The Effects of Vita-Lite and Blue Light Pre-Acclimation on Sexual Reproduction in the Green Snow Alga, *Chloromonas* sp.-D (Chlorophyceae, Volvocales), Using Different Photoperiods

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ABSTRACT

The effects of premating light regimes on sexual reproduction and the production of spherical cells in *Chloromonas* sp.-D, a unicellular green snow alga, were studied using strains 582C and 582D isolated from snowpacks associated with mixed hardwood-softwood forests in Whetstone Gulf State Park, Tughill Plateau, NY. Two pre-acclimation regimes were used, Vita-Lite as controls (530-700 nm peak) and blue light as experimentals (440 nm peak) prior to the mating experiments. In blue light, an increase in the number of matings and spherical cells (spheres) produced in the life cycle was observed as the photoperiod increased, but a plateau occurred in the production of spheres between 20 and 24 hours of continuous light. This implies that longer photoperiods of blue light are more optimal for sexual reproduction in *Chloromonas* sp.-D than shorter ones. Under Vita-Lite, there was a significant increase in the number of matings and spheres with the extended 20:4 photoperiod compared to the shorter 14:10 photoperiod as well. Under blue light, significantly more matings and spheres occurred than under Vita-Lite using the same irradiance level of 95 µmol photons m^{-2} s^{-1} for the 14:10 and 20:4 photoperiods. The results of these experiments suggest that *Chloromonas* sp.-D, known only from the Tughill Plateau, NY, is not reproducing optimally at this site where it grows and reproduces under an approximate 14:10 photoperiod in early April. However, in the upper 10 cm of snow in the Tughill Plateau, a blue light irradiance level of 95 µmol photons m^{-2} s^{-1} occurs, which is optimal for this species. When these conditions are combined with a 14:10 photoperiod, the Tughill Plateau appears to be sub-optimal for mating and production of spherical cells. *Chloromonas* sp.-D may reproduce more optimally under blue light (95 µmol photons m^{-2} s^{-1}) with an extended photoperiod (>20:4 hours, light:dark) in higher latitude field sites such as Lake Bienville, Québec, in eastern North America where other species of *Chloromonas* are known in snow associated with coniferous forests.

Key words: *Chloromonas*, light, photoperiod, sexual reproduction, snow algae.

INTRODUCTION

Most snow algae belong to the Order Volvocales in the green algae, which is characterized by flagellate or swimming stages (Hoham and Duval 2000). The interrelationships between snow algal life cycles and their surrounding physical and chemical factors have been reviewed (Hoham and Duval 2000; Hoham and Ling 2000) and include discussions on hydrological processes. These green algal flagellates occur in snow at the time when liquid water, nutrients and dissolved gases are available and solar irradiance penetrates through the snowpack (Hoham et al. 1998). These algae require liquid water in order to swim around the more solid snow crystals, they position

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themselves according to irradiance levels and spectral composition, and they eventually cause visible colors in the snowpacks of green, orange or red (Hoham and Duval 2000).

Light is an important environmental cue for phototrophs including many algae. Changes in the wavelength and intensity of light often elicit a response in the cell cycle and mating strategy of these organisms. Life histories of *Chloromonas pichinchae* (Hoham 1975), *C. nivalis* (Hoham and Mullet 1977; 1978), *C. brevispina* (Hoham *et al.* 1979), *C. polyptera* (Hoham *et al.* 1983) and *C. rubroleosa* (Ling and Seppelt 1993) were examined from snowpacks in the field, while the mating process in *Chloromonas* sp.-D was investigated in the laboratory (Hoham *et al.* 1997; Hoham *et al.* 1998). Mating studies in the closely related *Chlamydomonas* (Beck and Treier 1991; Quarmby and Hartzell 1994) provide insight into the cell cycle of *Chloromonas* (Hoham *et al.*1997).

Chlamydomonas and *Chloromonas* are algal representatives with a zygotic meiosis life cycle (Hoshaw 1961; Hoham 1980). In this type of life cycle, the zygote is the only diploid cell, all other cells are haploid. The vegetative cell through mitosis may produce additional vegetative cells through zoospore production or may form gametes. Gametes fuse with other gametes to form diploid zygotes, which subsequently undergo meiosis. In mitotic division, haploid biflagellate oblong cells divide to form cell packs of 2, 4 or 8 oblong or spherical cells within the common parental cell wall which dissolves releasing the biflagellate daughter cells (Hoham 1975; Harris 1989). Haploid spherical cells were also observed in *Chlamydomonas eugametos* and in *Chloromonas* sp.-D. In both species, an increase of spherical cells was noted through time when grown on nutrient deprived media, but the mechanism of formation of spherical cells was thought to be through cell packs in *Chloromonas* sp.-D as it was for oblong cells (Hoham *et al.* 1997). In addition, *Chloromonas* sp.-D rarely produces abnormal V-shaped mitotic divisions where haploid biflagellate oblong cells divide longitudinally (Behrstock *et al.* 1998, pers. comm.). Similar V-shaped configurations were reported from field material in the snow alga, *Chlamydomonas nivalis*, in Poland (Kawecka and Drake 1978).

