Evaluation of photosynthetic processes in Antarctic mosses and lichens exposed to controlled rate cooling: Species-specific responses

Yeray Folgar-Cameán[1], Miloš Barták[2*]

[1]*Faculty of Biology, University of Santiago de Compostela, Rúa Lope Gómez de Marzoa, 15782, Santiago de Compostela, Spain*
[2]*Laboratory of Photosynthetic Processes, Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

Abstract

Antarctic regions involve a great variety of habitats characterized by environmental stressors and life forms of autotrophic organisms with unique survival and functioning mechanisms. Lichens and mosses from these regions, similarly to high altitude alpine locations, have evolved physiological adaptations to perform photosynthesis at subzero temperatures. In this study we applied linear cooling technique in order to analyze interspecific differences in primary photosynthetic processes in Antarctic species affected by low and subzero temperature stress. We exposed *Sanionia uncinata*, *Rhizoplaca aspidophora*, *Ochrolechia frigida*, *Cladonia* sp., *Himantormia lugubris* and *Umbilicaria decussata* to the cooling from 20 to -35°C at a constant rate of 2°C min^{-1}. Fluorometric parameters were measured during the cooling experiments: F_V/F_M - potential yield of photosynthetic processes in photosystem II, and F_0 - minimal chlorophyll fluorescence. All the species showed S-curves for F_V/F_M in response to decreasing temperature and interspecific differences in the parameters of S-curve equation. Critical temperature for F_V/F_M was found -35°C for *U. decussata*, while the other species ranged between -16 to -20°C. The changes of F_0 with thallus temperature decrease were species-specific. F_0 decrease followed by an increase was found with cooling from 20 to -20°C, and from -20 to -35°C, respectively, in the majority of cases. These results suggest that the experimental moss and lichen species from Antarctica have a high resistance to freezing temperatures. The underlying physiological mechanisms are constitutive features of Antarctic lichens and mosses. They are a crucial part of the adaptation and short-term acclimatory changes in ecophysiological performance of the organisms in harsh polar environments.

Key words: temperature stress, chlorophyll fluorescence, linear cooling, Antarctic species, cold adaptation, cold resistance

DOI: 10.5817/CPR2019-1-10

Received February 27, 2019, *accepted* April 30, 2019.
[*]Corresponding author: M. Barták <mbartak@sci.muni.cz>
Acknowledgements: The authors express their thanks for the support provided by the ECOPOLARIS project (CZ.02.1.01/0.0/0.0/ 16_013/0001708) and the CzechPolar2 (LM2015078) infrastructure (EEL laboratory, Masaryk University, Brno). Sample collection at King George Island was supported by the Grant No. INACH RT2716 provided during the Chilean Antarctic expedition in January 2019.

List of symbols and abbreviations: F_V/F_M – potential quantum yield of photosynthetic processes in photosystem II, LHC II – light-harvesting complex II, LT50 – lethal temperature at which chlorophyll fluorescence parameters reach 50% of their maxima, PS II – photosystem II, RC – reaction centre, T1 – temperature at which temperature-dependent inhibition of the parameter starts, T2 – critical subzero temperature at which the parameter reaches zero.

Introduction

The Antarctic continent is characterized by several environmental factors that may be considered stressful for both aquatic and terrestrial organisms (Convey 1996). Light is limited on a seasonal, as well as a daily, basis from 24h daylight in midsummer to 24h darkness in midwinter. Terrestrial environments are generally extremely dry since most water is locked up as snow and ice. Other stressors may include low nutrient availability, unstable substrates, ultraviolet radiation and osmotic stress (Convey 1996, Convey 2001, Wharton et al. 2002). Although these stressors have been usually considered separately, they clearly have important interactions that produce short growing seasons (Wharton 2003).

Antarctica is mostly dominated by non-vascular plants, predominantly mosses and lichens, with a few liverwort species and some species of flowering plants (Convey 2001, Øvstedal and Lewis Smith 2001). There is also a significant microbial flora including photosynthetic prokaryotes, unicellular algae, and microfungi (Peat et al. 2007). Different features enable bryophytes and lichens to survive under the apparently severe stress of Arctic and Antarctic environments. Such features include, first, general characteristics of these plants that confer fitness under polar conditions and, second, species or population specific adaptations which may therefore have evolved in response to local selection pressures (Longton 1988).

