratic regression was a poor fit ($R^2 = 0.041$, $p = 0.425$), but for the other two it was a stronger fit (CU 722B: $R^2 = 0.408$, $p < 0.001$; CU 725B: $R^2 = 0.355$, $p < 0.001$). However, point estimates of optimal pH were not obtained for any strain because their best-fit curves were all concave-up.

Discussion

STANDARDIZING PROCEDURES AND pH EXPERIMENTS

Without a standard experimental design, it is difficult to compare pH studies conducted on algae. There is substantial variation with respect to sample replicate number, axenic vs. non-axenic cultures, pH drifting range, and periodic cell counts. Most studies have used triplicate flasks (Hoham and Mohn, 1985; Pederson and Hansen, 2003; Lundholm et al., 2004); however, Olaveson and Stokes (1989) and this study implemented quadruplicate flasks to reduce sampling error and better distinguish between treatment groups. Axenic (Hoham, 1975; Hoham and Mohn, 1985; Olaveson and Stokes, 1989) and non-axenic cultures (Pedersen and Hansen, 2003; Lundholm et al., 2004) have been employed, but axenic cultures are preferable because bacteria or fungi may affect algal growth and ambient pH values (Hoham and Duval, 2001). The pH adjustment from established pH points varied among experiments, which allowed shifts in pH of 0.2, 0.1, and 0.01 (Hoham and Mohn, 1985; Olaveson and Stokes, 1989; Pedersen and Hansen, 2003). This study allowed for a pH adjustment of 0.05 to limit pH variability and differences in the amount of NaOH and HCl added between flasks. Some studies conducted periodic cell counts during their experiments (Olaveson and Stokes, 1989; Lundholm et al., 2004), which was not done in this study because it reduces the volume of the media in the cultures and changes the ratio between media and air.

Lighting, temperature, degree of agitation, culture containers, and culture media varied in previous studies (Hoham and Mohn, 1985; Olaveson and Stokes, 1989; Graham et al., 1996; Pedersen and Hansen, 2003; Lundholm et al., 2004), and some of these differences related to more specific requirements of the species studied. Test tubes with cotton stoppers have been used to regulate air exchange (Graham et al., 1996), but in this study, beakers