Gametogenesis in *Chlamydomonas* is initiated by nitrogen starvation, which in combination with darkness, causes sexually incompetent pre-gametes to mature into sexually competent gametes with the addition of blue light (Gloeckner and Beck 1995; Pan *et al.* 1996; Gloeckner and Beck 1997). Sexual reproduction in *Chlamydomonas* is initiated when the flagella of gametes from opposite mating types (mt+ and mt-) come into contact through agglutination (Kooijiman *et al.* 1988), which results in their adhesion, entwining and eventual pairing (Lewin 1956; Van Winkle-Swift 1977; Tomson *et al.* 1990). In *Chloromonas* sp.-D, gametes involved in syngamy are either oblong or spherical, matings can be isogamous or anisogamous, and quadriflagellate zygotes are produced (Hoham *et al.* 1998). In snow algae, zygotes loose their flagella and develop into thick-walled resting zygospores that withstand desiccation after snowmelt. Zygospore germination does not occur until the following year at the time of snowmelt. The daughter cells released from the zygospores produce cell packs, and the cells released from the cell packs further divide to increase the algal population in the snowpacks (Hoham 1980).

In its natural habitat, *Chloromonas pichinchae* is found in the top 10 cm of snowpacks where blue (440 nm) irradiance levels are higher than other parts of the visible spectrum (Hoham 1975), and blue light also promotes optimal mating in *Chloromonas* sp.-D (Hoham *et al.* 1998). Blue light activates a carotenoid trigger in *Chlamydomonas reinhardtii* that causes the accumulation of glutamate-1-semialdehyde aminotransferase (gsa) mRNA, which results in a sudden increase in chlorophyll production (Matters and Beale 1995). It has been hypothesized that a correlation exists between chlorophyll production and increased mating in *Chlamydomonas* (Lewin 1956). Although blue light increases mating competence in *Chlamydomonas,* the ability to mate is quickly lost when cells are subjected to darkness (Lewin 1956; Kooijman *et al.* 1988). Also, snow algae may accumulate just below the surface of snowpacks where damage from UV light is reduced (Bidigare *et al.* 1993; Thomas and Duval 1995). Since blue light penetrates readily through snowpacks, *Chloromonas* sp.-D may experience optimal mating under light conditions that would exist about 10 cm below the surface of the snowpack where it lives and reproduces in early April in the Tughill Plateau, NY (Hoham *et al.* 1998).

In addition to blue light, field studies provide evidence that irradiance levels also affect gametogenesis. Studies on *Chlamydomonas moewusii* indicate a dependence on a particular light intensity for sexual activation (Lewin 1956). Similar results were found in *Chlamydomonas reinhardtii,* where a critical irradiance of 0.098 μmol photons m^{-2} s^{-1} was needed for at least 90

minutes (Beck and Acker 1992). Previous studies on *Chloromonas* sp.-D suggest that optimal mating occurs at an irradiance level of 95 μmol photons m^{-2} s^{-1} (Hoham *et al.* 1998). As with blue light, this irradiance level occurs in the top 10 cm of snowpacks where this species is found when it reproduces (Hoham *et al.* 1998). Although an optimal irradiance level and wavelength for mating in *Chloromonas* sp.-D is known from laboratory experimentation, an optimal photoperiod was not determined for this species. Studies of related species have investigated the effects of varying photoperiods on the cell cycle. Hoshaw (1961) found that *Chlamydomonas* experienced maximum growth under a 16:8 hours (hrs), light (L):dark (D) regime. An illumination period greater than 12 hours per day is necessary for maximum mating for the non-flagellated green alga, *Closterium acerosum* (Ueno and Sasaki 1978).

Chloromonas sp.-D has been observed only in the Tughill Plateau, NY (43.8°N, 75.5°W; Figure 1) (Hoham *et al.* 1993). Mating in this species occurs in early April when the photoperiod is approximately 14:10 hrs, L:D (14.5:9.5 including twilight); however, mating may occur in late March, when the photoperiod approaches 13:11. Other species of *Chloromonas* are found at higher altitudes and latitudes when the photoperiod is extended during late spring and early summer at the time of mating. *Chloromonas polyptera* is found at high altitudes in the western United States, where snowmelt occurs later in June and July and the photoperiod is approximately 18:6 when mating occurs (Hoham *et al.* 1983). *Chloromonas pichinchae* and *Chloromonas brevispina* are also found in the western United States (Mt. Rainier National Park, WA) where a photoperiod of approximately 16:8 occurs during mating (Hoham 1975; Hoham *et al.* 1979). In eastern North America, the northern most location where species of *Chloromonas* have been located is Lake Bienville, Quebec (55°N, 73°W; Figure 1). The photoperiod is approximately 21:3 including twilight in late June when these species undergo life cycle development (Begin 2000,

