Polar Biology (2019) 42:685–701
https://doi.org/10.1007/s00300-019-02464-w

ORIGINAL PAPER

Temperature effects on photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum*: a chlorophyll fluorescence study

Michaela Marečková[1] · Miloš Barták[1] · Josef Hájek[1]

Received: 24 May 2018 / Revised: 17 October 2018 / Accepted: 4 February 2019 / Published online: 4 March 2019

Abstract

Chlorophyll fluorescence is an important indicator of a photosynthetic energy conversion in chloroplast photosystem II and responds sensitively to stress factors affecting photosynthesizing organisms. Three different methods were employed to identify the most sensitive fluorescence parameters responding to thallus temperature decrease within Antarctic lichen *Dermatocarpon polyphyllizum*: (1) Fast chlorophyll fluorescence transient (OJIP with parameters characterizing photosystem II functioning) (2) Slow Kautsky kinetics supplemented by saturation pulses (to evaluate quantum yield of photosynthetic processes in photosystem II, as well as maximum quantum PSII efficiency and non-photochemical and photochemical quenching), and (3) Linear cooling from + 22 to − 40 °C (to determine change in Φ_{PSII} and the critical temperature for PSII). A K-step (usually documented at highly stressed organisms) was found in OJIPs measured at + 22 °C at 0.22–0.40 ms and attributed to the negative effect of high temperature on PSII functioning, PSII donor side limitation in particular. At subzero temperature (− 0.5, − 5 °C), an L-step was detected at 0.05 ms and related to a low temperature-induced decrease in connectivity between light-harvesting complexes and PSII. An increase of DI_0/RC (the flux of dissipated excitation energy) was reported for the first time in lichens. The OJIP-derived parameters, DI_0/RC and Phi_D_0 (quantum yield of energy dissipation) in particular, indicated that they might be used for the detection of early events in low temperature-affected lichens. Linear cooling data determined the critical temperature (− 12 °C) for primary photosynthetic processes (Φ_{PSII}) in *Dermatocarpon*.

Keywords *Diplosphaera* sp. · OJIP · K-step · Kautsky kinetic · Linear cooling · Photosystem II

Abbreviations

ChlF	Chlorophyll fluorescence
DA	Dark adapted
KK	Kautsky kinetics
LA	Light adapted
LHC	Light-harvesting complexes
OJIP	Fast chlorophyll fluorescence transient
PI	Performance (vitality) Index
PS	Photosystem
RC	Reaction center

Introduction

Lichens from polar regions are well adapted to low temperature and able to photosynthesize even at subzero temperatures. Primary photosynthetic processes related to PSII and a chloroplast thylakoid membrane are still effective at freezing temperatures, as reported by several chlorophyll fluorescence studies in polar lichens (Barták et al. 2007; Hájek et al. 2016; Míguez et al. 2017). This is because of several adaptive mechanisms, such as and including osmotically active compounds that decrease the freezing point of the cytoplasm and ice-nucleation proteins preventing the creation of ice crystals with sharp edges that could damage the cell organelles.

Chlorophyll fluorescence (ChlF) is a non-invasive method resulting in numerous indicators of photosynthetic energy conversion. Temperature response curves of chlorophyll fluorescence parameters are routinely used throughout the Plant Kingdom. In lichens, the effect of low and freezing temperature on chlorophyll fluorescence parameters has been studied as well (see e.g. Hájek et al. 2016). Despite the fact that