Temperature stress varies on both a continental and local scale. The maritime Antarctic comprises parts of the Antarctic Peninsula and nearby islands. This has a cold maritime climate with mean monthly air temperatures above 0°C for several months during summer and rarely falling below –15°C in winter (Block 1994, Holdgate 1970, 1977). The remaining areas are part of the continental Antarctic with mean monthly air temperatures rarely above 0°C in summer and winter means as low as –25°C even in coastal areas (Block 1994). When plants are exposed to low and subzero temperatures, water molecules move out from the cell to increase the intracellular osmotic potential and form rather extracellular than intracellular ice. This situation causes a considerable desiccation stress (Burke et al. 1976) and changes in the conformation of biomolecules (Crowe et al. 1990). Such changes alter the arrangement of proteins with thylakoid membranes, resulting in a reduction of the light absorption surface on the membranes and in an increase in self-absorption of emitted fluorescence. Proteins conformation during frozen periods inhibits the energy transfer between LHC II and PS II, leading to a reduction in the number of functional PS II centres (Lovelock et al. 1995). In consequence, less photosynthesis is performed under these conditions.

Freezing and low temperature resistance in species from alpine and/or polar ecosystems has been studied within the last few decades in lichens (Hájek et al. 2009a, Barták et al. 2007), algae and cyanobacteria (Davey 1989), mosses (Lovelock et al. 1995) and fungi (Robinson 2001). However, there are only limited number of studies that have focused on the responses of photosynthetic processes to changing

temperature. They can be divided into two groups: (1) repeated measurements at distinct temperatures (Linkosalo et al. 2014), and (2) continuous measurements of chlorophyll fluorescence during a constant-rate controlled temperature fall (Hájek et al. 2016).

In this study, we evaluated the interspecific differences in the resistance of Antarctic poikilohydric organisms to cold stress and freezing temperatures. The species were exposed to linear cooling combined with simultaneous measurements of chlorophyll fluorescence parameter and signal (F_V/F_M and F_0). We hypothesized that the experimental species would show high resistance to low temperatures as part of their adaptation to perform photosynthesis in harsh polar environments.

Material and Methods

Sample collection and handling

Six different Antarctic species were used in this study: one moss, *Sanionia uncinata*, and five lichens, *Rhizoplaca aspidophora*, *Ochrolechia frigida*, *Cladonia* sp., *Himantormia lugubris* and *Umbilicaria decussata*. Except of *U. decussata*, collected at the James Ross Island, all the species were collected in the King George Island. It is the largest island of the South Shetland archipelago, lying 120 km (75 miles) of the coast of Antarctica in the Southern Ocean. It is important to highlight that *H. lugubris* is the only Antarctic endemic species, restricted to the South Shetlands, from those mentioned above (Lamb 1964).

The King George Island is located close to the Antarctic Peninsula and considered a moist and humid place in maritime Antarctica. The species were collected at different places, mainly at the Fildes Peninsula and the Ardley Peninsula (*see* Table 1). The two localities represent the largest ice-free regions in maritime Antarctica, and they show higher biodiversity than continental Antarctica. This typical small-scale spatial region includes two Antarctic Special Protected Areas (ASPAs), covering approximately 30 km^2 of the Fildes Peninsula, Ardley Island, and adjacent islands (Braun et al. 2012).

The James Ross Island is located near the northeastern extremity of the Antarctic Peninsula, from which it is separated by the Prince Gustav Channel. The island is irregularly shaped and extends 64 km in a north–south direction. Northern part of James Ross Island represents one of the largest deglaciated areas in Antarctica. The area is rich in vegetation oasis located in wet places (lake and stream margins, seepages). They are dominated by mosses and lichens since vascular plants are not present at the James Ross Island. For our study, thalli of *U. decussata* were collected from volcanic boulders located below the northern slopes of the Berry Hill mesa.

Species	Site Abbr.	Coordinates	Altitude
Sanionia uncinata	KGI	62°10′10.4′′S, 58°51′13.4′′W	25 m a.s.l.
Rhizoplaca aspidophora	KGI	62°10′10.3′′S, 58°51′9.1′′W	36 m a.s.l.
Ochrolechia frigida	KGI	62°12′40′′S, 58°55′54′′W	30 m a.s.l.
Cladonia sp.	KGI	62°12′41′′S, 58°55′49′′W	40 m a.s.l.
Himantormia lugubris	KGI	62°12′15′′S, 58°57′35′′W	41 m a.s.l.
Umbilicaria decussata	JRI	63°48′28′′S, 57°50′37′′W	143 m. a.s.l.

Table 1. Overview of the sampling localities in the King George Island (KGI) and the James Ross Island (JRI), Antarctica.

Cooling protocols and chlorophyll fluorescence

At least three samples of each species were placed into a PLANER Kryo 560-16 (Great Britain) cooling chamber linked to a flask of liquid nitrogen and cooled from 20°C, room temperature, to -35°C at a constant rate of -2°C min^{-1}. This protocol has been frequently used in the studies focused on cryostability/cryoresistance of polar algae and lichens (Hájek et al. 2009b, Hájek et al. 2016, Šabacká and Elster 2006). During the cooling experiment both temperature and photosynthetic parameters were constantly measured. Sample and chamber temperatures were measured by inbuilt thermocouples. Basic chlorophyll fluorescence parameters (*see* below for their specification) were measured by a PAM 2000 fluorometer (H. Walz, Germany), the probe of which was placed inside the cooler chamber a few millimeters above the samples. F_V/F_M values were obtained by repeated saturation pulses of 5000 µmol m^{-2} s^{-1} for 0.8 s each 30 s. Wet samples (hydrated 24h) were predarkened for 5 min. before the cooling period to allow photosystem II reaction centres reach fully open state (oxidized, F_0 measurements) and obtain the real fluorescence maximum after the saturation pulse application (Lichtenthaler 1988).

