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Physiological Characteristics and Related Biochemical Parameters of Snow Algae from King George Island, Antarctica

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Abstract – Red and green snow caused by snow algal blooms is common on glaciers and snowfields worldwide. Reddish and greenish snow samples containing algae were collected at the vicinity of penguin rookeries on King George Island (62°13'S, 58°47'W, near the King Sejong Station), Antarctica in February 2017 to investigate their physiology. Eight pigments and six fatty acids were detected from the samples. No difference in pigment and fatty acid (FA) composition was found between reddish and greenish snow samples. In contrast, spectral profiling and mycosporine-like amino acids (MAAs) were different between reddish and greenish snow. Particularly in greenish snow, a high absorbance between 450–600 nm was observed. The average MAA concentration was 316.0 μg g^{-1} in greenish snow, which was higher than that of reddish snow (278.2 μg g^{-1}). The MAA to Particulate organic carbon (POC) ratio (mg (g C)$^{-1}$) for reddish snow (6.2 mg (g C)$^{-1}$) was higher than that of greenish snow (2.6 mg (g C)$^{-1}$). These results suggest that reddish and greenish snow are considered to be the same species based on pigment and FA composition. Compared with photoprotective pigments, MAAs offer snow algae a more effective photoprotection strategy to promote tolerance of natural levels of ultraviolet radiation (UVR).

Keywords – reddish snow, greenish snow, snow algae, pigments, fatty acids, mycosporine-like amino acids, King George Island, Antarctica

1. Introduction

Snow algae can thrive when snow starts to melt during the spring and summer in polar regions (Lutz et al. 2016 and references therein). They sustain not only bacterial communities but also other heterotrophic organisms such as protozoa and small animals on glaciers and snowfields (Thomas and Duval 1995; Takeuchi 2013 and references therein). In particular, the patches, usually red and green in color, but sometimes orange or gray in color by the dominant pigments, are critical players in glacial surface habitats (Ling and Seppelt 1990; Spijkerman et al. 2012; Lutz et al. 2014). Algal blooms can change snow albedo. According to Lutz et al. (2016), snow with red pigmented algae can reduce the albedo of snow by 13 percent compared to clean snow during an entire melt season in melting Arctic glaciers. The surface albedo of red snow is lower than that of green snow based on several studies (Lutz et al. 2014, 2016). This implies that induced algal blooms by snow melt may lead to reduced albedo and accelerated melting processes.

Generally, these colored snows are a ubiquitous phenomenon at alpine snow surfaces, glaciers, and persistent snowfields worldwide (Müller et al. 1998; Remias et al. 2005; Takeuchi et al. 2006). For decades, distinct habitats and the environmental characteristics of colored snows have been investigated (Müller et al. 1998; Remias et al. 2013; Lutz et al. 2015; Holzinger et al. 2016; Hodson et al. 2017). Snow algae have adapted to different environmental conditions such as pH, conductivity, low temperatures, and high levels of irradiation at the snow surface. As a consequence, a variety of biochemical compounds (pigments, fatty acids, and mycosporine-like amino acids; MAAs) of snow algae have been examined in several studies (Duval et al. 2000; Spijkerman et al. 2012; Lutz et al. 2016). For example, red snow algae have secondary pigments like the carotenoid astaxanthin, which is their protection against intensive radiation exposure (Bidigare et al. 1993; Remias et

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al. 2016). Therefore, snow algae can be considered a good indicator for monitoring climate change.

The Antarctic Peninsula (AP) region is the fastest warming area of the Southern Ocean (Rückamp et al. 2011; Pritchard et al. 2012). Surface air temperature has increased at an average rate of 0.5°C per decade in the AP (Turner et al. 2014). Reported rapid changes in air temperature, ice shelf, and marine ecosystems in the AP have led to the retreat/acceleration of some glaciers and consequent transit in the microbiome (Rott et al. 2002; Moline et al. 2004; Rückamp et al. 2011; Moon et al. 2015; Sahade et al. 2015). In particular, the western Antarctic Peninsula (WAP) is more rapidly changing than eastern Antarctica (Pritchard et al. 2012). Our study area is located on King George Island (KGI), which is the largest of the South Shetland Islands in the WAP. According to Rückamp et al. (2011), areal loss was estimated at approximately 1.6% of the ice cap of the KGI between 2000 and 2008 based on satellite data. Sahade et al. (2015) reported that a shift in benthic community could be affected by the consequences of ongoing climate change (e.g., increased sediment runoff by glacier retreat) in the KGI. A detailed knowledge of snow algal biochemical compounds is crucial for understanding their survival strategies underlying rapid climate changes, since these algae are potentially exposed to harsh conditions. Hence, we present specific spectral absorptions, pigments, fatty acids, and MAAs of red and green snow algae on KGI, Antarctica.