✉ Josef Hájek
jhajek@sci.muni.cz

[1] Department of Plant Physiology and Anatomy, Institute of Experimental Biology, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic

lichen temperature optima for photosynthesis are above 0 °C, many lichens perform photosynthesis at below-zero temperature. Kappen and Schroeter (1997) measured active photosynthesis of *Usnea* and *Umbilicaria* species at temperatures as low as − 20 °C. Other lichen species (e.g. *Umbilicaria antarctica, Xanthoria elegans*) also showed detectable photosynthetic activity at − 10 °C (according to the measured parameters F_V/F_M-maximum quantum efficiency of photosystem II, Φ_{PSII}-effective quantum yield of photosystem II and NPQ-photochemical quenching, Barták et al. 2007). The most apparent decrease of chlorophyll fluorescence (ChlF) parameters for these lichen species was found at − 5 °C, the temperature reported to be critical for ice nucleation in fully hydrated lichen thalli during gradual freezing (Schroeter and Scheidegger 1995; Barták et al. 2007). Below that temperature, a substantial inhibition of photochemical processes of photosynthesis begins. This goes along with an increase of non-photochemical quenching (Hájek et al. 2001), when the photosystem dissipates excess excitation energy absorbed with light-harvesting complexes (LHC) of PSII. At freezing temperature, photosynthetic linear electron transport through thylakoid membrane is limited. It results in reduced formation of ATP and NADPH in the biochemical part of the photosynthetic process (Barták et al. 2007). Since the utilization rate of ATP and NADPH in photosynthetic and non-photosynthetic processes is also temperature-dependent, simultaneous measurement of chlorophyll fluorescence parameters (effective quantum yield in particular) and the rate of CO_2 exchange is beneficial. In previous decades, several studies used a combined approach in analyzes focused on lichen/photobiont responses to low-temperature stress (Kappen et al. 1998; MacKenzie et al. 2001; Lange 2002; Piccotto and Tretiach 2010).

In chlorophyll fluorescence studies in lichens, two major groups of methods are used: (1) fast chlorophyll fluorescence transient (OJIP, e.g. Ilík et al. 2006; Lazár 2009), and (2) slow chlorophyll fluorescence induction (or Kautsky kinetic, e.g. Barták et al. 2007; Hájek et al. 2012). These two procedures could be considered complementary, since OJIPs predominantly refer to the intrinsic PSII processes, while slow Kautsky kinetics relate to the chloroplastic linear electron transport chain.

The first group of methods is fast chlorophyll fluorescence (OJIP curves). It is measured within the first 2 s after light exposure on a pre-darkened sample. In OJIP phase, chlorophyll *a* fluorescence rise from a minimal value F_0 (O), when reaction centers are fully oxidized, through intermediate inflections (I and J) to a peak F_P (P) with gradual reduction of plastoquinone Q_A and Q_B. Photochemical phase (O to J rise of ChlF) is strongly affected by the amount of absorbed photons and is not very sensitive to temperature changes (Strasser et al. 1995; Stirbett et al. 2014; Mishra et al. 2015). It is generally accepted, that the O–J phase is related to the balance of PSII primary electron acceptor reduction (Q_A) and its reoxidation by Q_B (Boisvert et al. 2006). Sometimes, small K-step is present and measured at 0.3 ms (between O and J) in highly stressed organisms. The K-step reflects a dissociation of oxygen-evolving complexes and diverse energy distribution as well as a progressive decrease in photochemical processes rates (Xue et al. 2011; Gururani et al. 2012; Martinazzo et al. 2012). The K-step formation is directly caused by an electron outflow from P680 (PSII primary donor) to PSII acceptors, which over-compensates the electron inflow from the donor side of photosystem II to P680 (Kalaji et al. 2016). A relative variable fluorescence in the K-step is generally more sensitive to stress than basic fluorescence parameter F_V/F_M (maximum quantum efficiency of PSII photochemistry, Brestic et al. 2013). The J–I–P phase is called "thermal", since it is more sensitive to temperature changes than the O–J photochemical phase. Inflection point I reflects a transient steady-state of electron transport. At this point, plastoquinone pool reduction by photosystem II and its oxidation by photosystem I (Cyt b_6/f) are in balance (Schansker et al. 2005). In certain photosynthesizing organisms, such as foraminifers (Tsimilli-Michael et al. 1999) or lichens (Ilík et al. 2006), a well-distinguishable split of the peak P (maximal ChlF) into two steps (H, G) was observed. The H-step is caused by removal of limitation on the acceptor side of photosystem I (PSI), probably resulting from cyclic electron flow around PSI. The G-step follows after a fluorescence decrease which is caused by the plastoquinone pool (and Q_A) re-reduction (Ilík et al. 2006).