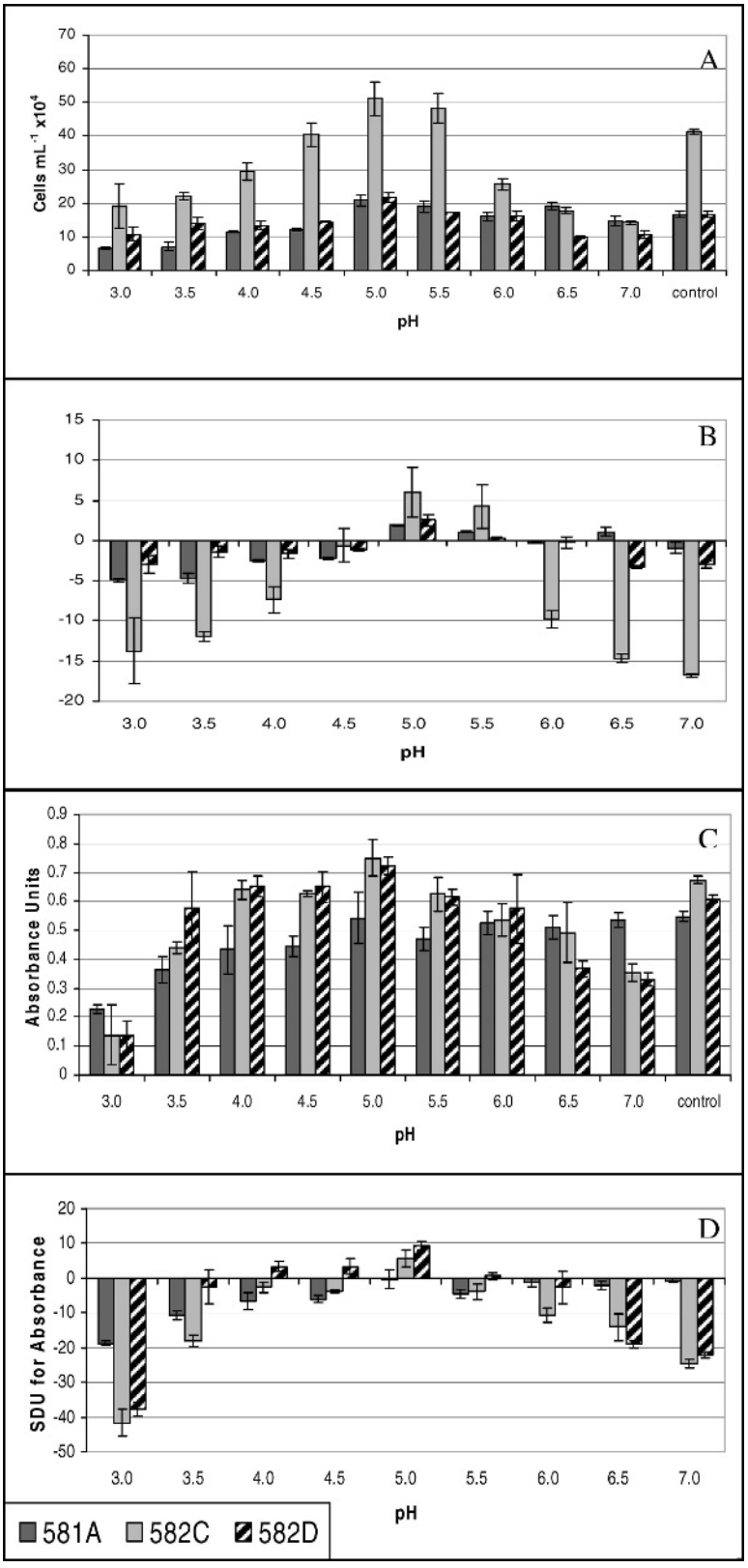


FIGURE 6. ***Cr. tughillensis*** **strains CU 581A, CU 582C, and CU 582D. (A) Mean cell counts (±1 SE) (*n* = 4 except CU 582C, pH 3.5 where *n* = 3 for A and B and CU 582D, pH 3.5 and 6.0 where *n* = 3 for A and B). (B) Mean cell counts (±1 SE) standardized to the control mean cell counts using standard deviation units (SDU). (C) Absorbance data (440 nm) (±1 SE) (*n* = 4 for C and D). (D) Mean absorbance data (440 nm) (±1 SE) standardized to the control mean absorbance data using SDU.**

TABLE 2

Results of two-way ANOVAs on mean cell count (A and B) and absorbance (C and D) data for *Chloromonas chenangoensis* with strain and pH as main effects.

		Source	df	*F*
Mean cell count	(A) Unstandardized	strain	2	75.445***
		pH	11	12.908***
		strain*pH	22	1.149
		error	105	
	(B) Standardized	strain	2	13.030***
		pH	10	16.471***
		strain*pH	20	1.308
		error	96	
Absorbance	(C) Unstandardized	strain	2	31.989***
		pH	11	13.793***
		strain*pH	22	2.070***
		error	107	
	(D) Standardized	strain	2	3.353*
		pH	10	19.157***
		strain*pH	20	2.777***
		error	98	

For *F*, *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

covered flasks to allow for gas exchange, reduce airborne contamination, and maximize irradiance penetration.

Laboratory experiments show that freshwater algae are tolerant to a wide range of pH. The unicellular euglenoid, *Euglena mutabilis*, commonly found in acidic mine drainage waters, had highest growth between pH 3.0 and 4.0 in a growth range of pH 2.0–9.0 (Olaveson and Stokes, 1989). The green alga *Chlamydomonas acidophila* revealed a broad pH tolerance for growth and photosynthesis at pH 1.5–7.0 (Gerloff-Elias et al., 2005). Maximum rates of sporulation and germination in the cyanobacteria *Anabaena fertilissima* and *Anabaenopsis arnoldii* occurred at an alkaline pH of 7.0–10.5 and 7.0–8.5, respectively (Reddy, 1984). Some algae may grow in both acidic and alkaline conditions such as the filamentous green alga, *Mougeotia*, which had a laboratory optimum of pH 8.0 but a field pH optimum of ~5.2 (Graham et al., 1996). In a molecular phylogeny study of two species of the filamentous green alga, *Klebsormidium*, it was found that *K. acidophilum* from mine-contaminated streams (pH < 3.0) was derived from *K. dissectum*, a species that grew best in less acidic environments at pH 4.8–6.2 (Novis, 2006).