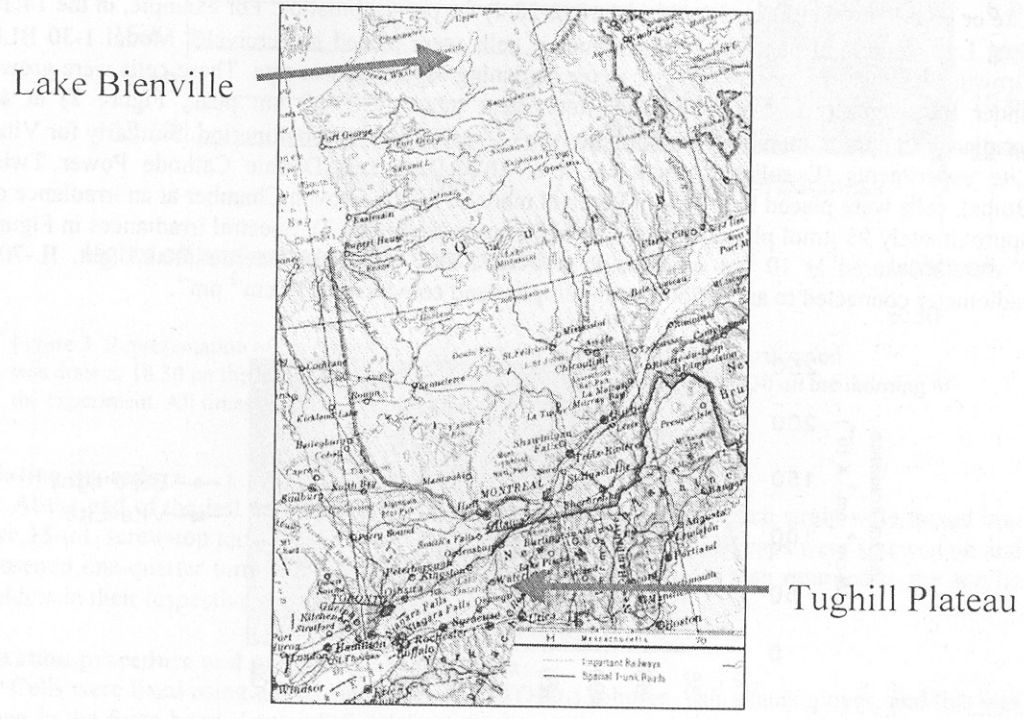

Figure 1. Sites of *Chloromonas* sp.-D, Tughill Plateau, NY, and *Chloromonas* spp., Lake Bienville, QU.

pers. comm). Cryophylic (cold loving) species of *Chloromonas* and *Chlamydomonas* were identified from northwest Svalbard (78-79°N; 10-11°E), where the photoperiod is 24:0 at the time of sexual reproduction (Müller *et al.* 1998).

In field observations of *Chloromonas nivalis*, the minimal amount of time needed between zygospore germination and sexual reproduction in populations derived from this germination was seven days (Hoham and Mullet 1977; 1978), which exposed the biflagellate daughter cells to

seven days of a particular photoperiod prior to mating. For *Chloromonas brevispina* in the Laurentian Mountains, Quebec, a similar time of 7-10 days was observed for sexual populations to appear in snow after zygospore germination (Hoham and Germaine 1988, pers. comm.). The study presented here examined acclimation of *Chloromonas* sp.-D to blue light and to Vita-Lite for seven days prior to mating experiments in the laboratory. It was hypothesized that optimal mating and production of spheres (many of which are involved in mating) in *Chloromonas* sp.-D would occur under blue light at 95 μmol photons m^{-2} s^{-1} under a shorter photoperiod such as 14:10 hrs, L:D, which is similar to the photoperiod found in the Tughill Plateau, NY, at the time of sexual reproduction in this species.

MATERIALS AND METHODS

Culture collection sites and preparations for mating

Chloromonas sp.-D strains 582C and 582D, which are cross-mating strains, were collected from Whetstone Gulf State Park, Tughill Plateau, NY, in April 1988. Prior to experiments, strains were maintained on M-1 plus nitrogen agar medium (Hoham *et al.* 1979) for 3 weeks and subsequently transferred to M-1 minus nitrogen agar medium for 3 weeks. All transfers were done in a sterile Laminar flow chamber, and plated cultures were placed in a walk-in Percival model CTR-66 Growth Chambers at 4°C. Plant and Aquarium Wide Spectrum bulbs (F40PL/AQ) under a 16:8 (hrs, L:D) photoperiod were used during the first five weeks of this six-week time period.

Pre-experiment pre-acclimation to light

For the final seven days of the minus nitrogen growth period, cells were exposed to the Vita-Lite or blue light photoperiod (either 14:10 or 20:4) for pre-acclimation. For example, in the 14:10 (hrs, L:D) blue-light photoperiod experiments, cells were placed in Percival® Model 1-30 BLL Growth Chambers for one week prior to the beginning of the experiments. These cells were grown under blue light (GE F20T12.B-Blue fluorescent tubes; 430-460 nm peak, Figure 2) at an irradiance of approximately 95 μmol photons m^{-2} s^{-1} using a 14:10 photoperiod. Similarly for Vita-Lite experiments (Durolite International, Inc. 40W Duro-Test Double Cathode Power Twist Bulbs), cells were placed in a walk-in Percival model CTR-66 Growth Chamber at an irradiance of approximately 95 μmol photons m^{-2} s^{-1} (530-700 nm peak; Figure 2). Spectral irradiances in Figure 2 were measured at 10 nm intervals in μW cm^{-2} nm^{-1} using the International Light, IL-700 radiometer connected to an IL-760 photomultiplier and converted to W cm^{-2} nm^{-1}.

