Effects of short-term low temperature stress on chlorophyll fluorescence transients in Antarctic lichen species

Michaela Marečková[1, 2], Miloš Barták[1*]

[1]*Laboratory of Photosynthetic Processes, Department of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*
[2]*Plant Cytogenomics Group, CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

Abstract

Chlorophyll fluorescence is an effective tool for investigating characteristics of any photosynthesizing organisms and its responses due to different stressors. Here, we have studied a short-term temperature response on two Antarctic green algal lichen species: *Umbilicaria antarctica*, and *Physconia muscigena*. We measured slow chlorophyll fluorescence transients in the species during slow a cooling of thallus temperature from 20°C to 5°C with a 10 min. acclimation at each temperature in dark. The measurements were supplemented with saturation pulses for the analysis of chlorophyll fluorescence parameters: maximum yield of PS II photochemistry (F_V/F_M), effective quantum yield of PS II photochemistry (Φ_{PSII}) and non-photochemical quenching (NPQ). In response to decreasing thallus temperature, we observed species-specific changes in chlorophyll fluorescence levels P, S, M, T reached during chlorophyll fluorescence transient as well as in the shape of the chlorophyll fluorescence transients. With a decrease in temperature, the time at which M and T chlorophyll fluorescence levels were reached, increased. These changes were attributed to redox state of plastoquinon pool, changes in Calvin-Benson cycle activity, non-photochemical quenching components, state transition in particular. In this study, we present some chlorophyll fluorescence ratios (P/M, M/T, P/T) and chlorophyll fluorescence increase rates (FR_1, *i.e.* O to P, and FR_2 - *i.e.* S to M) as the parameters reflecting direct temperature effects on chloroplastic apparatus of lichen alga sensitively. We proposed that species-specific changes in the slow phase of chlorophyll fluorescence transients could be potentially used as indicators of low temperature effects in photosynthetic apparatus of lichen algal photobionts. Interspecific differences in response to low temperature might be evaluated using the approach as well.

Key words: photosynthetic processes, polyphasic kinetics, chlorophyll fluorescence parameters, James Ross Island, Galindez Island

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[*]Corresponding author: Miloš Barták <mbartak@sci.muni.cz>
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Introduction

It is well established that cellular membranes are a prime target of damages caused during low and freezing temperature events and that membrane composition changes during cold acclimation. Therefore, many studies have focused on the effects on the thylakoid membranes when exposed to low/subzero temperature. In such studies, the response of thylakoids membranes, their photosynthetic processes in particular, to sustained low-temperature treatments was measured non-invasively, typically by the analysis of chlorophyll fluorescence (ChlF) emission. Among a wide spectrum of methods using chlorophyll fluorescence technique in higher plants, slow (Kautsky) chlorophyll fluorescence transient has been used much less frequently then ´traditional´ parameters such a *e.g* potential (F_V/F_M) and effective quantum yield of photosynthetic processes in photosystem II (Φ_{PSII}) – Bolhar-Nordenkamf et Oquist (1993). However, complex analysis of ChlF transient was presented by for *Arabidopsis thaliana* (Mishra et al. 2014), and Antarctic lichens (Mishra et al. 2015). The latter study suggested usefulness of ChF transient analysis to discriminate physical effects of low temperature on lichens and individual species responses, their tolerance and/or avoidance mechanisms. Moreover, relative contribution of PS II and PS I to overal ChlF emission is also reported to change during cooling treatments Agati et al. (2000). Therefore, slow ChlF transient and its analysis seems to be an efficient tool to study short-term responses of chloroplastic photosynthetic appartus to low temperature.

In this study, we focused on ChlF transient in lichens, *i.e.* slow (Kautsky) chlorophyll fluorescence transient. Slow ChlF transient is measured on dark-adapted plant material. When exposed to continuous light for several minutes, photosynthetizing organisms show rise to P peak followed by a polyphasic SMT phase. Riznichenko et al. (1996) mentioned that changes in chlorophyll fluorescence signal in PSMT part of the Kautsky kinetics represent combined effect of photochemical and non-photochemical processes taking place in photosynthetic apparatus. The PSMT phase of ChlF transient was first systematically studied in the laboratory of Govindjee (Papageorgiu et Govindjee 1968, 2011) – for review *see* Stirbet et al. (2014). During PSMT phase, photochemical quenching causes the decrease of chlorophyll fluorescence signal due to reoxidation of reduced QA thanks to photosynthetic electron transport chain. Generally, SMT phase of chlorophyll fluorescence signal forming the induction curve as well as the appearance of additional maxima are caused by stimulation of dark reactions of Calvin cycle of CO_2 fixation (*see* e.g. Seaton et Walker 1990). SMT part is polyphasic, because several co-acting processes interact. Non-photochemical fluorescence quenching results from these processes as formation of transthylakoidal proton gradient (Noctor et Horton 1990), phosphorylation of the light-harvesting complex (*see* e.g. Allen 1992), oxidation of plastoquinone pool (Vernotte at al. 1979) and photoinhibition (Krause 1988).