Optimal light, optimal temperature, and life cycles have been studied for *Cr. tughillensis* and *Cr. chenangoensis* (Hoham et al., 1998, 2000, 2006) (Table 3). Until this study, the optimal pH was not known for either species. However, both species showed different responses to changes in pH, and population growth estimates measured through cell counts and absorbance data were consistent within each species. Growth was best for *Cr. tughillensis* at acidic pH levels and for *Cr. chenangoensis* at weakly alkaline pH levels. The results are discussed separately for each species.

DATA INTERPRETATION

Chloromonas tughillensis

Strains differed with respect to growth, changes in pH affected cell division rates, and strains responded differently to changes in pH. However, the estimated pH optimum for each strain was consistent. By averaging the point estimates in the cell count and absorbance experiments, the apparent pH optimum for this species was near 5.3. This is consistent with field conditions (ambient pH = 5.0–5.3) and with data for other snow algal species (Table 4). Specifically, the pH optima of strains CU 582C (pH 4.9–5.1) and CU 582D (pH 5.0) corresponded with those found for strains of *Cr. rosae* v. *psychrophila* (pH 4.0–5.0 published as *Cr.* sp. for New York strains, and pH 4.5–5.0 published as *Cr. polyptera* for Arizona strains) (Hoham and Mohn, 1985).

Interestingly, the pH optimum of strain CU 581A was substantially higher than ambient field conditions with a pH optimum of 5.8–6.1. Discrepancies between field pH and laboratory pH optima have been reported previously. For example, the optimum laboratory pH for *Cr. pichinchae* (5.5–6.5) was higher than pH recorded from snow during field collections (3.8–5.2) (Hoham, 1975, 1980). It is not clear why strains and species such as these have not adapted locally to their pH environment, but these differences may be due to physiological and (or) genetic differences between strains. Cells of strain CU 581A are significantly smaller than those of strains CU 582C and CU 582D (Hoham et al., 2006), and smaller cells are better suited to grow at higher pH because their larger surface-area-to-volume ratio allows them to better regulate their intracellular pH (Lundholm et al., 2004).

Chloromonas chenangoensis

Strains differed with respect to population growth, changes in pH affected cell division rates, and cell count data suggested that strains may respond differently to changes in pH. When using a pH range of 3.0–8.0 (Fig. 7), the cell count and absorbance data clearly showed that population growth was greatest in weakly alkaline conditions (pH > 7.0) but not in acidic conditions (pH 5.0), which was the case for *Cr. tughillensis*. The absorbance data suggested that all strains grew relatively well in a strongly acidic environment (Fig. 7D), whereas the cell count data did not support this (Fig. 7B). While absorbance data indicated growth peaks at pH 3.5–4.5 and pH 7.0–8.0, the accuracy of absorbance data was less clear than data from cell counts because of the presence of other organisms (principally yeasts and bacteria) in these non-axenic cultures. These bacteria and yeasts may have affected the absorbance values for all three strains in the strongly acidic conditions, and the association between pH and absorbance was not significant ($p > 0.05$).

The single point estimate of optimal pH derived from the cell count data suggested that cell division rates were highest for strain CU 722B at a pH of 9.02 when using a pH range of 3.0–8.0. In addition, the cell count data suggested that the optimal pH for all three strains was at a pH > 7.0 with a peak at 7.5 (Fig. 7B). When the pH range was expanded to include points 8.5 and 9.0 for strain CU 722B (Figs. 8A, 8B), the optimal pH was 7.0–8.0. This was in stark contrast to most other research with snow algae (Table 4); however, both *Cr. polyptera* and *Desmotetra* sp. grew in the field at a pH between 6.7–8.1 and 6.8–7.8, respectively (Ling and Seppelt, 1998; Ling, 2001).