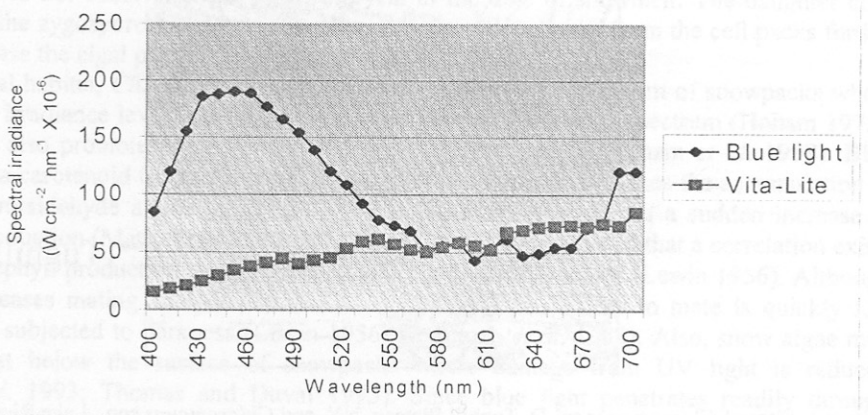

Figure 2. Transmission peaks for blue light and Vita-Lite bulbs used in experiments.

Equalization procedure and light:dark regimes

Sterile demineralized water, glass and plastic pipettes, Petri-plates, test tubes, graduated cylinders and 125-mL Erlenmeyer flasks were pre-chilled to 4°C in a growth chamber. For the

first 10 hours of the experiment, the agar plates were placed under Vita-Lite or blue light. Using sterile technique at 4°C, the cells were washed from agar plates with demineralized water using plastic disposable pipettes, and aliquots were placed into Erlenmeyer flasks. Cell suspensions were transferred into graduated cylinders, diluted to 70 mL with sterile demineralized water and this was the minimal amount of solution needed to perform three trials for each experiment. Cell concentrations were enumerated using hemocytometers and equalized to 1.5 x 10^6 cells mL^{-1} for each strain. No fewer than 23.3 mL of the cell suspensions were poured into three test tubes, one for each trial, with a loose cap for gas exchange. A test tube of each strain was placed on a white acrylic holder that angled the tubes approximately 20° from the horizontal (Hoham *et al.* 1998). Holders were placed under Vita-Lite at 4 °C using triplicate trials, and photoperiods tested for pre-acclimation were 14:10 and 20:4 (hrs, L:D). For the blue light regime, tubes were placed in three chambers at 4°C, and photoperiods tested for pre-acclimation were 12:12, 14:10, 16:8, 18:6, 20:4, 22:2 and 24:0. Figure 3 is a representation of the light:dark cycles used in the experiments showing at what times the lights would be turned on or off for the photoperiod being tested.

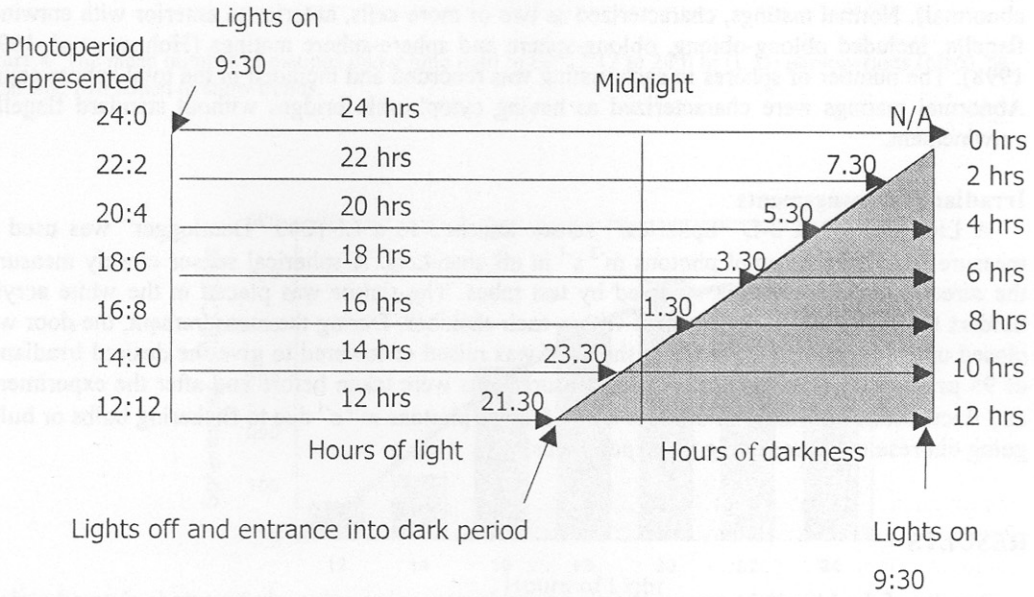

Figure 3. Representation of the light:dark cycle used for each experiment. Equalization was done at 18.30 on the last night of pre-acclimation. Mating was done at 9.30 on the morning of the experiment. All times are on a 24 hour clock.

Mating procedure

At the end of the last dark cycle on day seven (Figure 3), 4 mL of each strain were mixed into five 15-mL screw-top test tubes to begin mating. After mixing, test tube caps were screwed on and loosened one-quarter turn to allow for gas exchange. The tubes were then returned to the acrylic holders in their respective growth chambers.

Fixation procedure and precautionary measures

Cells were fixed using a 4% osmium tetroxide (OsO_4) solution, using latex gloves, and this was done in the fume hood. For each fixation, one tube was removed from each growth chamber, set on ice in a sealed container and transported to the hood. A 3-mL aliquot was removed from each tube and transferred into a Petri-plate. Whatman® 90 mm filter paper (qualitative circle) was applied to the underside of the Petri-plate lid using Time Tape. Approximately 5-6 drops of OsO_4 were pipetted evenly over the filter paper in the fume hood. The lid with OsO_4-containing filter paper was then placed over the bottom plate to begin fixation. These Petri-plates were left on ice in a tightly sealed container, which was tilted 5° in the growth chamber for a 30-minute fixation period. The slant enabled the algal aliquot to pool together and the OsO_4 fumes to penetrate the cells and liquid evenly. The sealed container was checked for leaks by looking for blackened

oxidation areas on the cover outside of the seal. Once fixed, the contents on the Petri plates were transferred to 15 mL screw-top test tubes in the fume hood. This procedure was performed at 0.5, 2, 4, 6 and 8 hours after mating began.