P, S, M peaks are denoted to components of non-photochemical quenching (qN), *i.e.* energy-dependent quenching (qE), state transition quenching (qT) and photoinhibitory treatment (qI) - (Kodru et al. 2015). Since the proportion of the qN components are species- and treatment-specific, it may be suggested that the entire PSMT chlorophyll fluorescence transient is due to a superimposition of several processes in which qE (energy-dependent NPQ of Chl fluorescence), as well as state changes play an important role. Typically, qI does not take much share, however, the possible involvement of several mechanisms related to photoinhibition and consequent changes of ChF transient during the M to T decline must be considered.

Chl fluorescence decrease ratio or the vitality index (R_{Fd}) was suggested and introduced into chlorophyll fluorescence literature in the 1980s (Lichtenthaler et al. 1986). For calculation of R_{Fd}, F_P and F_S chlorophyll fluorescence levels are used in the formula $R_{Fd} = (F_M - F_S)/F_S$. F_P chlorophyll fluorescence level denotes peak signal reached within first 1-2 s of illumination of predarkened plant material. F_S denotes a steady state chlorophyll fluorescence reached typically after 5 min. of continuous illumination. Whenever a constant F_S level is reached, reduction and the oxidation rate of quinones became theoretically equal. R_{Fd} is resulted of $(F_M - F_S)/F_S$ and has been demonstrated to be highly correlated with CO_2 fixation (Flexas et al. 2002, Lichtenthaler et al. 2005). Therefore, R_{Fd} values depend on the whole photosynthetic electron transport chain, rate of synthesis and utilization of ATP and NADPH.

In lichens, slow ChlF transient was used to characterize sensitivity to photoinhibition (*e.g.* Conti et al. 2014) and freezing stress (Mishra et al. 2015). Similarly, Nabe et al. (2007) studied sorbitol effect on shape of PSMT phase of transient in a liverworth (*Marchantia polymorpha*) and a moss (*Bryum argenteum*). In this study, we focused on several lichen species and hypothesized that species-specific differences in shape of slow ChlF transients would be indicative of species-specific responses of selected Antarctic lichens to low temperature. Therefore, we measured ChlF in several lichen species and analyzed parameters derived from the ChlF transient shape and ChlF signals. We hypothesised that they would be well related to selected ChlF parameters resulting from application of saturation pulse technique and quenching analysis. We evaluated species-specific sensitivity of primary photosynthetic processes of two chlorolichens to low temperature. We suggested some new ChlF parameters to detect early responses of photosynthetic apparatus of lichen photobionts in response short-term acclimatory changes to low temperature.

Material and Methods

Sample collecting and handling

In experiments, two lichen species were used: *Physconia muscigena* and *Umbilicaria antarctica* (Fig. 1). They were collected in two different regions neighbouring to the Antarctic peninsula. *P. muscigena* was collected at the James Ross Island from a long-term research plot (63° 48′ 03′′ S, 57° 52′ 50′′ W, *see* Barták et al. 2015 for details) located close to Mendel (Czech Antarctic research station). *Umbilicaria antarctica* was collected in the Maritime Antarctic at the Galindez Island in a close vicinity of Ukrainian station Vernadsky (65° 14′ 43′′ S, 64° 15′ 24′′ W).

Physconia muscigena is a foliose chlorolichen quite common in the Antarctics. Thallus of *P. muscigena* is pale gray-brown to dark brown. Thallus upper surface might be coated with pruina that disappear when thallus is rehydrated. Then thallus color turns into a brighter green. At James Ross Island, *P. muscigena* is quite common in moist habitats. It grows also on wet soil surface or over mosses. *U. antarctica* is a typical macrolichen with a foliose morphotype of thallus. The species is quite abundant in maritime Antarctica. Specifically at the Galindez island, it grows at rock surfaces close to Woozle Hill. It is found also at neigbouring islands (Green Island, Berthelot Island) and at the Antarctic peninsula west coast (Cape Tuxen,

Waddington Bay - M. Barták, unpublished information).

Prior to the chlorophyll fluorescence measurements, dry thalli were allowed to rehydrate for 48 h. After rehydration and full activation of primary photochemical processes (tested by F_V/F_M, whenever the parameter reached maximum value, the thalli were considered optimally rehydrated).