The strains of *Cr. chenangoensis* were collected in 2001 when the pH of the snow packs was measured at 6.7–7.6 ($n = 11$). Since 2001, these strains have been maintained in culture in modified M-1 medium (Hoham et al., 2006) at pH 5.0–5.3. Consequently, these cultures have not adapted to the acidic pH of this medium, and the combined results clearly showed that population growth was substantially decreased at a pH near 5.0. It is not clear why these cultures have not adapted to perform better at pH 5.0. The pH range of 3.0–8.0 used initially in this study was based on pH values recorded in the field prior to and when snow algae appear (Table 4; Schofield and Trojnar, 1980). The neutral to weakly alkaline pH recorded from residual snow patches in central NY

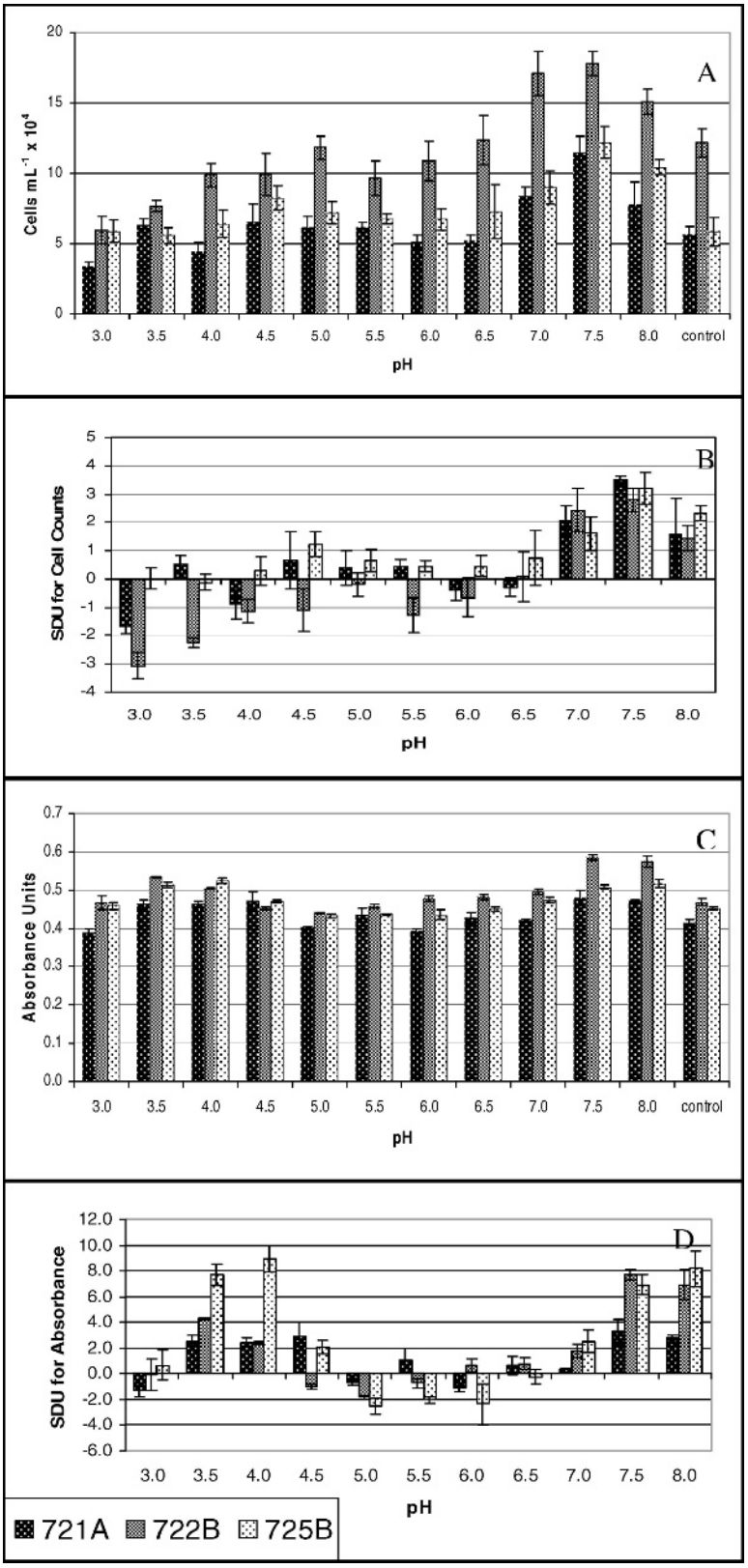


FIGURE 7. ***Cr. chenangoensis*** **strains CU 721A, CU 722B, and CU 725B. (A) Mean cell counts (±1 SE) (*n* = 4 except CU 721A, pH 7.5 where *n* = 3 for A and B; and CU 722B, pH 3.5 and 8.0 where *n* = 3 for A and B). (B) Mean cell counts (±1 SE) standardized to the control mean cell counts using standard deviation units (SDU). (C) Absorbance data (440 nm) (±1 SE) (*n* = 4 except CU 721A and CU 722B, pH 3.5 where *n* = 3 and CU 722B, pH 8.0 where *n* = 2 for C and D). (D) Mean absorbance data (440 nm) (±1 SE) standardized to the control mean absorbance data using SDU.**