2. Materials and Methods

Study site and sampling

Two different colored snow samples containing snow algae were collected in the vicinity of penguin rockeries on KGI (62°13'S, 58°47'W, near the Korean research station; King Sejong Station) Antarctica on February 20th, 2017 (Fig. 1a and b). One of the snow samples was obtained from reddish colored patches, and another one was sampled from greenish colored snow (Fig. 1b). All the samples were transported to the laboratory and immediately stored at -80°C after collection until analyses.

Particulate organic carbon and stable carbon isotope

Particulate organic carbon (POC) and nitrogen (PON) and the abundance of ^{13}C ($\delta^{13}C$) of the snow samples were determined in the Finnigan Delta+XL mass spectrometer at the University of Alaska Fairbanks after overnight HCl fuming to remove carbonate on the filters.

Biochemical compound (pigments, fatty acids, and MAAs) analyses

For the analysis of pigment composition, samples were extracted in 100% acetone (3 mL), and 50 μL (1 mg/mL) apo-8-carotennoate (an internal standard) was added before extraction. The extracts were sonicated by an ultrasonicator (30 s, 50 W; Ulsso Hi-tech ULH-700s: Seoul, Korea) and then

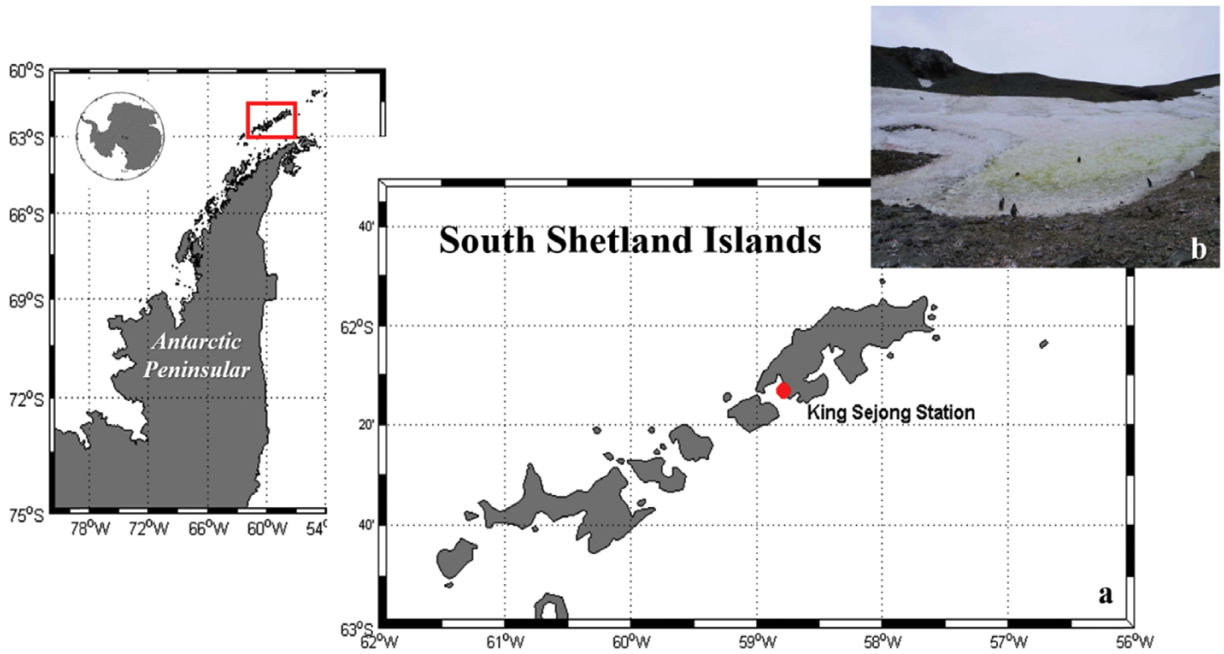

Fig. 1. Study site for colored snow sampling ion King George Island, February 20th, 2017

stored for 24 h at 4°C until analysis. To remove the debris, the extract (1 mL) was filtered by a syringe filter (PTFE 0.2 μm Hydrophobic). Quantitative and qualitative analyses of pigments were performed by high-performance liquid chromatography (HPLC). The pigments were analyzed using the method reported by Zapata et al. (2000). The pigment compounds were separated using a column (Waters symmetry C 8 column; 150 mm × 4.6 mm, 3.5 μm) with mobile phase A (methanol: 50%, acetonitrile: 25%, and aqueous pyridine solution: 25%) and B (methanol: 20%, acetonitrile: 60%, and acetone: 20%). The pigments were identified and confirmed by comparison with the retention times of standards (DHI Water and Environment, Hørsholm, Denmark). Pigment concentrations were calculated from the peak areas in the chromatogram using an equation according to Park (2006).