Then, the lichen samples were subjected to the measurements of chlorophyll fluorescence imaging at different temperatures so that ChlF transient could be recorded and ChlF parameters determined. Lichen thalli were pre-darkened for 5 min. inside the measuring compartment of a FluorCam HFC-010 fluorometer (Photon Systems Instruments, Czech Republic). Then Kautsky curves (transients) supplemented with analysis of quenching mechanisms were measured. The method consisted of measurements of chlorophyll fluorescence curves induced by an actinic light supplemented with saturation pulses given during at dark (F_M induction), and when the actinic light was on (F_M' induction). Potential (F_V/F_M), effective quantum yield (Φ_{PSII}) and non-photochemical quenching (NPQ) were calculated.

Fig. 1. Experimental species used in this study: *Umbilicaria antarctica* (upper panel), *Physconia muscigena* (lower panel).

Temperature treatment

In our study, we exploited slow ChlF transient approach to evaluate a short-term response the two Antarctic chlorolichens to decreasing thallus temperature (20, 15, 10 and 5°C) with a 10 min. acclimation at each temperature (Fig. 2). For such short-term temperature treatment, a ConBrio Cooling unit (ConBrio, Czech Republic) linked to Labio thermostat (Labio, Czech Republic) were used.

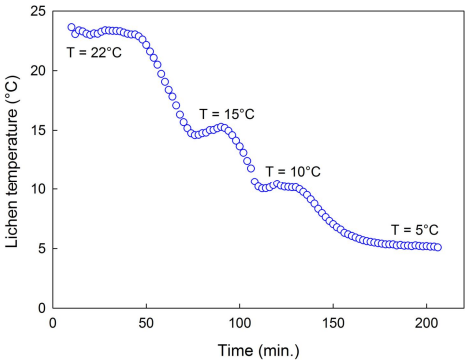

Fig. 2. Record of thallus temperature during experimental cooling of lichens to a target temperature, *i.e.* 22, 15, 10 and 5°C.

Lichen thalli were placed into a Petri dish and fixed. The Petri dish was placed on cooled aluminium plate (a part of ConBrio Cooling Unit and acclimated for 10 min. at each above-specified temperature. During acclimation and ChlF measurements, thallus temperature was measured by a hairy Cu-Co thermocouple in 2 min. interval and stored into a data logger (Edge Box, Environmental Measuring Systems, Brno, Czech Republic). On-line control of thallus temperature was allowed thanks to a EMS software.

Measurements of Chlorophyll fluorescence

At each temperature, measurements of ChlF were made using a HFC-010 fluorometer and a FluorCam07 software (Photon Systems Instruments, Brno, Czech Republic). Kautsky kinetics supplemented with quenching analysis was used that comprised of a saturation pulse applied in dark-adapted state to induce F_M followed by a 10 s of dark adaptation. Then, the samples were exposed to actinic light (AL = 4) for 300 s and a polyphasic time course of ChlF emission was recorded. When a steady state ChlF was reached (typically after 300 s), another saturation pulse was applied to induce F_M' level of ChlF, *i.e.* maximum value in light-adapted material. After switching of actinic light, background ChlF (F_0') was recorded for 20 s. Then, another saturation pulse was given in order to induce F_M'' value. Standard ChlF parameters were calculated by a FluorCam software. From those, F_V/F_M, Φ_{PSII}, NPQ, Fs, R_{Fd} (*sensu* Lichtenthaler et al. 2005), their dependence on experimental temperature, respectively, are presented in this study.

Records of ChlF transients for particular species and experimental temperature were analyzed. ChlF levels P, S, M, T were identified as well as the times at which they were reached. From linear part of ChlF transient between (1) O to P, and (2) M to S, chlorophyll fluorescence increase rate (FR) was calculated according to Smillie et Hetherington (1984). Effects of experimental temperature on the above-specified ChlF parameters were then evaluated.

Results and Discussion

Slow ChlF transients showed temperature-dependent changes in their time courses (Fig. 3) as well as parameters derived from the transients: R_{Fd} F_P/F_S ratio, FR and induction time at which M peak was achieved. These parameters were well related to ChlF parameters obtained by a saturation pulse method: F_V/F_M, Φ_{PSII} (effective quantum yield of photosynthetic processes in PS II), NPQ (non-photochemical quenching) since they were found temperature-dependent (Fig. 4), similarly to our earlier studies. It was, therefore, concluded that species-specific changes in the slow ChlF transients can be potentially used as indicators of low temperature stress in photosynthetic apparatus of lichen algal photobionts, altered redox state of plastochinone pool, and energy-dependent quenching in particular.