It is well established that high temperature stress often reveals a K-step in OJIP curves (see e.g. Brestic et al. 2013). Therefore, OJIPs represent a useful tool to study PSII thermostability, usually by estimating a critical temperature at which K-step appears. At such temperature, a serious disorganization of PSII structure and thylakoid membrane components appears, and loss of photosynthetic apparatus main functions occurs (Brestic et al. 2013).

In studies focused on low-temperature stress, OJIPs are employed as indicators both in higher plants (Kalaji et al. 2016) and lichens (Marečková and Barták 2017). Long-term low-temperature stress causes a decrease in chlorophyll content and oxygen-evolving complex efficiency on the PSII donor side. Such changes affect overall PSII effectivity and low-temperature-induced inhibition on donor and acceptor sides (Bao et al. 2010). Lichen thallus temperature decline to freezing values induces a decrease of variable ChlF, although ChlF parameter responses are very species-specific (Mishra et al. 2015). The most widely used parameter from OJIP transients is the Performance Index (PI, also called Vitality Index). This reflects both PSII and PSI functionality, and therefore, provides quantitative information about the general state of plants. PI is an integrative parameter that includes three independent characteristics: (1) concentration

of reaction centers per chlorophyll (2) electron movement efficiency of trapped excitation energy into the electron transport chain beyond Q_A and (3) probability that absorbed photon will be trapped by reaction centers (Strasser et al. 2004; Zivcak et al. 2008; Kalaji et al. 2016). According to many studies, PI appears to be very sensitive to any stress (Strasser et al. 2000, 2004; Żurek et al. 2014; Kalaji et al. 2016). In our previous study of *Dermatocarpon polyphyllizum* (Marečková and Barták 2017), we measured and analyzed OJIPs taken at a wide range of above-zero temperatures. We recorded a K-step presence with samples exposed to + 22 °C and attributed this to temperature stress at the donor side of photosystem II and inhibition of an oxygen-evolving complex (OEC).

The second group of methods includes slow Kautsky kinetics, typically supplemented with several saturation pulses and consequent analysis of quenching mechanisms, measured during a pre-darkened sample's exposure to several minutes of continuous light. At first, initial fluorescence (F_0) is measured with a dim measuring light, followed by strong actinic light which raises chlorophyll fluorescence to maximum level (F_M). At this state, only photosystem II is involved. Subsequently, photosystem I begins to operate and drains electrons from PSII, and chlorophyll fluorescence decreases to steady-state F_S (Roháček et al. 2008). At the steady state, another saturation pulse is applied typically, and, consequently, chlorophyll fluorescence rises to a maximum (F_M'). The F_M' value is used for a calculation of effective quantum yield of photosynthetic processes in PSII and the evaluation of quenching mechanisms, non-photochemical quenching in particular (Lichtenthaler et al. 2005).

With the third method (linear cooling), measurements are continuous while the temperature changes quickly (2.0 °C min^{-1}). This allows us to evaluate sample cryoresistance and determine the critical temperature evincing primary photosynthetic process full inhibition.