Lipids were extracted with dichloromethane:methanol (2:1 v/v) after the addition of a C_{21} saturated fatty acid (21:0) as an internal standard according to the method described in Hama and Handa (1987). After removal of the debris, the extracted lipids in the dichloromethane phase were separated from the water-methanol phase, saponified using 0.5 M methanolic potassium hydroxide, and then methylated with boron trifluoride methanol (BF_3-MeOH) with heating at 80°C for 30 min. The concentrations of the fatty acid methyl esters (FAMEs) were determined by gas chromatography and a flame ionization detector (GC-FID; HP 6890 GC system; Agilent) with a fused-silica capillary column (INNOWAX, 30 m length, 0.25 mm internal diameter; Agilent) using helium as the carrier gas. The injector and detector temperatures were set at 300°C, and the oven temperature was programmed from an initial value of 40°C (1 min) before increasing to 200°C at a rate of 10°C min^{-1}, and then to 250°C at a rate of 2°C min^{-1}. Ultimately, the oven temperature was increased to 300°C at a rate of 10°C min^{-1} and subsequently held at 300°C for 5 min. The fatty acids were identified by comparison of the retention times of standards (37 component FAME mixture; Supelco, USA) and from mass spectra acquired using a combined gas chromatograph mass spectrometer (GC-MS QP2010; Shimadzu, Japan). The GC-MS was equipped with a fused-silica capillary column (VB-5, 30 m length, 0.25 mm internal diameter; Valco Bond, USA), and the temperature setting was identical to that used for the GC-FID. An ion source temperature of 200°C, a repeat scanning speed of 0.5 s, and a mass to charge ratio (m/z) range of 50–450 were used for the analysis of the chemical ionization spectra. The reagent gases used in this analysis were isobutane.

MAAs were extracted and calculated according to Sinha et al. (2003) and Ha et al. (2014b). To analyze the MAA contents, algae samples were placed in 3 mL of 100% methanol (v/v) and sonicated with an ultrasonicator (30 s, 50 W; Ulsso Hi-Tech: Seoul, Korea). After resting overnight at 4°C, the extract was filtered through 0.2 μm pore size syringe filters (PTFE Hydrophobic). A rotary evaporator (CVE-200D; EYELA) was used to remove the extraction solvent. Then, 100 μL of chloroform was added into the mixture (dried sample + 500 μL of distilled water) to remove lipids and pigments, followed by centrifugation at 10,000 rpm for 10 min. An aliquot (400 μL) of the supernatant was injected into the HPLC system (Agilent Technologies, 1200 series, Wilmington, DE, USA) to quantitatively analyze the MAA contents. The chromatographic conditions were similar to those described by Sinha et al. (2003) and Ha et al. (2014b), and compounds were identified by co-chromatography with standards.

Spectral profiling of snow algae

Samples for the absorption analysis of algae were extracted in 100% methanol. The spectral absorption of snow algae from 200 to 900 nm was determined by spectrophotometer (Agilent Cary 8454 UV-Visible Spectrophotometer).

3. Results and Discussion

Particulate organic carbon and nitrogen, and $\delta^{13}C$ of reddish and greenish snow

Stable isotope analysis of organic materials has been employed as a useful tool for investigating food web structure (Peterson and Fry 1987; Wada et al. 1987; Layman et al. 2012). In particular, the carbon isotopic composition ($\delta^{13}C$) of organisms has been widely used in elucidating the origin and food sources of organisms in a food web. The average $\delta^{13}C$ values from our reddish and greenish snow samples were -27.0‰ (S.D. = ± 0.2‰) and -27.9‰ (S.D. = ± 0.2‰), respectively. The $\delta^{13}C$ value for red snow (-29.7‰) observed by Lutz et al. (2015) in Feiringbreen, Svalbards was relatively lower than our data, while our $\delta^{13}C$ values are consistent with their value for green snow (-27.7‰). In comparison, Lutz et al. (2016) reported an averaged $\delta^{13}C$ value of bulk organic matter (-26.4 ± 0.2‰) in red snow from northern Sweden and Svalbard, which is slightly higher than our data. Collected bulk snow algae samples from several sites within the Cascade Volcano Arc in western North America had $\delta^{13}C$ values ranging from -24.1‰ to -26.9‰ (Hamilton and Havig 2017).