Cell counting procedures and statistical analysis

Through time, fixed cells settle to the bottom of the test tubes. To counter this effect, cells were resuspended by rolling the test tubes on a counter top to restore homogeneity and ensure equally distributed cell types. Cells were observed and enumerated through Zeiss® phase-contrast microscopes using the line transect counting technique. Counts were made by three individuals during each collection period to avoid statistical bias. A total of 400 counts is the standard number accepted for a 10% measurement error (Guillard 1975). Once cells were tabulated, statistics were performed using the JMP statistical package. Standard Least Square tests were performed, standard errors (S.E.) were calculated for the means and $P<0.05$ was considered significant.

Cells were categorized and tabulated using the following descriptions: oblong flagellates and non-flagellates, spherical flagellates and non-flagellates, cell packs with 2, 4 or 8 oblong or spherical daughter cells, quadriflagellate zygotes, v-configurations and matings (normal and abnormal). Normal matings, characterized as two or more cells, anterior to anterior with entwined flagella, included oblong-oblong, oblong-sphere and sphere-sphere matings (Hoham *et al.* 1997; 1998). The number of spheres in each mating was recorded and included in the total sphere count. Abnormal matings were characterized as having cytoplasmic bridges without standard flagellar entwinement.

Irradiance measurements

A Licor LI-1935a 3-D "Spherical" sensor attached to a LI-1000 "Datalogger" was used to measure irradiance in µmol photons m^{-2} s^{-1} in all chambers. A spherical sensor closely measures the direct and reflected light received by test tubes. The sensor was placed in the white acrylic holders to obtain the irradiance level within each chamber. During the measurement, the door was closed until readings stabilized, and the shelf was raised or lowered to give the desired irradiance of 95 µmol photons m^{-2} s^{-1}. Irradiance measurements were taken before and after the experiments and fluctuations in irradiance of more than 15 µmol photons m^{-2} s^{-1} due to flickering bulbs or bulbs going out resulted in repeating the experiments.

RESULTS

Results of the blue light pre-acclimation experiments using seven photoperiods showed a clear trend (Figures 4 and 5). The number of matings increased with increasing photoperiod (Figure 4), while the number of spheres increased up to the 20:4 photoperiod where the number leveled off (Figure 5). These results indicate that longer photoperiods may be more optimal than shorter photoperiods for total matings and spheres for *Chloromonas* sp.-D strains 582C and 582D.

For the effects of blue light on the number of matings and spheres, pre-acclimation under blue light was compared to pre-acclimation controls under Vita-Lite using a reduced photoperiod, 14:10, and an extended photoperiod, 20:4. Matings (Figure 6; Table 1) and spheres (Figure 7; Table 2) were significantly greater when the cells were acclimated and mated under blue light as opposed to Vita-Lite for both 14:10 and 20:4 photoperiods.

The effects of an extended photoperiod (20:4) for both blue light and Vita-Lite pre-acclimations were compared separately. For both treatments, the extended photoperiod resulted in significantly greater matings (Figure 8; Table 1) and production of spheres (Figure 9; Table 2). Under blue light, the production of spheres leveled off at the longer photoperiods starting at 20:4 (Figure 5), and there were no significant differences in total matings and production of spheres between the 20:4, 22:2 and 24:0 photoperiods ($P>0.05$; Table 3). However, total matings and production of spherical cells were significantly greater in all three of these longer photoperiods when compared to the 14:10 photoperiod ($P<0.05$; Table 3).

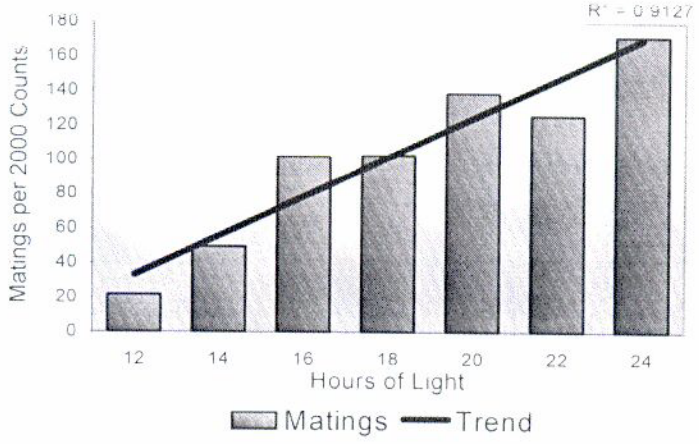

Figure 4. The mean number of matings under blue light in the 12:12 to 24:0 hr (L:D) photoperiods (N = 6). A linear line was added to show trends.