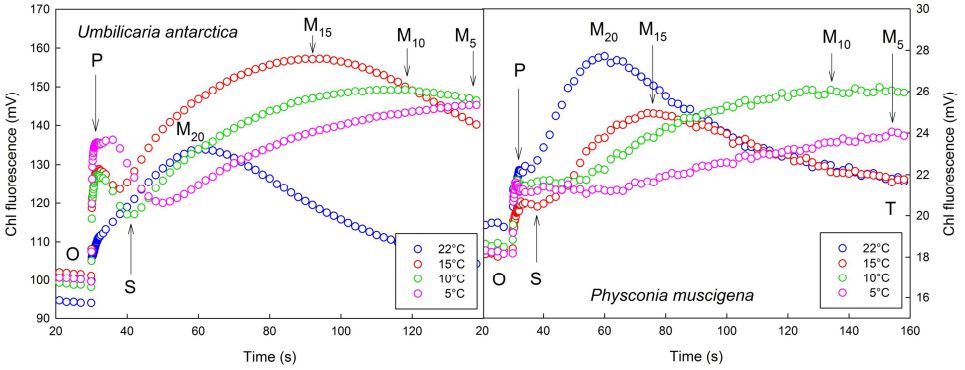

Fig. 3. Initial parts of slow chlorophyll fluorescence transient for *Umbilicaria antarctica* (left*)* and *Physconia muscigena* (right) with an indication of O, P, S, M levels. The transients for individual species are means of 4 replicates measured at each experimental thallus temperature decresing from 22 to 5°C with at least 20 minute acclimation at each temperature (22, 15, 10 and 5°C). Modified from Marečková et Barták 2016.

In both species, the PSMT phase exhibited temperature-dependent changes. Among them, the change in P/S, P/M, and S/M, and M/T ratios were apparent (*see* Table 1) documenting temperature effects of ChlF transient. With temperature decrease, the time, at which M level of Chl fluorescence was reached, increased dramatically (up to tens of seconds). This is contrasting to the evidence from higher plants exposed to low temperature where P, S and M ChlF levels are reached withing the first 5 seconds of exposition to light (Srand et Lundmark 1987). Moreover, in higher plants two M levels (peaks) can be distinguised (Roháček et Barták 1999). The fisrt one, denoted as M1, appearing typically at 4-10 s of exposition to light is attributed to changes in background ChlF (F_0') during the first few secons of the exposition to light. Typically, F_0' increases within this period in higher plants (Roháček 2002, Roháček et al. 2008). The second one, M2, appearing typically at 40-60 s of light exposition is related to the changes in carboxylation in Calvin-Benson cycle and attributed to a feedback limitation of photosynthetic linear electron flow due to accumulation of ATP and NADPH. Whenever ATP and NADPH consuption increases thanks to accelerated CO_2 fixation, ChlF tends to decrease from M2 peak to T (steady state) ChlF level. However, time at which M

peaks are reached within ChlF transients, seems to be highly dependent on a lichen species (*see* Conti et al. 2014, *Usnea antarctica*, *Stereocaulon vesuvianum*), actual extent of photoinhibition (Barták et al. 2012) and acclimation to particular experimental temperature. In our study, *U. antarctica* and *P. muscigena* showed M1 and M2 peaks only at 10 and 5°C while M1 was missing at the temperature above 10°C. This phenomenon will be focused in more details in further study.