Moreover, Bidigare et al. (1993) reported that red and green snow algae were -26.8‰ (S.D. = ± 0.2‰), with no large difference in $\delta^{13}C$. Although we found a difference in $\delta^{13}C$ between reddish and greenish snow samples in this study ($p < 0.05$, t-test), our values are within the range previously reported in snow algae samples.

At the site, average concentrations of total POC from reddish and greenish snow samples were 0.051 mg mg^{-1} (S.D. = ± 0.032 mg mg^{-1}) and 0.088 mg mg^{-1} (S.D. = ± 0.053 mg mg^{-1}), respectively (Fig. 2a). The PON content was lower in reddish snow (0.008 ± 0.005 mg mg^{-1}) than greenish snow (0.015 ± 0.009 mg mg^{-1}) (Fig. 2b). The carbon-to-nitrogen molar ratios (C/N) were 8.0 (± 0.9) and 7.0 (± 0.4) for reddish and greenish snow, respectively. These values are slightly higher than the Redfield ratio (6.6; Redfield 1958) and those reported by Lutz et al. (2015) for green snow samples (5.3) collected from Feiringbreen in Svalbard in the Arctic, but not for red snow (17.7). High C/N ratio values (10–39; mean ± S.D. = 19.0 ± 5.6) in red snow were reported by Lutz et al. (2016), who collected samples from 16 glaciers and snow fields across the Arctic. Therefore, the investigated biochemical parameters ($\delta^{13}C$ and C/N ratios) in reddish and greenish snow suggest that they should be considered as originating from algae and not under nitrogen-shortage conditions.

Algal C/N ratios are generally an indicator of nutrient-deficient conditions and increase under nitrogen limitation (Goldman et al. 1979; Steinhart et al. 2002). In this study, nutrients appear to be not limited for the algal growth based on the C/N ratios and geographical features in our sampling sites, since we collected all the samples at the vicinity of penguin rockeries. According to previous studies (Müller et al. 1998; Lutz et al. 2015), guano is rich in nitrogen, phosphate and potassium and provides essential nutrients for algal growth. In addition, the mean values of $\delta^{15}N$ for reddish and greenish snow were 4.1‰ (± 3.2‰) and 3.6‰ (± 1.5‰) in this study, respectively (not shown), which are higher than that of red snow (mean ± S.D. = -4.5 ± 2.1‰) from the Arctic glaciers and snow fields reported by Lutz et al. (2016).