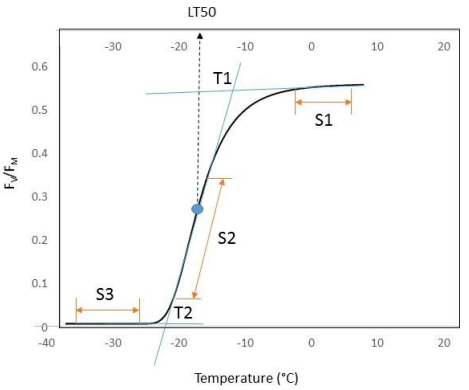

Fig. 1. S-curve showing temperature dependence of chlorophyll fluorescence parameter (F_V/F_M) recorded during linear cooling with graphical indications of the following variables: LT50 - lethal temperature at which the parameter reaches 50% of its maxima, S1 – slope of the relationship at linear part in above zero temperature, S2 – slope of the relationship at linear part in below zero temperature, T1 - temperature at which temperature-dependent inhibition of F_V/F_M starts, T2 - critical subzero temperature at which F_V/F_M reaches zero (modified from Hájek et al. 2016).

Processing of F_V/F_M and F_0

F_V/F_M and F_0 were normalized to maximum values (reached in high temperature) in order to compare species-specific response to the decrease in thallus temperature. Then, a 5 parameter logistic model was used to construct S-curves which best-fit the F_V/F_M data by non-linear regression (software SigmaPlot). The model used the following equation:

$$f(x) = C + \frac{D-C}{(1+\exp(B(\log(x)-\log(E))))^F} \qquad \textit{Eqn. 1}$$

where f(x) – is the value of F_V/F_M, x – represents temperature, B – is a Hill slope, C – is the minimum value of the dependent variable, D – is the maximum value of the dependent variable, E – is the inflection point, and F – is a parameter of the S-curve asymmetry.

The obtained S-curves were compared in terms of their shape and by the following parameters: (1) temperature of the sample at which F_V/F_M starts being affected by the stress (T1), (2) critical temperature at which F_V/F_M reaches 0 (T2), and (3) lethal temperature (LT50) or temperature at which F_V/F_M reaches 50% of its maxima. In addition, the slopes of the linear parts of the curves (S1, S2) were evaluated (*see*

Fig. 1). The data from F_0 were not analyzed by non-linear regression but T1 and T2 were calculated in order to compare inter-specific differences in both parameters.

Results and Discussion

All the experimental species showed a decrease in F_V/F_M with temperature decrease following a typical S-shaped curve (Fig. 2). The curves were based on three phases or segments: (1) the initial segment where the parameter shows its maximum with a slow linear decreasing usually from 20 to -5°C (Phase I); (2) a segment where the parameter decreases rapidly, typical in a range between -5 and -20°C (Phase II); and (3) the end part of the curve with a slow linear decrease close to zero and usually below -20°C (Phase III). Constant close-to-maximum values of F_V/F_M were found above 0°C in all the studied species. However, some of the species showed specific differences apart from the general trends. The Phase II in *O. frigida* was slightly shorter than in other species because of the rapid decrease of the parameter with temperature; in the case of *U. decussata*, this phase showed a slower decrease related with a larger range of the Phase II.

Species-specific differences in the shape of the S-curves were also reflected in their best-fit parameters (Table 3) and in specific temperatures derived from the logistic curve (Table 4). In contrast to C, D and E parameters that remained very similar among the species, the parameter B (Hill slope) was between 2 and 3 times higher in *S. uncinata* and *O. frigida* than in the other experimental species, respectively. The F parameter (S-curve asymmetry) showed also big inter-specific differences. On the other hand, T1 values were similar in all the species. They were found in the range from -5 to -7°C. However, T2 was clearly different in two species: *O. frigida*, with a higher value around -16°C, and *U. decussata*, with the lowest value at -35°C. For the critical temperature LT50 the values followed the same trend as T2.

	T1 (°C)	T2 (°C)
S. uncinata	-5.0	-19.0
R. aspidophora	-5.0	-21.0
O. frigida	n.d.	n.d.
Cladonia sp.	-7.0	-19.0
H. lugubris	-11.0	-19.0
U. decussata	-16.0	-37.0

Table 2. Parameters of F_0 relationship with temperature. T1 – temperature at which substantial increase of F_0 starts, T2 – temperature at which F_0 reaches its maximum. The values for *O. frigida* are not presented in this Table because of exclusively decreasing trend with missing increase of F_0 values in subzero temperature (*see* Fig. 3).