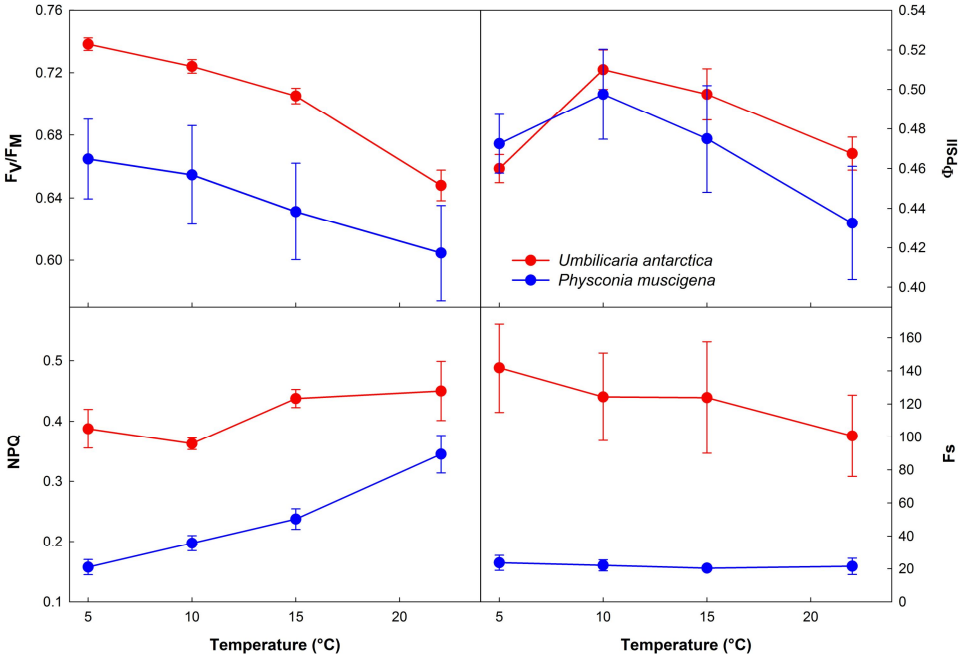

Fig. 4. Dependence of chlorophyll fluorescence parameters on thallus temperature: F_V/F_M – potential yield of photosynthetic processes in photosystem II, Φ_{PSII} – effective quantum yield of photochemical processes in photosystem II, NPQ – non-photochemical quenching, Fs – steady-state chlorophyll fluorescence (equivalent to T chlorophyll fluorescence level). Data points are means of 4 replicates. Error bars represent standard deviations.

In our study done in *P. muscigena* an *U. antarctica*, all the above-specified parameters related to particular ChlF levels reached during exposition to (actinic) light reflected temperature-induced changes in PSMT time courses. To attribute particular changes to individual factors is rather difficult since many interconnected dynamic processes take place. During SMT phase of ChlF transient, the Calvin–Benson cycle and several other correlated physiological processes are induced. Simultaneously, mechanisms forming non-photochemical quenching (qE, qT, qI) are activated. Before reaching T phase, different regulatory processes co-act until final steady-state chlorophyll fluorescence signal is reached (*i.e.* T level). Among them, state changes (transition from State 1 ↔ State 2), *i.e.* short-term regulatory mechanisms are effective (*see* Murata 2009 for review). They comprise light-dependent activation of the kinase which phosphorylates the light-harvesting complexes (LHCII), followed by the release of LHCII from photosystem II and its migration to photosys-

tem I (Lemeille et Rochaix 2011) where absorbed light energy is delivered to core of PS I. This leads to a decrease in ChlF signal. Such mechanism is well documented for green alga *Chlamydomonas reinhardtii*. (Minagawa 2011) cells where it allows to switch between photosynthetic linear and cyclic electron flow. The latter study investigated role of PS II core proteins (CPs) in *C. reinhardtii*. In lichen symbiotic alga of genus *Trebouxia*, however, state 1 - state 2 transition has not yet been studied. It is present in the species since state1-state 2 transition is considered common mechanism in higher plants and green algae.

In *U. antarctica* and *P. muscigena*, M ChlF level was found higher then P level. When such phenomenon is observed in cyanobacteria, it is attributed to a subsequent State 2 → State 1 change taking place during the S to M rise Tsimili-Michael et al. (2009), Kaňa et al. (2012). For unicellular green algae such phenomenon is reported (*see e.g.* Finazzi et al. 2001 – *Chlamydomonas reinhardtii*) and related to migration of LHCII from PS I to PS II after LHC dephosphorylation. For *Trebouxia* sp., however, it still has not been experimentally proven. Moreover, value of ChlF level at P point was in majority of cases close to T ChlF level, which brought some difficulties with R_{Fd} evaluation. Since, the T ChlF level was even higher than the P ChlF level at 5°C (resulting in negative R_{Fd} values), application of R_{Fd} as an indicator of physiological state of a lichen (in response to temperature – direct, short – term effect) is disputable. R_{Fd}, however, might be used in evaluation of other stressors effects in lichens, such as *e.g.* heavy metals impact (Takacs et al. 1999), partial hydration of thalli, and high light stress (Valladares et al. 1995).