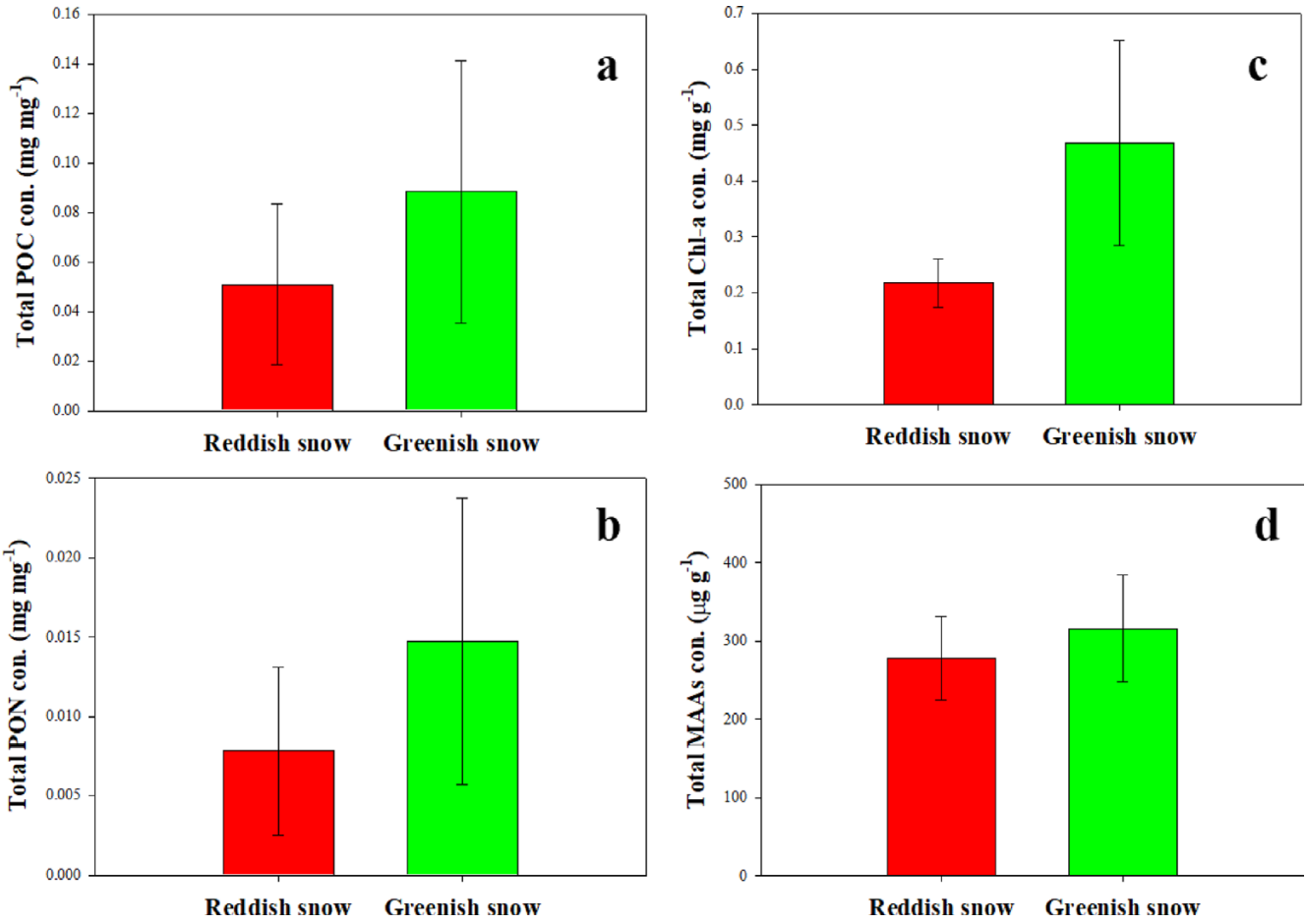

Fig. 2. Biochemical compounds concentrations at different snow covers (a: total POC, b: total PON, c: total Chl-a, and d: total MAAs)

Pigments of reddish and greenish snow

Generally, pigment compositions of algae can be useful biomarkers for phytoplankton biomass and species (Barlow et al. 1993; Jeffrey et al. 1997; Yacobi and Ostrovsky 2012). In the present study, eight pigments were identified from reddish and greenish snow samples (Fig. 3). Chlorophyll a (Chl-a) concentrations ranged from 186.8 to 248.6 μg g^{-1} (with a mean of 166.8 μg g^{-1}) and 337.8 to 597.5 μg g^{-1} (with a mean of 467.7 μg g^{-1}) for reddish and greenish snow, respectively, on a dry weight basis (Fig. 2c). The concentrations of chlorophyll b (Chl-b) ranged from 102.0 to 231.6 μg g^{-1} (with a mean of 166.8 μg g^{-1}) and 182.7 to 322.8 μg g^{-1} (with a mean of 467.7 μg g^{-1}) for reddish and greenish snow, respectively. Lutein (Lut), diadinoxanthin (Diadino), and β-carotene (β-car) were 77.6 and 194.8 μg g^{-1}, 8.0 and 30.5 μg g^{-1}, and 11.9 and 28.8 μg g^{-1} for reddish and greenish snow, respectively. Other pigments contributed only a minor proportion of total pigments, with average concentrations of less than 50.0 μg g^{-1}. On average, 43.9% of all pigments were made up of Chl-a, followed by Chl-b (33.6%), whereas violaxanthin (Viola) were the least abundant pigments (0.8%) in the reddish snow (Fig. 3). In comparison, the most abundant pigment in the greenish snow was Chl-a (46.1%), followed by Chl-b (24.9%), Lut (19.2%), β-car (2.8%), Diadino (3.0%), Viola (2.0%), dinoxanthin (Dino; 1.1%), and neoxanthin (Neo; 0.9%) (Fig. 3). Diadino and Dino represent diatoms, haptophytes, pelagophytes, dictyochophytes, and some dinoflagellates, based on Jeffrey et al. (1997). Likewise, Lut and Neo are chlorophyte and prasinophyte signature pigments. Carotenes are dominant in chlorophytes and prasinophytes (Jeffrey et al. 1997). In particular, Chl-b and Lut are used as proxies for chlorophytes and Chl-a and β-car represent phytoplankton biomass (Yacobi and Ostrovsky 2012). Our results show that the snow algae community structure for reddish and greenish snow was dominated by chlorophytes based on pigment analysis.