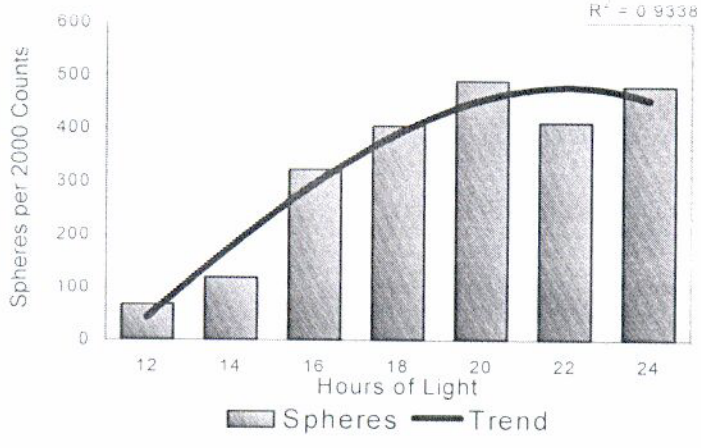

Figure 5. The mean number of spheres under blue light in the 12:12 to 24:0 hr (L:D) photoperiods (N = 6). A polynomial line was added to show trends.

Table 1. P-value comparisons between photoperiods and light regimes for total matings.

Matings	Blue Light (14:10)	Vita-Lite (20:4)
Blue Light (20:4)	<0.0001	<0.0001
Vita-Lite (14:10)	<0.0001	0.0319

Table 2. P-value comparisons between photoperiods and light regimes for total spheres.

Spheres	Blue Light (14:10)	Vita-Lite (20:4)
Blue Light (20:4)	<0.0001	<0.0001
Vita-Lite (14:10)	0.0007	<0.0001

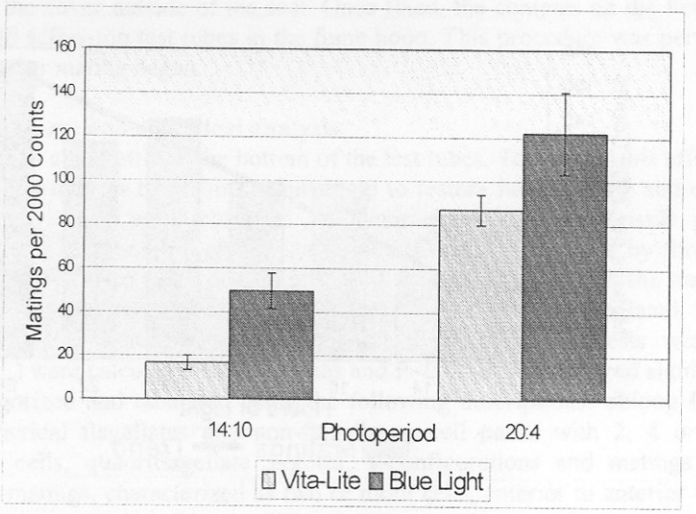

Figure 6. Comparisons of the mean number of matings in the 14:10 and 20:4 hr (L:D) photoperiods between blue and Vita-Lite (N=6).

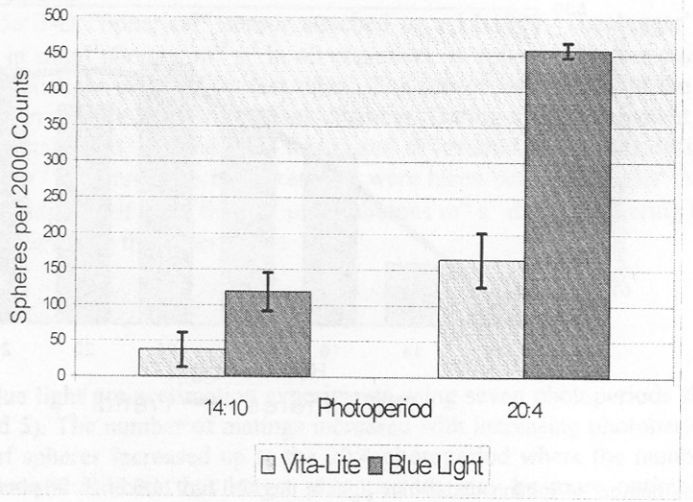

Figure 7. Comparisons of the mean number of spheres in the 14:10 and 20:4 hr (L:D) photoperiods between blue and Vita-Lite (N=6).

Table 3: Comparisons of mean matings and spheres under blue light pre-acclimation (N=6). All means are ± 1 S.E.

Photoperiod	Matings	Spheres
14:10	50 ± 4.2	119 ± 16.3
20:4	139 ± 2.6	491 ± 24.6
22:2	126 ± 5.6	411 ± 13.2
24:0	171 ± 9.3	480 ± 18.7

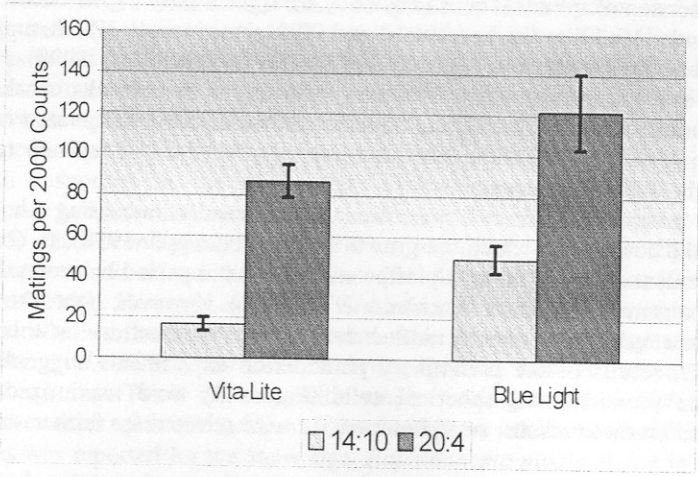

Figure 8. Differences in observed matings between photoperiods for blue light and Vita-Lite (N=6).