Umbilicaria antarctica	Temperature (°C)							
	5°C		10°C		15°C		22°C	
	Mean	Std	Mean	Std	Mean	Std	Mean	Std
P/S	1.020	± 0.011	0.516	± 0.504	0.513	± 0.357	0.472	± 0.067
P/M	0.896	± 0.014	0.455	± 0.441	0.451	± 0.312	0.415	± 0.060
S/M	0.878	± 0.015	0.447	± 0.432	0.443	± 0.305	0.407	± 0.059
M/T	0.980	± 0.014	0.497	± 0.483	0.493	± 0.342	0.454	± 0.065
R_{Fd}	-0.122	± 0.009	-0.057	± 0.066	-0.026	± 0.070	0.013	± 0.056
Physconia muscigena	Temperature (°C)							
	5°C		10°C		15°C		22°C	
	Mean	Std	Mean	Std	Mean	Std	Mean	Std
P/S	1.130	± 0.007	0.569	± 0.561	0.567	± 0.397	0.523	± 0.073
P/M	0.925	± 0.021	0.473	± 0.452	0.468	± 0.320	0.428	± 0.063
S/M	0.819	± 0.024	0.422	± 0.398	0.416	± 0.281	0.379	± 0.057
M/T	1.070	± 0.022	0.546	± 0.524	0.540	± 0.371	0.495	± 0.072
R_{Fd}	-0.011	± 0.002	-0.004	± 0.006	-0.002	± 0.007	0.002	± 0.005

Table 1. Ratio parameters derived from slow chlorophyll fluorescence transients, their P, S, M, T levels in particular.

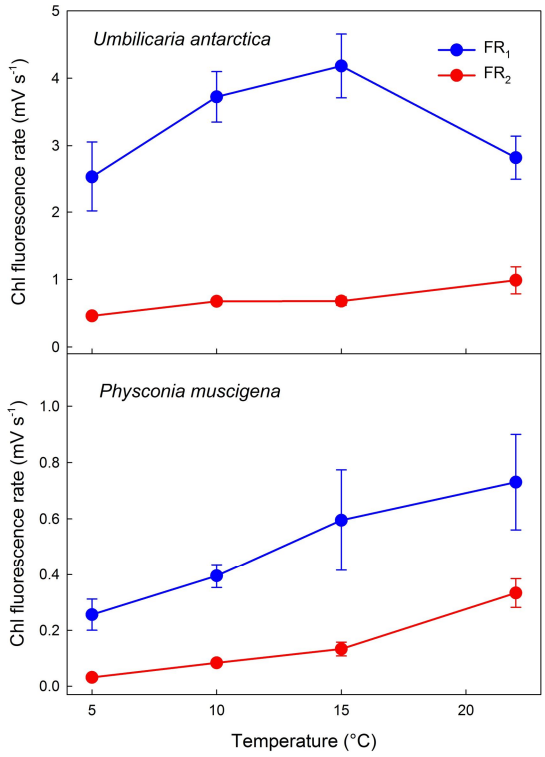

Fig. 5. Rates of chlorophyll fluorescence increase (FR_1, FR_2) measured on lichens *Umbilicaria antarctica* (upper panel) and *Physconia muscigena* (lower panel) at the temperature decreasing from 22 to 5°C (particular experimental temperatures were 22, 15, 10 and 5°C). Data points are means of 4 replicates. Error bars represent standard deviations. FR_1 represents the increase between O and P chlorophyll fluorescence levels. FR_2 represents the increase between S and M chlorophyll fluorescence level.

Conclusions

ChlF transient, their O, P, S, M, T chlorophyll fluorescence levels, and derived parameters respectively, can be used easily as early indicators of direct effects of temperature on primary photosynthetic processes in chlorolichens. Redox state on plastoquinone pool and the involvement on mechanisms forming non-photochemical quenching leads to the changes in P, S, M, T ChlF levels and the times at which they are reached. In this paper, we suggest to use the ratios P/M, P/T, M/T as indicators of short-term temperature effect on photochemical processes of photosynthesis, as well as FR_1, FR_2, which are the rates of ChlF increase between O and P, and S and M ChlF levels (Fig. 5). All the above-mentioned parameters should be related to traditional ones, such as *e.g.* F_V/F_M, Φ_{PSII}, NPQ in order to evaluate direct effects of a short-term temperature treatment on particular photochemical processes in thylakoid membrane of chloroplast of symbiotic alga.

The results presented in this study showed that Φ_{PSII} indicated temperature optimum of photosynthetic processes at 10°C for both species. Short-term treat-

ment at low temperature (5°C) did not induce activation of non-photochemical quenching, *i.e.* there is no demand for protective mechanisms at 5°C. This conclusion is consistent with an earlier study done in *U. antarctica* (Barták et al. 2007). In presented study focused on *P. muscigena* and *U. antarctica* F_V/F_M showed an increase with temperature fall, *i.e.* no temperature-induced limitation of capacity of primary photosynthetic processes in photosystem II was apparent. The two experimental species exhibited similar responses (trends) in O, P, S, M, T changes with temperature fall, however, species-specific differences were apparent. The same was true for ChlF pa-rameters F_V/F_M, Φ_{PSII}, NPQ.