The red color is due to the carotenoid pigments found in algal cells. In general, the accumulation and production of secondary carotenoids protect cells under unfavorable environmental conditions (Bidigare et al. 1993; Lemoine and Schoeffs 2010; Lutz et al. 2016). According to Lutz et al. (2016), red snow contains a high content of secondary carotenoids (~70–90%), which are synthesized by snow algae as a protective mechanism against the high levels of irradiation. Similarly, Remias et al. (2005) found that a relatively high concentration (approximately 20 times) of carotenoid astaxanthin compared to the Chl-a was observed in *Chlamydomona nivalis* cells from the Austrian Alps (Rettenbach glacier and Ötztal, Obergurgl). According to Holzinger et al. (2016), a high absorbance between 400–550 nm was observed in snow algae (*Chlamydomonas nivalis* and *Chlainomonas* sp.) due to naturally occurring secondary carotenoids. In our study, spectral profiling was different between reddish and greenish snow samples (Fig. 4). Generally, a high absorbance was found in the wave band between 400 and 600 nm, and an additional peak was approximately 680 nm in reddish and greenish snow samples. However, a higher absorbance between 450–550 nm was observed in greenish snow than reddish snow (Fig. 4). Unlike previous research results, β-carotene related carotenoids was higher in greenish snow (28.8 μg g^{-1}) compared to reddish snow (11.9 μg g^{-1}), and astaxanthin was absent in reddish snow in our study. Based on the ratios of each pigment to Chl-a (w/w) in the different colored snow

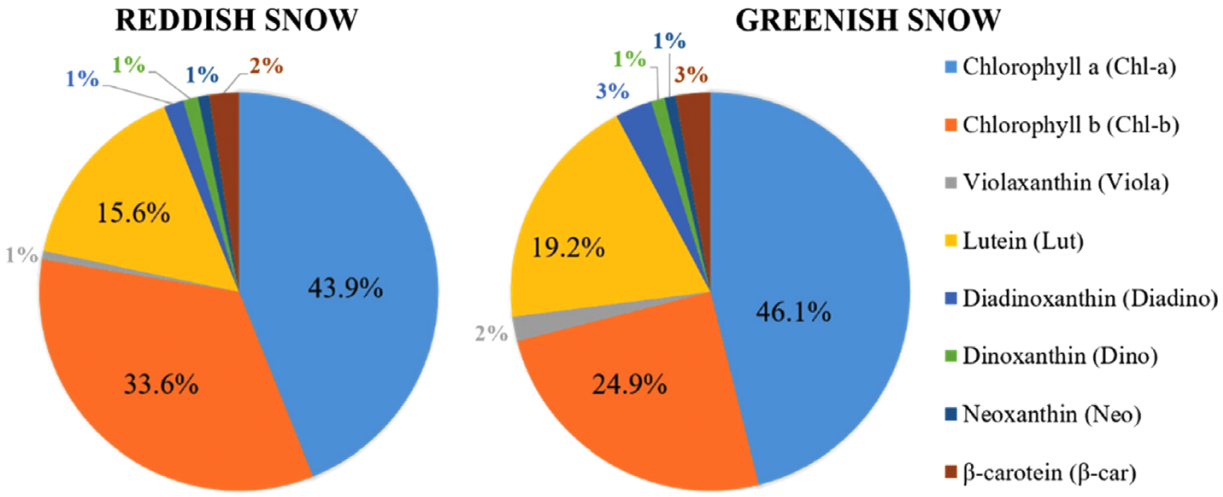

Fig. 3. Pigment composition of reddish and greenish snow

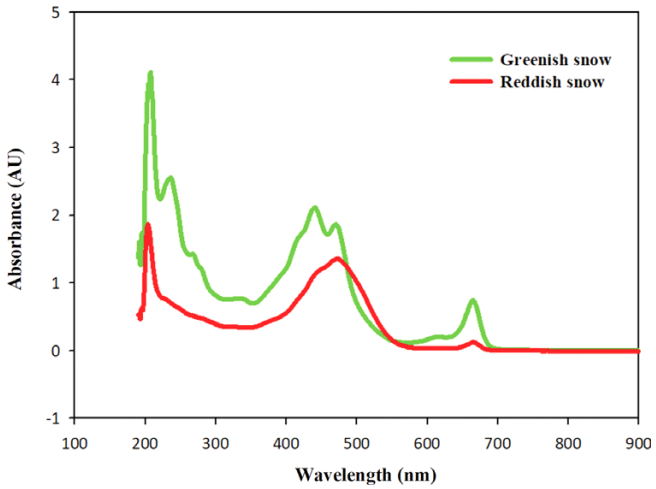

Fig. 4. Absorption spectra of reddish and greenish snow with extract in 100% methanol

Table 1. The ratio of each pigment to Chl-a for the reddish and greenish snow samples. Pigment abbreviations are defined in Fig. 3