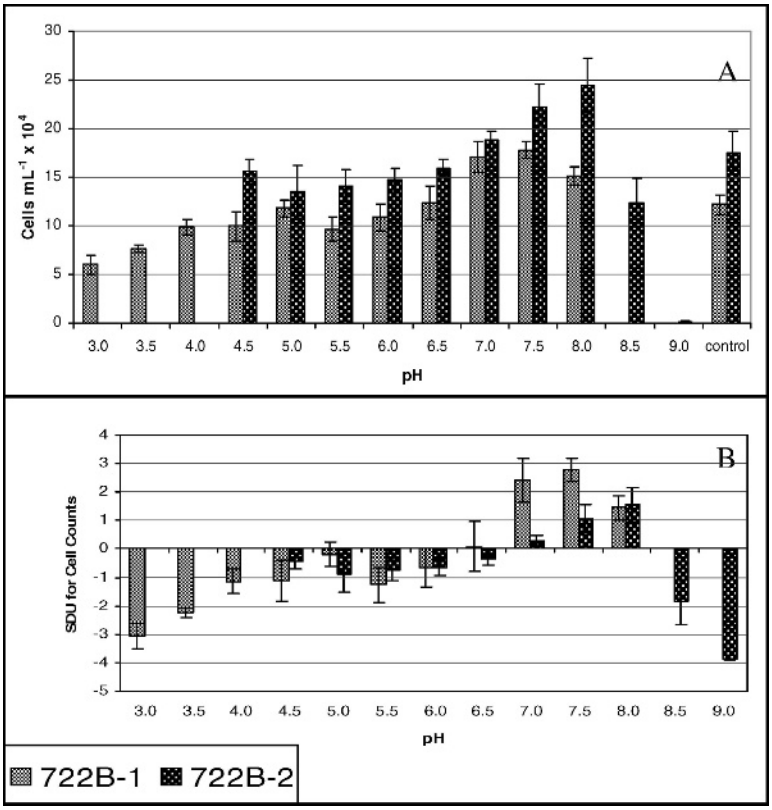


FIGURE 8. Comparison between two experiments for *Cr. chenangoensis* strain CU 722B. (A) Mean cell counts (±1 SE) for pH 3.0–8.0 (CU 722B-1) and 4.5–9.0 (CU 722B-2) ($n = 4$ except CU 722B-1, pH 3.5 and 8.0 where $n = 3$ for A and B; and CU 722B-2, pH 4.5 and 8.5 where $n = 3$ for A and B). (B) Mean cell counts (±1 SE) standardized to the control mean cell counts using standard deviation units (SDU).

may relate to the limestone-based topography found there, and the laboratory pH optimum of 7.0–8.0 for strain CU 722B correlated with the pH values recorded from the field.

Conclusions

Even though the two species of snow algae presented in this paper are neither alpine nor polar in their distribution, the subalpine to temperate snow habitat where they exist is very similar to habitats occupied by polar and alpine species. All of these species, regardless of habitat, are subjected to the same extreme parameters of low temperature, high irradiance levels except for shade-tolerant species, low nutrient concentrations, high acidity except for a few species, and desiccation after snow melt (Hoham and Duval, 2001).