Temperature-response curves of F_0 showed a general trend of increase with low temperature stress in subzero temperature (Fig. 3). The lichen *O. frigida* was the only experimental species in which F_0 decreased with low temperature. The temperature at which the increase of F_0 starts (T1) was species-specific as shown in Table 2; in contrast, T2 was reached at about -20°C in the majority of cases. *U. decussata* showed the highest T2 value at -37°C.

	S. uncinata	*R. aspidophora*	*O. frigida*	*Cladonia* sp.	*H. lugubris*	*U. decussata*
B	-33.40 ± 6.25	-19.51 ± 3.26	-43.79 ± 8.00	-18.48 ± 1.55	-16.46 ± 1.17	-8.96 ± 2.76
C	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.01	0.02 ± 0.01	0.05 ± 0.03
D	0.95 ± 0.01	0.94 ± 0.01	0.96 ± 0.01	0.96 ± 0.01	0.97 ± 0.01	0.96 ± 0.04
E	29.12 ± 0.81	27.51 ± 2.10	29.65 ± 0.87	28.03 ± 1.14	22.93 ± 1.49	25.86 ± 4.73
F	0.40 ± 0.12	0.94 ± 0.43	0.65 ± 0.24	1.00 ± 0.23	1.95 ± 0.66	0.55 ± 0.33

Table 3. Parameters of the S-curves fitting F_V/F_M to species temperature relationship (*see* Eqn. 1). B – is a Hill slope, C – is the minimum value of the dependent variable, D – is the maximum value of the dependent variable, E – is the inflection point, and F – is a parameter of the S-curve asymmetry.

The F_V/F_M ratio is considered to represent the maximum quantum yield of PS II (Malkin and Kok 1966, Butler and Kitajima 1975, Genty et al. 1989). It has been reported that its decrease is indicative of the decline in the efficiency of photochemistry of PS II (Butler 1978). A pronounced decrease in our data was shown below -5°C in all the species. This result is consistent with the values for T1 obtained in previous studies (Barták et al. 2003, Hájek et al. 2016) and also corresponds closely to the temperature reported as critical for ice-nucleation in hydrated lichen thalli (Schroeter and Scheidegger 1995, Haranczyk et al. 2003). The ability to form extracellular ice at these temperatures provides a useful protective mechanism for lichens (Moffett et al. 2015). Freezing temperature causes the movement of intracellular water along a water potential gradient out of the cells resulting in changes in the biomolecules conformation (Crowe et al. 1990). The biophysical mechanisms involved into lichen freezing resistance have been reviewed recently by Harańczyk et al. (2017). They comprise (i) the decrease in hydration rate of freezing of loosely bound water pool, (ii) active transfer of freezing, (iii) share of loosely bound water pool to non-freezing one, (iv) presence of tightly bound non-freezing water fraction, and (v) non-co-operative immobilization of supercooled bound water.

	T1 (°C)	**T2 (°C)**	**S1**	**S2**	**LT50**
S. uncinata	-7.2	-19.2	y = 0.0012x + 0.9475	y = 0.0769x + 1.4925	-13.3
R. aspidophora	-6.1	-19.7	y = 0.0017x + 0.9230	y = 0.0647x + 1.3069	-13.0
O. frigida	-6.7	-16.1	y = 0.0001x + 0.9608	y = 0.0984x + 1.6158	-11.6
Cladonia sp.	-5.7	-18.6	y = 0.0001x + 0.9542	y = 0.0721x + 1.3641	-12.3
H. lugubris	-7.2	-20.1	y = 0.0012x + 0.9674	y = 0.0720x + 1.4784	-14.0
U. decussata	-7.2	-35.0	y = 0.0012x + 0.8762	y = 0.0289x + 1.0748	-22.3

Table 4. Parameters and slopes derived from S-curves (F_V/F_M). LT50 – lethal temperature at which chlorophyll fluorescence parameters reach 50% of their maxima, T1 – temperature at which a substantial decline of F_V/F_M starts, T2 – temperature at which decline of F_V/F_M reaches zero, S1 – slope of the relationship at linear part above zero temperature, S2 – slope of the relationship at linear part below zero temperature.