Pigment to Chl-a ratio	Reddish snow	Greenish snow
Chl-b/Chl-a	0.77	0.54
Viola/Chl-a	0.02	0.04
Lut/Chl-a	0.36	0.42
Diadino/Chl-a	0.04	0.07
Dino/Chl-a	0.03	0.02
Neo/Chl-a	0.02	0.02
β-car/Chl-a	0.05	0.06

samples, reddish snow had lower values in all pigments than greenish snow except for the ratios of Dino/Chl-a and Chl-b/Chl-a (Table 1). Most likely, the higher absorbance between 450–550 nm in greenish snow appears to be induced by Chl-b rather than carotenoids because the Chl-b content of greenish snow was approximately 3 times higher than that of reddish snow. Although our results are not consistent with previous studies, pigments dominated by chlorophylls (Chl-a and Chl-b) and xanthophyll (Viola, Lut, and Neo) in reddish and greenish snow samples suggest relatively low light stress conditions of snow algae. Xanthophyll pigments play an important role in the photosynthetic light-harvesting complexes of algae, which dissipate excess light energy (Demmig-Adams and Adams 1996), and Viola is shifted to antheraxanthin and zeaxanthin during light stress (Goss and Jakob 2010). However, both antheraxanthin and zeaxanthin were not found in all the samples in the present study.

Fatty acid compositions in reddish and greenish snow samples

Similar to the pigment apparatus, a lipid metabolism is also influenced by environmental factors such as extremely high light intensities and nitrogen deficiency (Leya et al. 2009; Spijkerman et al. 2012). In particular, the fatty acid (FA) composition of algae is a potential biomarker of nutritional quality for consumers, and algal taxonomic composition is an important determinant for material transfers and energy pathways in food webs (Sahu et al. 2013). In addition, synthesized polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA; C20:5ω3) and docosahexaenoic acid (DHA; C22:6ω3), by microalgae are normally used in nutraceutical and pharmaceutical applications (Pereira et al. 2004; Christian et al. 2009). The FA compositions for reddish and greenish snow samples are presented in Table 2. Total FA concentrations of reddish and greenish snow samples on a dry weight basis were 430.9 μg g^{-1} and 889.2 μg g^{-1}, respectively. Likewise, the total FA concentration on a per-carbon basis (mg FA gC^{-1}) in greenish snow samples (10.1 mg FA gC^{-1}) was slightly higher than reddish snow samples (8.4 mg FA gC^{-1}). These values for the FA contents in this study are substantially lower than those from 8 different snow algal communities (50–300 mg FA gC^{-1}) reported by Spijkermann et al. (2012). In our samples, the prominent FAs were Palmitic acid (C16:0), Oleic acid (C18:1ω9), and Stearic acid (C18:0). Generally, C16:0 and C18:1ω9 are known to be major fatty acids in dinoflagellates and members of Chlorophyceae and Cyanophyceae (Ahlgren et al. 1992; Sahu et al. 2013). In this study, we found high percentages of C16:0, C18:0, and C18:1ω (> 80%) in our reddish and greenish snow samples. This is consistent with the results of Bidigare et al. (1993) who found that the composition of FAs mainly consisted of C16:0, C18:0, and C18:1ω9 in red (72%) and green (60%) cells from Hermit Island near Palmer Station, Antarctica.

Unexpectedly, no clear difference in FA composition was observed between the different colored snow samples in the present study. The FA compositions of the reddish and greenish snow samples were dominated by saturated fatty acids (SFAs; 88.6% for reddish and 83.1% for greenish snow) followed by monounsaturated fatty acids (MUFAs; 11.4% for reddish and 16.9% for greenish snow) (Table 2). PUFAs were not detected in our snow samples. In general, PUFAs play a significant role in photoprotection, maintaining membrane fluidity and preventing intracellular ice crystal formation in organisms against extreme environmental conditions (i.e., high light

Table 2. Fatty acid composition of reddish and greenish snow. Fatty acid compounds are reported as the concentration of total fatty acids. Individual identified fatty acids are reported as well as total saturated (SFA) and monounsaturated (MUFA) fatty acids

Contents of products (common name)	Reddish snow (μg/g d.w)	Reddish snow (%)	Greenish snow (μg/g d.w)	Greenish snow (%)
Myristic acid (C14:0)	18.2	4.2	46.7	5.3
Palmitic acid (C16:0)	214.3	49.7	491.5	55.3
Oleic acid (C18:1ω9)	41.9	9.7	111.3	12.5
Octadecenoic acid (C18:1ω11)	7.1	1.7	39.4	4.4
Stearic acid (C18:0)	144.3	33.5	188.3	21.2
Arachidic acid (20:0)	5.2	1.2	11.9	1.3
Total	430.9	100	889.2	100
SFAs	382.0	88.6	738.4	83.1
MUFAs	49.0	11.4	150.7	16.9