Several methods based on chlorophyll fluorescence measurement were used to identify the most sensitive parameters responding to thallus temperature. We assumed that a combination of sensitive parameters could be used in lichen ecophysiological studies as a proxy for photosynthesis in response to temperature. Three groups of methods were used: (1) fast (2) slow chlorophyll fluorescence curves measured at different temperature, and (3) chlorophyll fluorescence monitored during linear cooling from 18 to − 40 °C. Since the fast chlorophyll fluorescence curve reflects the processes in reaction centers and the photosystem II complex, we hypothesized that electron transport (quantum yield for electron transport-Phi_E_0, efficiency of electron transport-Psi_E_0 and electron transport flux-ET_0/RC) would be the most affected, and therefore, sensitive to temperature change. Slow Kautsky kinetics reflected the entire chloroplast photosynthetic electron transport chain, co-acting processes of non-photochemical quenching of absorbed light energy, and, last but not least, biochemical processes of photosynthesis, in particular ATP and NADPH utilization in carbon dioxide fixation. Hence, we expected a decrease of PSII maximal quantum yield and effective quantum yield with temperature decrease and a high sensitivity of Φ_{PSII} to temperature. We hypothesized that a positive relation would exist between OJIP- and KK-derived parameters under non-stressed conditions. Whenever temperature effects generate stress to PSII and primary photosynthetic processes, then the relation between OJIP- and KK-derived parameters should be imbalanced. Consequently, our study focused on chlorophyll fluorescence parameters measured in *D. polyphyllizum* by both techniques within the temperature range of + 22 °C to − 5 °C. Linear cooling represented a supplementary method used primarily in critical temperature evaluation, i.e., freezing temperature in which photosynthetic processes are fully inhibited. In our study, critical temperature was used for interspecific comparison of Antarctic lichens and their photobionts evaluated by previous procedure (Hájek et al. 2012, 2016).

Material and methods

Species

Dermatocarpon polyphyllizum (Nyl.) Blomb. & Forssell is a lichen species occurring typically in the Earth's polar regions. The species belongs to the lichen family Verrucariaceae. It usually grows on the rock surfaces in higher altitudes or in polar regions. Lichen databases report the species from Iceland, Svalbard, Scandinavia, and islands along the west and east coast of the Antarctic Peninsula as far south as Alexander Island (71°00′S, 70°00′W, e.g. Australian Antarctic Data Centre-biodiversity database, A Global Information System for Lichenized and Non-Lichenized Ascomycetes, see References, Other sources). *D. polyphyllizum* has a dark brown foliose thallus. Very little is known about the algal photobiont species of the genus *Dermatocarpon*, although the green alga *Diplosphaera chodatii* (Trebuxiophyceae) was identified as photobiont of the few members of this genus (Fontaine et al. 2012).

For our study focused on low-temperature effects on primary photosynthetic processes, several different thalli of *Dermatocarpon polyphyllizum* lichen were collected at James Ross Island, Antarctica, in February 2017 and March 2018. The northernmost part of James Ross Island represents one of the largest deglaciated areas along the Antarctic Peninsula. The monthly air temperature range is from 2.5 °C (January) to − 15.3 °C (July) measured 2 m above ground, the monthly air temperature at the surface reaches up to 15.0 °C. The snow cover is usually melted from December

to February (Láska et al. 2011). The collection site was located on the long-term research plot (LTPR) in the neighborhood of the Czech station Johann Gregor Mendel-for detailed information of the LTRP vegetation cover see Barták et al. (2015). After collection, *D. polyphyllizum* samples were dried under natural conditions and then stored in dry state at + 10 °C. After transfer to the Masaryk University laboratories (Brno, Czech Republic), samples were stored in a freezer at − 20 °C for 3–4 months. Samples collected in March 2018 were stored for only a few weeks before measurement and showed similar results. Before the experiments, all dry thalli samples were allowed to rehydrate in between two sheets of wet filter paper for at least 48 h at a temperature of + 10 °C while exposed to dim light (10 µmol m^{-2} s^{-1} of photosynthetically active radiation).

Fast chlorophyll fluorescence (OJIP) curves

Rehydrated samples of *Dermatocarpon polyphyllizum* were stored at + 10 °C. Round disks (8 mm in diameter) were cut from the samples, and upper cortex was then carefully removed. This uncovered the photobiont layer and allowed higher ChlF signals. A small square of filter paper was put under the disk to prevent sample dehydration. Three disks of *Dermatocarpon* were placed into a Petri dish with demineralized water. The Petri dish was situated into the cooling unit and acclimated to a particular experimental temperature (see below) for 10 min while exposed to light.