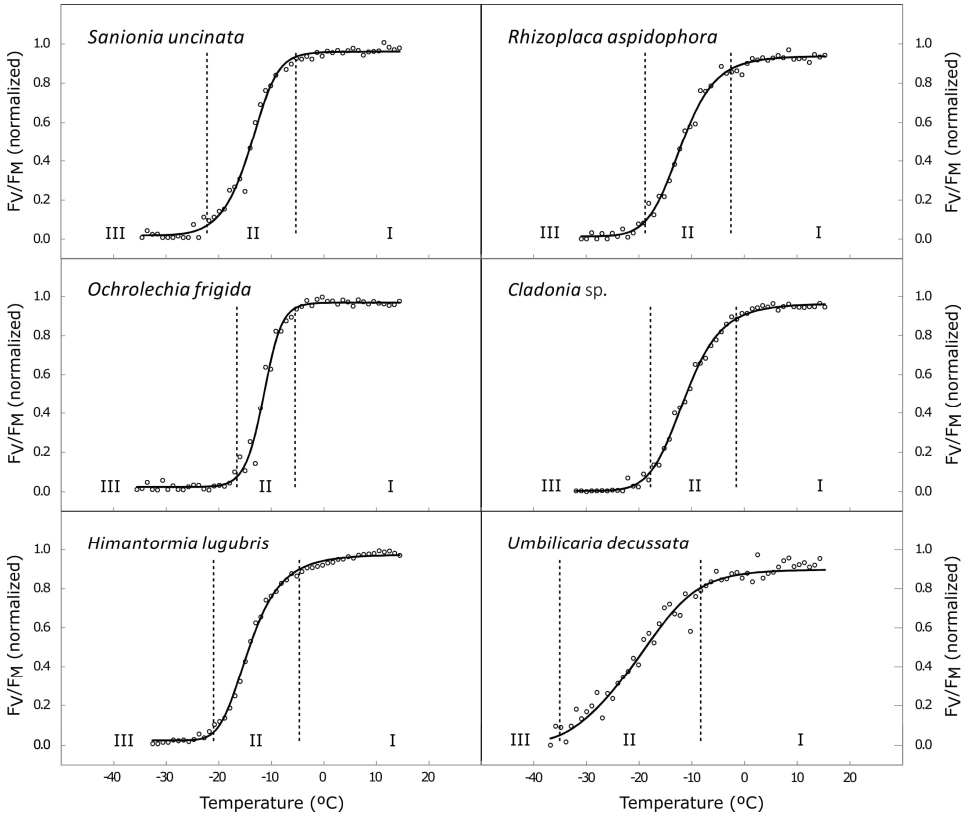

Fig. 2. Temperature-response curves of F_V/F_M (potential yield of photochemical processes in PS II, normalized to maximum value) recorded during linear cooling of experimental species from 20 to -35°C at a rate of 2°C min^{-1}. The three characteristic *Phases* are represented on the graphs by I, II and III. Data points are means of at least 3 replicates.

This process helps freezing tolerant organisms to avoid the formation of potentially destructive intracellular hexagonal ice crystals. The mechanism stimulates the growth of non-lethal ice crystals in the extracellular space (Burke et al. 1976, Harańczyk et al. 2003). Moreover, ice nucleation in lichen and moss cells protoplast is usually observed at higher temperatures than the lowest temperature at which photosynthetic processes occur (Nash et al. 1987, Kieft 1988, Kieft and Ahmadjian 1989). In our study, F_V/F_M reached 0 or close to 0 values in the temperature range -16 to -35°C, depending on the species. This range is much lower than that reported for some mosses (Buchner and Neuner 2010, Atanasiu 1971). For lichens, however, the range of critical temperature for F_V/F_M was similar to those reported in earlier studies in *Usnea antarctica*, *Xanthoria elegans* (Barták et al. 2007), *Umbilicaria cylindrica* and *Usnea aurantiaco-atra* (Hájek et al. 2016), *i.e.* from -20 to -30°C. Critical temperature (T2) for F_V/F_M might be associated with formation of ice crystals in photobiont cells in such temperature. This can be supported by the critical point of about -20°C found recently by Bacior et al. (2019) who reported the ice melting temperature in *Turgidosculum complicatulum* (-20°C, DSC and H-NMR spec-

tra measured during linear heating from frozen state). Moreover, a similar range of critical temperature (about -20°C) is reported in the studies related to the net photosynthesis activity (*e.g.* Kappen et al. 1996). Our results indicate that *O. frigida* (high T2) might be less resistant to freezing temperatures than the other species. On the contrary, *U. decussata* performed primary photosynthetic processes at the lowest temperature, T2 of -35°C, which can be related to its higher resistance, on photosynthetic processes in particular, to subzero temperature (*see* Fig. 2).

The decrease in sample temperature below -15°C was accompanied by an increase of the minimum chlorophyll fluorescence (F_0). Such change in F_0 seems to be connected with the blockage of RC II and the redistribution of excitation energy (Pospíšil et al. 1998). This causes the emission of the excess energy from LHCs as chlorophyll fluorescence. All our experimental species support this assumption except *O. frigida*. For unknown reasons, the species showed a decrease in F_0 with temperature decrease. In order to obtain a better understanding of this behavior, further experiments should be done in forthcoming studies.