d.w: dry weight

intensity, UV radiation, and low temperature) (Whitelam and Codd 1986; Spijkerman et al. 2012). According to Řezanka et al. (2008), PUFAs account for > 75% of total FAs in *Chloromonas brevispina* collected from green patches in the Bohemian Forest (Czech Republic). Lutz et al. (2015) found that PUFAs accounted for 49% of total FAs in red snow, which was sampled on Feiringbreen in Svalbard. A similar finding for the absence of PUFAs was observed in green snow from Svalbard in Lutz et al. (2015), who explained that high nutrient availability and water film in green snow could lead to lower light stress. The most striking difference, in comparison to Lutz et al. (2015), was the similar proportion of the C18:1ω9 (10–13%) for the total FA pool in all the samples, whereas C16:3, C16:4, C18:2, and C18:3 were absent in the reddish snow sample in this study. High concentrations of C18:1ω9 (30–50 mg FA gC^{-1}) were observed among FAs in orange and red snow samples obtained from Spitsbergen, Svalbard (Spijkerman et al. 2012). In their laboratory experiments, the content of C18:1ω9 was increased in field samples and snow algal strains grown under nitrogen limited and high light conditions (Spijkerman et al. 2012). Therefore, our results are not representative of nutrient-limited and high light intensity conditions. However, absent PUFAs in these samples might be due to the small sample amount.

Mycosporine-like amino acid concentration of reddish and greenish snow

Mycosporine-like amino acids (MAAs) with a maximum absorbance between 310 and 365 nm are ultraviolet radiation (UVR) energy-absorbing small secondary metabolites in various organisms such as algae, cyanobacteria, and fungi (Häder et al. 1998; Karentz 2001; Whitehead et al. 2001; Volkmann and Gorbushina 2006; Wada et al. 2015). In this study, the MAAs concentration was different between the reddish and greenish snow samples (Fig. 2d). The average total MAA concentrations of the reddish and greenish snow samples were 278.2 μg g^{-1} (± 53.5 μg g^{-1}) and 316.0 μg g^{-1} (± 68.7 μg g^{-1}), respectively (Fig. 2d). However, the MAA to POC ratio for the reddish snow samples (6.2 mg (g C)$^{-1}$) was approximately 2 times higher than that of greenish snow samples (2.6 mg (g C)$^{-1}$). Likewise, the MAAs for Chl-a-specific concentrations for the reddish snow samples (1.4 mg (μg Chl-a)$^{-1}$) were also approximately 3 times higher than those of the greenish snow samples (0.5 mg (μg Chl-a)$^{-1}$).

There is little information on the concentrations of MAAs in snow algae, but some information is available for various algal communities. Based on previous reports (Neale et al. 1998; Llewellyn and Harbour 2003), MAAs of algae are known to be associated with the level of UVR. Therefore, differences in levels of MAAs may reflect a level of protection from UVR-induced damage in these snow algae samples. This suggests that reddish snow was more exposed to UVR than greenish snow. However, low photoprotective pigment contents (e.g., β-car, Diadino, Viola, and Lut) were found in this study. According to Ha et al. (2014a), organic carbon is initially fixed to produce a photoprotective pigment (Diadino) and then produce UV-absorbing MAAs within the cell based on ^{13}C-labeling experiments of *Porosira glacialis*. Therefore, these results on the observed data indicate synthetic pathways of photoprotective compounds (pigment and MAAs) involved in algal metabolism. Algae may have accumulated MAAs as a result of selecting a UV-absorbing MAA strategy rather than pigment to survive in a UVR exposure environment.

4. Conclusion

The colors of snow might be determined by pigments and used in the taxonomy of algae in fields. As a part of the life cycle, snow algae change their pigment composition. The FA composition of the different colored snow shows that SFAs were dominated by C16:0 and C18:0, whereas the MUFAs, including C18:1ω9 and C18:1ω11, were less dominant in this study. As confirmed by the C/N ratio, low contents of photoprotective pigments, and no detection of PUFAs, algae in the reddish and greenish snow samples were inferred as the same species and in non-limiting nutrient and low stress conditions. Although greenish snow has a higher total MAA concentration than reddish snow, the ratios of MAA to POC were opposite. These results suggest that reddish and greenish snow are regarded as a stage of MAA production after the metabolic pathway of pigment formation. Greenish snow made a higher contribution to carbon storage than reddish snow, which is the major contributor to both organic matter and carbon cycling in snowfields based on $\delta^{13}C$ and POC data. However, investigated parameters cannot fully explain the difference in the different colored snows with physiological adaptations in this study. Therefore, analysis of the temporal patterns of physiological parameters for colored snow and other environmental factors (such as slope and water rivulets), and metabolic pathway-related biochemical compounds should be investigated further, since biochemical compounds of snow algae are important for understanding their unique life in extreme habitats and for determining the effect of algae on global climate change.

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