References

AGATI, G., CEROVIC, Z. G. and MOYA, I. (2000): The effect of decreasing temperature up to chilling values on the in vivo F685/F735 chlorophyll fluorescence ratio in *Phaseolus vulgaris* and *Pisum sativum*: The role of the photosystem I contribution to the 735 nm fluorescence band. *Photochemistry and Photobiol*ogy, 72: 75-84.

ALLEN, J. F. (1992): Protein phosphorylation in regulation of photosynthesis. *Biochimica et Biophysica Acta*, 1098: 275–335.

BARTÁK, M., HÁJEK, J. and OČENÁŠOVÁ, P. (2012): Photoinhibition of photosynthesis in Antarctic lichen *Usnea antarctica*. I. Light intensity- and light duration-dependent changes in functioning of photosystem II. *Czech Polar Reports*, 2: 42-51.

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,* 31:47–51.

BARTÁK, M., VÁCZI, P., STACHOŇ , Z. and KUBEŠOVÁ, S. (2015): Vegetation mapping of moss-dominated areas of northern part of James Ross Island (Antarctica) and a suggestion of protective measures. *Czech Polar Reports*, 5: 75-87.

BOLHAR-NORDENKAMPF, H., ÖQUIST, G. (1993): Chlorophyll fluorescence as a tool in photosynthesis research. *In*: D. O. Hall, J. M. O. Scurlock, H. R. BolharNordenkampf, R. C. Leegood, S.P. Long (eds.): Photosynthesis and Production in a Changing Environment, Chapman & Hall, London, pp. 193-206.

CONTI, S., HAZDROVÁ, J., HÁJEK, J., OČENÁŠOVÁ, P., BARTÁK, M., SKÁCELOVÁ, K. and ADAMO, P. (2014): Comparative analysis of heterogeneity of primary photosynthetic processes within fruticose lichen thalli: Preliminary study of interspecific differences. *Czech Polar Reports*, 4: 149-157.

FINAZZI, G., BARBAGALO, R. P., BERGO, E., BARBATO, R. and FORTI, G. (2001): Photoinhibition of Chlamydomonas reinhardtii in State 1 and State 2. Damages to the photosynthetic apparatus under linear and cyclic electron flow. *The Journal of Biological Chemistry*, 276: 22251-22257.

FLEXAS, J., ESCALONA, J. M., EVAIN, S., GULÍAS, J., MOYA, I., OSMOND, C.B. and MEDRANO, H. (2002): Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO_2 assimilation and stomatal conductance during water-stress in C3 plants. *Physiologia Plantarum*, 114: 231-240.

KAŇA, R., KOTABOVÁ, E., KOMÁREK, O., ŠEDIVÁ, B., PAPAGEORGIOU, G. C., GOVINDJEE and PRÁŠIL, O. (2012): The slow S to M fluorescence rise in cyanobacteria is due to a state 2 to state 1 transition. *Biochimica et Biophysica Acta,* 1817: 1237-1247.

KRAUSE, G.H. (1988): Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiologia Plantarum*, 74: 566-574.

KODRU, S., MALAVATH, T., DEVADASU, E., NELLAEPALLI , S., STIRBET, A., SUBRAMANYAM, R. and GOVINDJEE (2015): The slow S to M rise of chlorophyll a fluorescence reflects transition from state 2 to state 1 in the green alga *Chlamydomonas reinhardtii*. *Photosynthesis Research,* 125:219-231.

LEMEILLE, S., ROCHAIX, J.-D. (2011): State transitions at the crossroad of thylakoid signaling pathways. *Photosynthesis Research*, 106: 33-46.

LICHTENTHALER, H. K., BUSCHMANN, C. and KNAPP, M. (2005): How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio R_{Fd} of leaves with the PAM fluorometer. *Photosynthetica*, 43: 379-393.

LICHTENTHALER, H. K., BUSCHMANN, C., RINDERLE, U. and SCHMUCK, G. (1986): Application of chlorophyll fluorescence in ecophysiology. – Radiatiom Environment. *Biophysics*, 25: 297-308.

LICHTENTHALER, H. K., LANGSDORF, G., LENK, S. and BUSCHMANN, C. (2005): Chlorophyll fluorescence imaging of photosynthetic activity with the flash-lamp fluorescence imaging system. *Photosynthetica*, 43: 355-369.

MAREČKOVÁ, M., BARTÁK, M. (2016): Interspecific differences in photosynthetic parameters of Antarctic foliose lichens at low temperature as evaluated by chlorophyll fluorescence parameters. Students in Polar and Alpine Research, Brno.

MINAGAWA, J. (2011): State transitions - the molecular remodeling of photosynthetic supercomplexes that controls energy flow in the chloroplast. *Biochimica et Biophysica Acta*, 1807: 897-905.