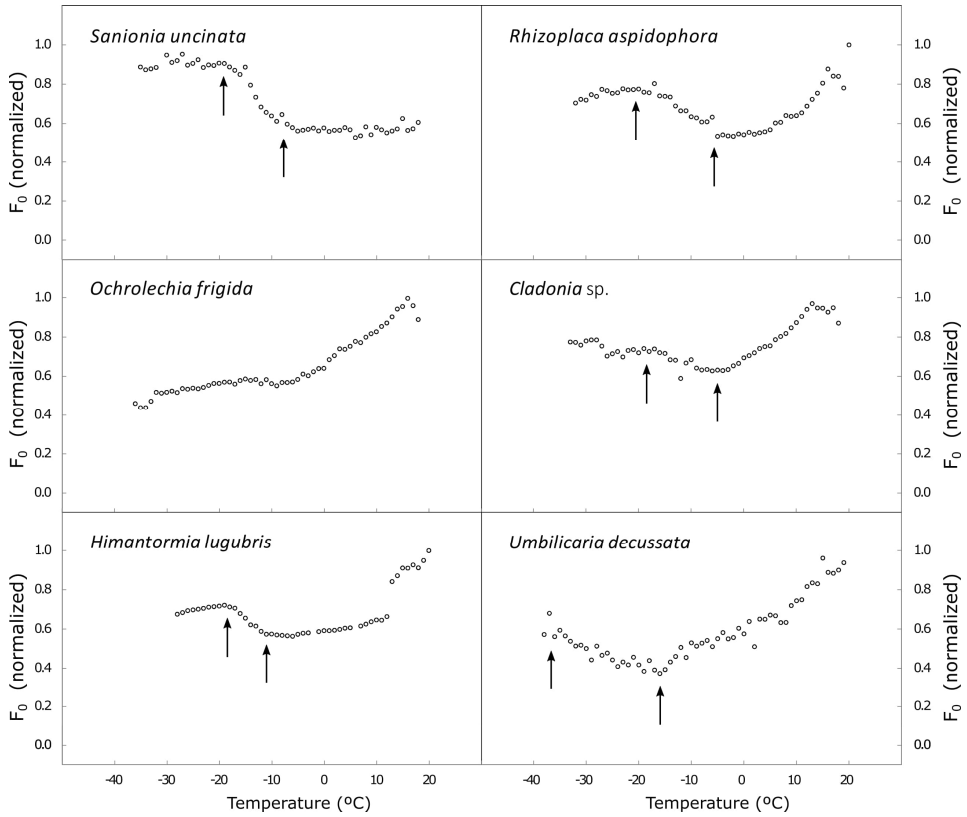

Fig. 3. Temperature-response curves of F_0 (minimum chlorophyll fluorescence, normalized to maximum value) recorded during linear cooling of experimental species from 20 to -35°C at a rate of 2°C min^{-1}. T1 and T2 are indicated by arrows with the exception of *O. frigida* in which T1 and T2 were not distinguishable. Data points are means of at least 3 replicates.

The comparison of both chlorophyll fluorescence parameters shows that in the majority of cases F_V/F_M ratio reaches 0 in the same temperature in which the increase of F_0 shows its maximum. Similar behavior of the chlorophyll fluorescence parameters was documented in several heat stress studies (Fork et al. 1987, Ludlow 1987, Al-Khatib and Paulsen 1989). This can be related to high temperature-induced inhibition of the PS II in a species-specific temperature. However, the temperature at which F_V/F_M starts to decrease (T1) seems not to be correlated with the temperature at which F_0 starts to increase (*see* Table 2 and Table 4).

Lichen and moss resistance to subzero temperatures has been usually studied by repetitive freezing-thawing cycles (Mishra et al. 2015). However, our research supports the idea that the linear cooling method with simultaneous chlorophyll fluorescence measurements is a faster alternative enabling large-scale experiments studying the responses of a great number of species. In addition, the obtained results suggest that our experimental species are well adapted to freezing temperatures. Such adaptation is a crucial part of their resistance. It helps the studied species to perform short-term acclimatory changes in response to fluctuations of temperature in the field. The studied species have a high potential in ecophysiological responses to harsh polar environments.

References

AL-KHATIB, K., PAULSEN, G. M. (1989): Enhancement of thermal injury to photosynthesis in wheat plants and thylakoids by high light intensity. *Plant Physiology*, 90(3): 1041-1048.

ATANASIU, L. (1971): Photosynthesis and respiration of three mosses at winter low temperatures. *Bryologist*, 74(1): 23-27.

BACIOR, M., HARAŃCZYK, H., NOWAK, P., KIJAK, P., MARZEC, M., FITAS, J. and OLECH, M. A. (2019): Low-temperature immobilization of water in Antarctic *Turgidosculum complicatulum* and in *Prasiola crispa*. Part I. *Turgidosculum complicatulum*. *Colloids and Surfaces B: Biointerfaces*, 173: 869-875.