For snow algae, the optimum pH of *Cr. tughillensis* of 4.9–6.1 is similar to that reported for other species, but the pH of 7.0–8.0 for *Cr. chenangoensis* is the first report of an alkaline optimum pH. Even though *Cr. polyptera* and *Desmotetra* sp. were collected in alkaline snow, an optimum pH has not been determined for these species. The pH of snow may also result in the natural selection of snow microbes. Strains of *Cr. rosae* v. *psychrophila* from the Adirondack Mountains, NY, had an optimum growth in pH 4.0–5.0 compared to 4.5–5.0 for strains from the White Mountains, Arizona (Hoham and Mohn, 1985). These differences were significant ($p < 0.05$), which suggested that the Adirondack snow algae were adapting to the more acidic precipitation characteristic of eastern North America; however, it is not known whether alkaline snow is selecting for species.

Snow communities include a combination of food chains and food webs at micro- and macrobiotic levels (Hoham and Duval, 2001), and algae are the primary producers which all life forms in snow depend on. Snow microbial communities that include *Cr. tughillensis* (pH 5.0–5.3) and *Cr. chenangoensis* (pH 6.7–7.6) may be different enough in pH to support life forms which are not similar. In addition to algae, organisms in snow microbial communities include bacteria, fungi, protozoa, rotifers, nematodes, tardigrades, oligochaetes, and copepods. Larger animals such as arachnids, insects, birds, and mammals also play an important role in the snow food web. Future research may reveal whether other microbial forms in snow are adapting to differences

TABLE 3

Some optimal laboratory conditions for the snow algae *Chloromonas tughillensis* and *Chloromonas chenangoensis*.

Parameter	*Cr. tughillensis*	*Cr. chenangoensis*	Reference
Spectral composition (nm)	430–460 (blue light)	430–460 (blue light)	Hoham et al. (1998, 2006)
Irradiance level (μmol photons m^{-2} s^{-1})	95	70–130	Hoham et al. (1998, 2006)
Photoperiod (hours, light:dark)	>20:4	>20:4	Hoham et al. (2000, 2006)
Temperature (°C)	2.5–5.0	<10.0	Hoham (unpublished)
Life cycle	Two mating types (heterothallic)	Self-mating strains (homothallic)	Hoham et al. (2006)
pH	4.9–6.1	7.0–8.0	This study

TABLE 4

The pH range and optima for snow algal taxa.

Species	Location	pH range (field)	pH optimum (lab)	Reference
Cd. nivalis[1]	Unspecified	4.1–5.8		Kawecka (1978)
Cd. spp.	Svalbard, Norway	4.4–6.2		Muller et al. (1998)
Cr. brevispina[2]	Washington State	5.0–5.1		Hoham et al. (1979)
Cr. chenangoensis	Chenango Valley, NY	6.7–7.6	7.0–8.0	This study
Cr. hohamii	White Mtns., AZ	4.9		Hoham et al. (1983)
Cr. nivalis	Washington State	5.0–5.1		Hoham and Mullet (1977, 1978)
Cr. pichinchae	Washington State	3.8–5.2	5.5–6.5	Hoham (1975, 1980)
Cr. polyptera	Windmill Is., Antarctica	6.7–8.1		Ling and Seppelt (1998)
Cr. rosae v. *psychrophila*	Whiteface Mtn., NY	4.9–5.2	4.0–5.0	Hoham and Mohn (1985)
Cr. rosae v. *psychrophila*	White Mtns., AZ	4.9	4.5–5.0	Hoham and Mohn (1985)
Cr. rubroleosa	Windmill Is., Antarctica	4.6–6.2		Ling and Seppelt (1993)
Cr. tughillensis	Tughill Plateau, NY	5.0–5.3	4.9–6.1	This study
Chlorosarcina sp.	Windmill Is., Antarctica	6.3–6.9		Ling (2002)
Desmotetra sp.	Windmill Is., Antarctica	6.8–7.8		Ling (2001)
Mesotaenium sp.	Windmill Is., Antarctica	4.5–5.7		Ling and Seppelt (1990)

[1] *Cd.* = *Chlamydomonas.*

[2] *Cr.* = *Chloromonas.*

in pH as documented for algae and if they are benefiting or inhibiting one another.

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