OJIP curves were measured using a FluorPen fluorometer (Photon Systems Instruments, Czech republic) at a particular temperature (from high to low, $T = +22, +18, +14, +12, +10, +7, +4, 0, -0.5$ and -5 °C) with 10 min acclimation each. Temperature was measured during the entire experiment by a Cu–Co thermocouple placed in a OJIP clip with lichen samples at 30 s intervals and stored in a data logger (Edge Box V12, Environmental Measuring Systems, Czech Republic). The actual disk temperatures were also recorded on-line through EMS software with a Cu–Co thermocouple previously noted.

The initial set of experiments was conducted with lichen samples exposed to light during a cooling period (18.3 µmol m^{-2} s^{-1}) and pre-darkened for 5 min before each measurement (LA-light adapted). In the other set of experiments, samples were darkened during the whole cooling experiment (DA-dark adapted). Previous testing determined that 5 min of pre-darkening was enough time for lichen species (during a darkening period F_0 and F_M were measured, and the optimum pre-darkening time was when F_0 and F_M values were consistent). Three samples from different lichen thalli were measured at each experimental setup.

Mean values of three measured OJIP transients were calculated, then double normalized between F_0 and F_K (0.3 ms) and between F_0 and F_J (10 ms) according to Oukarroum et al. (2007). The chlorophyll fluorescence data were normalized between O (0.02 ms) and K (0.3 ms) steps, as $W_{OK} = (F_t - F_0) / (F_K - F_0)$. Then, the difference $\Delta W_{OK} = W_{OK(Ti)} - W_{OK\ (Tcontrol)}$ was calculated and plotted against the time range of 0.02–0.3 ms. Resulting curves were analyzed to detect L-step (band) presence/absence at about 0.08 ms. The L-step was considered indicative of energy transfer from LHC to PSII centers (connectivity parameter).

From the OJIPs recorded at particular thallus temperature and LA/DA treatments, chlorophyll parameters evaluating PSII performance of symbiotic alga were calculated (see Table 1) as well as the time at which peak P was reached (t_P). Then, parameters were plotted in a radar plot to find the most sensitive parameters reflecting the effect of PSII photochemical reactions to temperature drops. In radar plots, changes were expressed between control (at + 18 °C) and +4 (before freezing) and − 5 °C (after freezing), respectively. The temperature + 18 °C was considered to be the optimum for *D. polyphyllizum*, because it provided the highest measured values of F_V/F_M ratio. For statistical testing the one-way ANOVA and Fisher's LSD test at $p < 0.05$ were used.

Slow chlorophyll fluorescence

The rehydrated thalli of *Dermatocarpon polyphyllizum* (five samples of different thali) were placed into a Petri dish with a low amount of demineralized water to prevent dehydration. Samples were put into a cooling unit (URAS cooler, Germany), where they were exposed to a stepwise decrease of temperature. At each temperature ($+22, +18, +14, +12, +10, +7, +4, 0, -0.5$ and -2.5 °C) samples were acclimated for 10 min before chlorophyll fluorescence measurement. Slow Kautsky kinetics supplemented with quenching mechanism analysis were measured using a FluorCam HFC-010 fluorometer (Photon Systems Instruments, Czech Republic)-for the method used with lichens, see e.g. Mishra et al. (2015). During acclimation and ChlF measurements, thallus temperature was measured by Cu–Co thermocouple at 30 s intervals and stored in a data logger (Edge Box V12, Environmental Measuring Systems, Czech Republic). The effect of thallus temperature decrease on slow Kautsky kinetics shape and derived ChlF parameters was evaluated. The following parameters were used: F_V/F_M-potential quantum yield of photochemical processes in PSII indicating capacity of photosynthetic processes in PSII, Φ_{PSII}-effective quantum yield of PSII indicating actual effectivity of photosynthetic processes in light-adapted sample, qP-photochemical quenching indicating the ratio of PSII reaction centers that are open, NPQ-non-photochemical quenching indicating the activation of the pathways, other than photosynthesis, involved into thermal dissipation of absorbed energy, energy