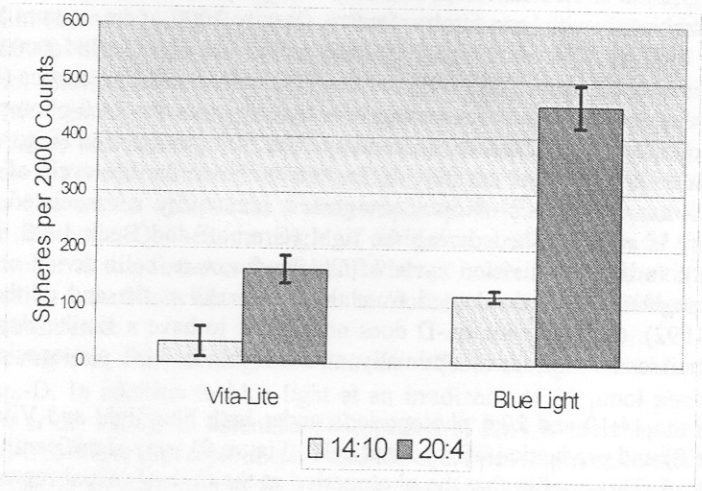

Figure 9. Differences in observed spheres between photoperiods for blue light and Vita-Lite (N=6).

DISCUSSION

Spectral composition and irradiance levels used in the laboratory experiments presented here are similar to those found in natural snowpacks where *Chloromonas* sp.-D lives. Blue light at an irradiance of 95 μmol photons m^{-2} s^{-1} occurs in the upper 10 cm of snowpacks in the Tughill Plateau, NY (Hoham *et al.* 1998). This implies that this species is mating optimally with respect to spectrum and photon irradiance there. Blue light at a irradiance of 95 μmol photons m^{-2} s^{-1} has been found in the top 10 cm of snowpacks at other sites (Hoham *et al.* 1983), and is likely common worldwide in snowpacks. Studies have indicated that algal cells may accumulate just below the snow surface in response to damaging UV levels (Thomas and Duval 1995). Models have suggested that 85% of incident UV would be absorbed in the upper 10 cm of wet snow (Perovich 1993). Green cells may be more susceptible to UV damage than red cells because they lack the special carotenoids that behave as UV-absorbing pigments (Thomas and Duval 1995). It is possible that the adaptation of this green alga to blue light at an irradiance level that occurs deeper in the snow pack for optimal mating may be a response to protect their photosynthetic pigments from UV radiation.

The results presented in this study for *Chloromonas* sp.-D show that sexual reproduction (Figure 6) and production of spherical cells (Figure 7) are significantly higher under blue light pre-acclimation than under Vita-Lite for both 14:10 and 20:4 photoperiods, which supports previous data that mating is optimal under blue light in this species (Hoham *et al.* 1998). However, their study only looked at a 16:8 photoperiod. This study shows that their results can be expanded to include all photoperiods from 12:12 to 24:0. With the optimal spectra and photon irradiance level determined, it is important to determine how photoperiod affects sexual reproduction in the alga with these other light factors held constant.

Growth in the green alga, *Scenedesmus,* was optimal under increasing photoperiod until reaching about 13:11 hours, after which the growth leveled (Nicklisch 1998). In *Chlamydomonas eugametos,* environmental factors indirectly affected the gametic period by increasing the growth rate of cells prior to gamete formation (Zachleder *et al.* 1991). However, light intensity, nitrogen, CO_2 and temperature did not directly influence sexual reproduction in this species. For *Chloromonas* sp.-D, results of the preliminary photoperiod experiments suggested that mating (Figure 4) and the production of spherical cells (Figure 5) were maximized under longer photoperiods. Based on these results, two photoperiods were selected for further study, 14:10 and 20:4.

The 14:10 photoperiod was selected because it most closely represents the photoperiod at the time of mating in late April in the Tughill Plateau. The 20:4 photoperiod was selected to represent a longer photoperiod because it occurs in late June – early July at Lake Bienville, Québec, which is the northernmost point in eastern North America where species of *Chloromonas* have been found in snow associated with coniferous forests (Begin 2000, pers. comm.). Species of *Chloromonas* have been discovered at higher latitudes where the photoperiod can approach 24:0 such as Svalbard, Norway (Müller *et al.* 1998) and the Windmill Islands, Antarctica (Ling 1996).

Experiments in this study showed that mating was highest under the 24:0 photoperiod (Figure 4) and production of spheres remained the same above the 20:4 photoperiod (Figure 5; Table 3). This suggests that a dark cycle does not play an important role in the life cycle of *Chloromonas* sp.-D. In *Chlamydomonas* and *Chloromonas*, pre-gamete immaturity is associated with the dark and gamete maturity is accomplished during the light (Gromoff and Beck 1993; Hoham 1975; Hoham *et al.* 1998). In the cell division cycle of *Chlamydomonas*, cells divide only during the dark period and daughter cells are released from the cell packs at the end of the dark period (Molendijk *et al.* 1992). *Chloromonas* sp.-D does not appear to have a similar dependence on a dark cycle, and so it could reproduce optimally at sites with a 24:0 photoperiod such as in Svalbard.