BARTÁK, M., VRÁBLÍKOVÁ, H. and HÁJEK, J. (2003): Sensitivity of photosystem 2 of Antarctic lichens to high irradiance stress: fluorometric study of fruticose (*Usnea antarctica*) and foliose (*Umbilicaria decussata*) species. *Photosynthetica*, 41(4): 497-504.

BARTÁK, M., VÁCZI, P., HÁJEK, J. and SMYKLA, J. (2007): Low-temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*. *Polar Biology*, 31(1): 47-51.

BLOCK, W. (1994): Terrestrial ecosystems: Antarctica. *Polar Biology*, 14(5): 293-300.

BRAUN, C., MUSTAFA, O., NORDT, A., PFEIFFER, S. and PETER, H. (2012): Environmental monitoring and management proposals for the Fildes Region, King George Island, Antarctica. *Polar Research*, 31: 18206.

BUCHNER, O., NEUNER, G. (2010): Freezing cytorrhysis and critical temperature thresholds for photosystem II in the peat moss *Sphagnum capillifolium*. *Protoplasma*, 243(1-4): 63-71.

BURKE, M., GUSTA, L., QUAMME, H., WEISER, C. and LI, P. (1976): Freezing and injury in plants. *Annual Review Plant Physiology*, 27: 507-528.

BUTLER, W. L., KITAJIMA, M. (1975): Fluorescence quenching in photosystem II of chloroplasts. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 376(1): 116-125.

BUTLER, W. L. (1978): Energy distribution in the photochemical apparatus of photosynthesis. *Annual Review of Plant Physiology*, 29(1): 345-378.

CONVEY, P. (1996): The influence of environmental characteristics on life history attributes of Antarctic terrestrial biota. *Biological Reviews*, 71(2): 191-225.

CONVEY, P. (2001): Antarctic ecosystems. *In*: S. Levin (ed.): Encyclopedia of biodiversity. Academic Press, San Diego, pp. 171-184.

CROWE, J., CARPENTER, J., CROWE, L. and ANCHORDOGUY, T. (1990): Are freezing and dehydration similar stress vectors? A comparison of modes of interaction of stabilizing solutes with biomolecules. *Cryobiology*, 27(3): 219-231.

DAVEY, M. C. (1989): The effects of freezing & desiccation on photosynthesis and survival of terrestrial Antarctic algae and cyanobacteria. *Polar Biology,* 10(1): 29-36.

FORK, D. C., SEN, A. and WILLIAMS, W. P. (1987): The relationship between heat-stress and photobleaching in green and blue-green algae. *Photosynthesis Research*, 11(1): 71-87.

GENTY, B., BRIANTAIS, J. M. and BAKER, N. R. (1989): The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 990(1): 87-92.

HÁJEK, J., VÁCZI, P. and BARTÁK, M. (2009a): Photosynthetic electron transport at low temperatures in the green algal foliose lichens *Lasallia pustulata* and *Umbilicaria hirsuta* affected by manipulated levels of ribitol. *Photosynthetica*, 47(2): 199-205.

HÁJEK, J., VÁCZI, P., BARTÁK, M., SMEJKAL, L. and LIPAVSKÁ, H. (2009b): Cryoproective role of ribitol in Xanthoparmelia somloensis. *Biologia Plantarum*, 53(4): 677-684.

HÁJEK, J., BARTÁK, M., HAZDROVÁ, J. and FORBELSKÁ, M. (2016): Sensitivity of photosynthetic processes to freezing temperature in extremophilic lichens evaluated by linear cooling and chlorophyll fluorescence. *Cryobiology*, 73(3): 329-334.

HARAŃCZYK, H., GRANDJEAN, J., OLECH, M. and MICHALIK, M. (2003): Freezing of water bound in lichen thallus as observed by 1H NMR. II. Freezing protection mechanisms in a cosmopolitan lichen Cladonia mitis and in Antarctic lichen species at different hydration levels. *Colloids and Surfaces B: Biointerfaces*, 28(4): 251-260.

HARAŃCZYK, H, CASANOVA-KATNY, A., OLECH, M. and STRZAŁKA, K. (2017): Dehydration and freezing resistance of lichenized fungi. *In*: V. Shukla, S. Kumar, N. Kumar (eds.): Plant Adaptation Strategies in Changing Environment. Springer, Singapore. 386 p. ISBN 978-981-10-6744-0.

HOLDGATE, M. W. (1970): Vegetation. *In*: M. W. Holdgate (ed.): Antarctic ecology. Academic Press, London, pp. 729-732.

HOLDGATE, M. W. (1977): Terrestrial ecosystems in the Antarctic. *Philosophical Transactions of the Royal Society of London series B*, 279: 5-25.