Table 1 Formulas and explanation of fast chlorophyll fluorescence (OJIP)-derived parameters used in study (according to Strasser et al. 2004)

$F_J \equiv F_2$ ms	Fluorescence intensity at the J-step (2 ms) of OJIP
$F_I \equiv F_{30}$ ms	Fluorescence intensity at the I-step (30 ms) of OJIP
$F_0 \cong F_{50}$ µs or $\cong F_{20}$ µs	Minimal fluorescence (all photosystem II reaction centers—PSII RCs are assumed to be open)
F_M	Maximal fluorescence, when all PSII RCs are closed
$F_V \equiv F_M - F_0$	Maximal variable fluorescence
F_V/F_M	Maximal quantum yield of PSII
F_V/F_0	Ratio of variable to minimal fluorescence
F_M/F_0	Ratio of maximal to minimal fluorescence
$ABS/RC = M_0(1/V_J)(1/Phi\_P_0)$	Absorption flux (of antenna chlorophylls) per RC
$TR_0/RC = M_0(1/V_J)$	Trapped energy flux (leading to quinone A$-Q_A$ reduction) per RC
$ET_0/RC = M_0(1/V_J)Psi\_E_0$	Electron transport flux (further than Q_A) per RC
$V_J = (F_J - F_0)/(F_M - F_0)$	Relative variable fluorescence at the J-step
$V_I = (F_I - F_0)/(F_M - F_0)$	Relative variable fluorescence at the I-step
$Psi\_0 = 1 - V_J = ET_0/TR_0$	Probability that a trapped exciton is used for electron transport beyond Q_A
$Phi\_Pav = Phi\_P_0$ (S_M/t_{FM})	Time to reach maximal chlorophyll fluorescence
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	The flux of dissipated excitation energy at time 0
$Phi\_D_0 = 1 - Phi\_P_0 = F_0/F_M$	Quantum yield (at $t = 0$) of energy dissipation
$Phi\_P_0 \equiv TR_0/ABS = [1 - (F_0/F_M)]$	Maximum quantum yield for primary photochemistry
$Psi\_E_0 \equiv ET_0/TR_0 = (1 - V_J)$	Efficiency (probability) for electron transport (ET), i.e. efficiency (probability) that an electron moves further than Q_A
$Phi\_E_0 \equiv ET_0/ABS = [1 - (F_0/F_M)]\psi E_0$	Quantum yield for electron transport (ET)
$Pi\_ABS = (RC/ABS)[Phi\_P_0/(1 - Phi\_P_0)]\ [Psi\_0/(1 - Psi\_0)]$	Performance Index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors

quenching associated with xanthophyll cycle pool, and conformational changes in chloroplast photosynthetic apparatus. For equations used in the calculations of the parameters, see Roháček (2010). For statistical testing the one-way ANOVA and Fisher's LSD test at $p < 0.05$ was used.