Comparisons of the 14:10 and 20:4 photoperiods under both blue light and Vita-Lite showed that mating (Figure 8) and production of spherical cells (Figure 9) were significantly higher under 20:4 than under 14:10. This implies that the photoperiod at the time of sexual reproduction in the Tughill Plateau is not optimal for mating in this species. If *Chloromonas* sp.-D were living in northern Québec near Lake Bienville, the photoperiod there in late June (21:3) may be more optimal for sexual reproduction in this species. Therefore, some other site with blue light at an irradiance level of 95 μmol photons m^{-2} s^{-1} and a longer photoperiod may be more optimal for this species.

Studies involving other algae have shown that growth and mating are positively influenced by a longer photoperiod. In the marine red alga, *Phycodrys rubens,* growth was maximized under longer photoperiods (16:8 vs. 12:12 hrs, L:D) when grown under temperatures of 15-18°C (Voskoboinikov *et al.* 1996). It was found that there was no strong selectional pressure to optimize growth rates in this species with respect to temperature and photoperiod, probably due to gene flow between latitudinal populations. Therefore, not all populations of this species were living optimally with respect to their environment. For the Mediterranean marine red alga, *Eupogodon planus,* the longer photoperiod of 14:10 increased sexual reproduction when compared to the shorter 10:14 photoperiod (Orfanidis *et al.* 1999). In the red algae, *Gelidium, Gelidiopsis* and *Pterocladia*, peak spore shedding was observed under long day conditions (16:8 hrs, L:D) (Ganesan *et al.* 1999). For six phytoplankton species, *Limnothrix redekei, Planktothrix agardhii, Scenedesmus acuminatus, Scenedesmus armatus, Stephanodiscus minutulus* and *Synedra acus,* it was found that a shorter photoperiod (6:18 vs. 12:12) caused a significant decrease in specific growth rates in all the algae (Nicklisch and Woitke 1999).

One should be cautious when applying laboratory results to field sites without considering other environmental factors. For example, *Chloromonas* sp.-D is known to inhabit snow that is associated with trees (Hoham *et al.* 1998). In a study of cryophilic algae of the American Southwest, it was found that in shaded areas, *Chloromonas nivalis* often replaced *Chlamydomonas nivalis*, which was more common in open exposures (Hoham and Blinn 1979). Since Svalbard and the antarctic have no tree cover, these sites may not be optimal for *Chloromonas* species such as *Chloromonas* sp.-D. Coniferous litter experiments indicated that leachates from conifers positively affected growth responses in the snow alga, *Chloromonas pichinchae* (Hoham 1976). If *Chloromonas* sp.-D had a similar response to coniferous litter, than these polar locations may not be optimal. Also, it has been hypothesized that trees may reduce light intensity allowing for more optimal irradiance levels to exist at the top of snowpacks located under trees (Bidigare *et al.* 1993; Hoham and Duval 2000). However, species of *Chloromonas* have been found in Svalbard where it was not reported whether these sites were shaded either by low-lying vegetation, rocks or other landforms (Müller *et al.* 1998). Near Lake Bienville, Québec, there is tree cover, which consists mainly of black spruces. These could provide ample shading to allow *Chloromonas* sp.-D to sexually reproduce under optimal conditions if leachates from conifers do not inhibit the growth of this snow alga as was reported for the snow alga, *Raphidonema nivale,* living in Washington State snowfields (Hoham 1980; Hoham and Duval 2000).

Another possible factor in latitudinal location is the angle of incidence of incoming solar radiation. The angle of incidence will affect the depth to which different wavelengths of light will penetrate in the snowpack. In the Tughill Plateau, NY, in late April, the angle of incidence is 57.55° at 12.00 hr (Lammi 2000). However, at Svalbard, the angle would be 34.26° at noon in late June (Lammi 2000). This angle would decrease the penetration of blue light and an irradiance level of 95 µmol photons m^{-2} s^{-1} may not occur in snowpacks there. At lower latitudes such as those found at Mt. Lemmon, Arizona, where *Chloromonas nivalis* is known to exist in snowpacks in early May (Hoham and Blinn 1979), the angle of incidence is 75.07° at 12.00 hr (Lammi 2000). This would allow for deeper penetration of blue light, but the high intensity of this incoming blue light in Arizona may not be optimal for *Chloromonas* sp.-D if it were found living there. In late June near Lake Bienville, Québec, the angle of incidence at 12.00 hr is 57.41° (Lammi 2000), which is very similar to that found in the Tughill Plateau in late April at the time of mating there. Thus hypothetically, Lake Bienville represents the most optimal site for *Chloromonas* sp.-D when photoperiod is considered.

This study completes our investigation into the effects of light on sexual reproduction in *Chloromonas* sp.-D. In addition to blue light at an irradiance of 95 µmol photons m^{-2} s^{-1} being optimal (Hoham *et al.* 1998), an extended photoperiod of 20:4 is more optimal than a reduced photoperiod of 14:10 for sexual reproduction and life cycle development in this species. Possibly, the Tughill Plateau is an optimal site for *Chloromonas* sp.-D with respect to photon irradiance level and spectral emission. However, based solely on photoperiod, this species may be living in a sub-optimal environment. This information on spectral composition, irradiance level and photoperiod for *Chloromonas* sp.-D is the most comprehensive analysis to date for any alga as to how these three factors interact with sexual reproduction and life cycle development.

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