KAPPEN, L., SCHROETER, B., SCHEIDEGGER, C., SOMMERKORN, M. and HESTMARK, G. (1996): Cold resistance and metabolic activity of lichens below 0°C. *Advances in Space Research*, 18(12): 119-128.

KIEFT, T. L. (1988): Ice nucleation activity in lichens. *Applied and Environmental Microbiology*, 54(7): 1678-1681.

KIEFT, T. L, AHMADJIAN, V. (1989): Biological ice nucleation activity in lichen mycobionts and photobionts. *Lichenologist*, 21: 355-362.

LAMB, I. M. (1964). Antarctic lichens: I. The genera *Usnea, Ramalina, Himantormia, Alectoria, Cornicularia. British Antarctic Survey Scientific Reports*, 38: 1-34.

LICHTENTHALER, H. K. (1988): *In vivo* chlorophyll fluorescence as a tool for stress detection in plants. *In*: H. K. Lichtenthaler (ed.): Applications of chlorophyll fluorescence in photosynthesis research, stress physiology, hydrobiology and remote sensing. Springer, Netherlands, pp. 129-142.

LINKOSALO, T., HEIKKINEN, J., PULKKINEN, P. and MÄKIPÄÄ, R. (2014): Fluorescence measurements show stronger cold inhibition of photosynthetic light reactions in Scots pine compared to Norway spruce as well as during spring compared to autumn. *Frontiers in Plant Science*, 5: 264.

LONGTON, R. E. (1988): Biology of polar bryophytes and lichens. Cambridge University Press. *CUP Archive*. 404 p.

LOVELOCK, C. E., JACKSON, A. E., MELICK, D. R. and SEPPELT, R. D. (1995): Reversible photoinhibition in Antarctic moss during freezing and thawing. *Plant Physiology*, 109(3): 955-961.

LUDLOW, M. M. (1987): Light stress at high temperature. *In*: D. J. Kyle, C. B. Osmond, C. J. Arntzen (eds.): Photoinhibition. Elsevier, Amsterdam, pp. 89–109.

MALKIN, S., KOK, B. (1966): Fluorescence induction studies in isolated chloroplasts I. Number of components involved in the reaction and quantum yields. *Biochimica et Biophysica Acta (BBA)-Biophysics including Photosynthesis*, 126(3): 413-432.

MISHRA, A., HÁJEK, J., TUHÁČKOVÁ, T., BARTÁK, M. and MISHRA, K. B. (2015): Features of chlorophyll fluorescence transients can be used to investigate low temperature induced effects on photosystem II of algal lichens from polar regions. *Czech Polar Reports*, 5(1): 99-111.

MOFFETT, B. F., GETTI, G., HENDERSON-BEGG, S. K. and HILL, T. C. J. (2015): Ubiquity of ice nucleation in lichen—possible atmospheric implications. *Lindbergia*, 38: 39-43.

NASH, T. H., KAPPEN, L., LOSCH, R., LARSON, W. and MATTHES-SEARS, U. (1987): Cold resistance of lichens with Trentepohlia or Trebouxia photobionts from the North American west coast. *Flora*, 179: 241-251.

ØVSTEDAL, D. O., LEWIS SMITH, R. I. (2001): Lichens of Antarctica and South Georgia. A Guide to Their Identification and Ecology. Cambridge University Press, Cambridge, 424 p.

PEAT, H. J., CLARKE, A. and CONVEY, P. (2007): Diversity and biogeography of the Antarctic flora. *Journal of Biogeography*, 34(1): 132-146.

POSPÍŠIL, P., SKOTNICA, J. and NAUŠ, J. (1998): Low and high temperature dependence of minimum F_0 and maximum F_M chlorophyll fluorescence *in vivo*. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1363(2): 95-99.

ROBINSON, C. H. (2001): Cold adaptation in Arctic and Antarctic fungi. *New Phytologist*, 151(2): 341-353.

ŠABACKÁ, M., ELSTER, J. (2006): Response of cyanobacteria and algae from Antarctic wetland habitats to freezing and desiccation stress. *Polar Biology*, 30(1): 31.

SCHROETER, B., SCHEIDEGGER, C. (1995): Water relations in lichens at subzero temperatures: structural changes and carbon dioxide exchange in the lichen *Umbilicaria aprina* from continental Antarctica. *New Phytologist*, 131(2): 273-285.

WHARTON, D. A., GOODALL, G. and MARSHALL, C. J. (2002): Freezing rate affects the survival of a short-term freezing stress in *Panagrolaimus davidi*, an Antarctic nematode that survives intracellular freezing. *CryoLetters*, 23(1): 5-10.

WHARTON, D. A. (2003): The environmental physiology of Antarctic terrestrial nematodes: A review. *Journal of Comparative Physiology B*, 173(8): 621-628.