MISHRA, A., HEYER, A. G. and MISHRA, K. B. (2014): Chlorophyll fluorescence emission can screen cold tolerance of cold acclimated *Arabidopsis thaliana* accessions. *Plant Methods*, 38:1-10.

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: 99-111.

MURATA, N. (2009): The discovery of state transitions in photosynthesis 40 years ago. *Photosynthesis Research*, 99: 155-160.

NABE, H., FUNABIKI, R., KASHINO, Y., KOIKE, H. and SATOH, K. (2007): Responses to desiccation stress in Bryophytes and an important role of dithiothreitol-insensitive non-photochemical quenching against photoinhibition in dehydrated states. *Plant Cell Physiology*, 48: 1548–1557.

NOCTOR G., HORTON, P. (1990): Uncouple titration of energy-dependent chlorophyll fluorescence quenching and photosystem II photochemical yield in intact pea chloroplasts. *Biochimica et Biophysica Acta*, 1016:228-234.

Papageorgiou, G. C., Govindjee (1968): Light-induced changes in the fluorescence yield of chlorophyll a *in vivo*. I. Anacystis nidulans. *Biophysical Journal*, 8: 1299-1315.

PAPAGEORGIOU, G. C., GOVINDJEE (2011): Photosystem II fluorescence slow changes – scaling from the past. *Journal of Photochemistry and Photobiology B: Biology*, 104: 258-270.

RIZNICHENKO, G., LEBEDEVA, G., POGOSYAN, S., SIVCHENKO, M. and RUBIN, A. (1996): Fluorescence induction curves registered from individual microalgae cenobiums in the process of population growth. *Photosynthesis Research* , 49: 151-157.

ROHÁČEK, K. (2002): Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationships. *Photosynthetica*, 40: 13-29.

ROHÁČEK, K., BARTÁK, M. (1999): Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. *Photosynthetica*, 37: 339-363.

ROHÁČEK, K., SOUKUPOVÁ, J. and BARTÁK, M. (2008): Chlorophyll Fluorescence: A wonderful tool to study plant physiology and plant stress. *In*: B. Schoefs (ed.): Plant Cell Compartments – Selected Topics. Research Signpost, Kerala, India, pp. 41-104.

SEATON G.G., WALKER, D.D. (1990) : Chlorophyll fluorescence as a measure of carbon metabolism. *Proceedings of Royal Society (London),* B242: 29-35.

SMILLIE, R. M., HETHERINGTON, S. E. (1984). A screening method for chilling tolerance using chlorophyll fluorescence in vivo. *In*: C. Sybesma (ed.): Advances in Photosynthesis Research. Proceedings of the VIth International Congress on Photosynthesis, Brussels, Belgium, August 1–6, 1983. Volume IV, Publisher Springer Netherlands, pp. 471-474. ISBN 978-90-247-2945-6, DOI: 10.1007/978-94-017-4971-8_104.

STIRBET, A., RIZNICHENKO, G. YU., RUBIN, A. B. and GOVINDJEE (2014): Modeling chlorophyll a fluorescence transient: Relation to photosynthesis. *Biochemistry (Moscow)*, 79: 291-323.

SRAND, M., LUNDMARK, T. (1987): Effects of low night temperature and light on chlorophyll fluorescence of field-grown seedlings of Scots pine (*Pinus sylvestris* L.). *Tree Physiology*, 3: 211-224.

TAKACS, Z., CSINTALAN, Z. and TUBA, Z. (1999): Responses of the lichen *Cladonia convoluta* to high CO_2 level and heavy metal treatment. *Zeitschrift für Naturforschung*, 54c: 797-801.

TSIMILLI-MICHAEL, M., STAMATAKIS, K. and PAPAGEORGIOU, G. C. (2009): Dark-to-light transition in *Synechococcus* sp. PCC 7942 cells studied by fluorescence kinetics assesses plastoquinone redox poise in the dark and photosystem II fluorescence component and dynamics during state 2 to state 1 transition.. *Photosynthesis Research*, 99: 243-255.

VALLADARES, F., SANCHEZ-HOYOS, A. and MANRIQUE, E. (1995): Diurnal changes in photosynthetic efficiency and carotenoid composition of the lichen *Anaptychia ciliaris*: Effects of hydration and light intensity. *The Bryologist*, 98: 375-382.

VERNOTTE, C., ETIENNE, A. L. and BRIANTAIS, J. M. (1979): Quenching of the system II chlorophyll fluorescence by the plastoquinone pool. *Biochimica et Biophysica Acta*, 545: 519-527.