Chlorophyll fluorescence parameters during a linear cooling

To increase ChlF signal and its detection, four disks of rehydrated *D. polyphyllizum* with removed upper cortex were prepared. A piece of wet paper was put under the disk so that the disk would not dehydrate during measurement of chlorophyll fluorescence parameters (see below). During the experiment exploiting linear cooling, thallus temperature was measured by a Cu–Co thermocouple in 30 s intervals and stored in a data logger (Edge Box V12, Environmental Measuring Systems, Czech Republic). The lichen disk was placed into a cooling chamber of Kryo-Planer (Great Britain), then gradually cooled with liquid nitrogen from + 22 °C to − 40 °C using a constant rate of 2.0 °C min^{-1} (for details see Hájek et al. 2016). During cooling, the disk was exposed to the light of 30 µmol $m^{-2}\,s^{-1}$ (PAR) and several chlorophyll fluorescence parameters were measured by a PAM-2000 fluorometer (Heinz Walz, Germany) using the method described in Hájek et al. (2016). Measurements were repeated with four disks of *D. polyphyllizum,* and mean values were calculated. Chlorophyll fluorescence parameters F_S (steady state chlorophyll fluorescence) and Φ_{PSII} (effective quantum yield of photochemical processes in photosystem II, according to Genty et al. 1989) were evaluated and plotted against thallus temperature.

Results

Fast chlorophyll fluorescence (OJIP) curve shapes

Differences in OJIP curve shape between DA (samples darkened during the whole experiment) and LA treatment (samples pre-darkened for only 5 min before measurements) were observed. In LA samples, chlorophyll fluorescence decreased with a temperature fall to − 5 °C by the factor of two (Fig. 1, upper panel), and it decreased to a lower extent in DA samples (Fig. 1, lower panel). The differences in chlorophyll fluorescence signals between LA and DA samples are dependent on the amount of chlorophyll in lichen thalli samples. Results can vary with lichen thallus age as well as with actual sample topology in lichen thallus. Most of the OJIP-derived parameters are ratios of fluorescence signals and were not affected by differences in chlorophyll content within samples. Temperature decrease caused a reduction of the time at which peak P was reached both in LA and DA

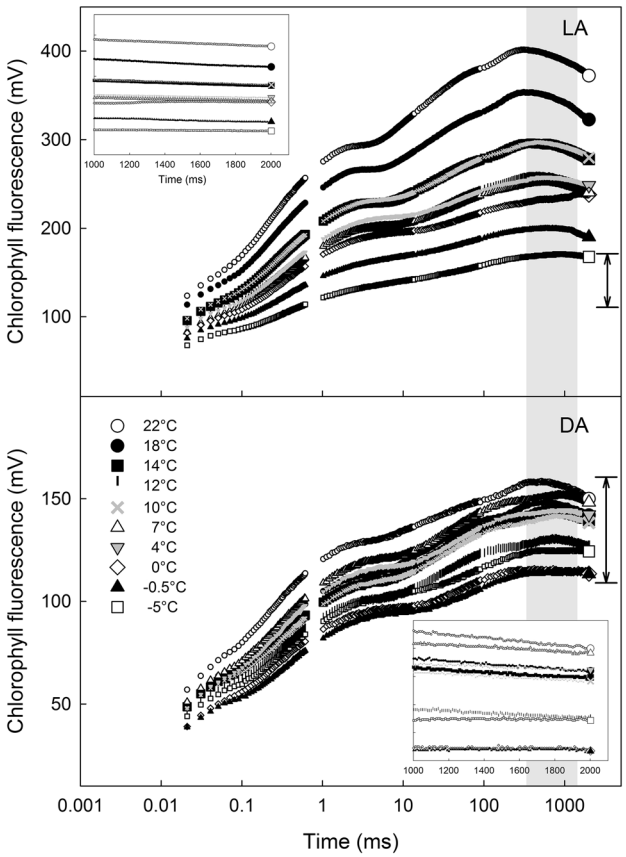

Fig. 1 Fast chlorophyll fluorescence (OJIP) curves measured in *Dermatocarpon polyphyllizum* at decreasing temperature (from + 22 °C to − 5 °C). Gray area indicates location of peaks P (from 400 to 1200 ms). For better comparison of both (LA and DA) graphs there are arrows showing the range of DA curves. Inset graphs indicate the linear decrease of chlorophyll fluorescence signals after peak P (1000–2100 ms). *Upper panel LA* light-adapted samples (exposed to light and pre-darkened for 5 min before measurement). *Lower panel DA* dark-adapted samples (in darkness during whole experiment)

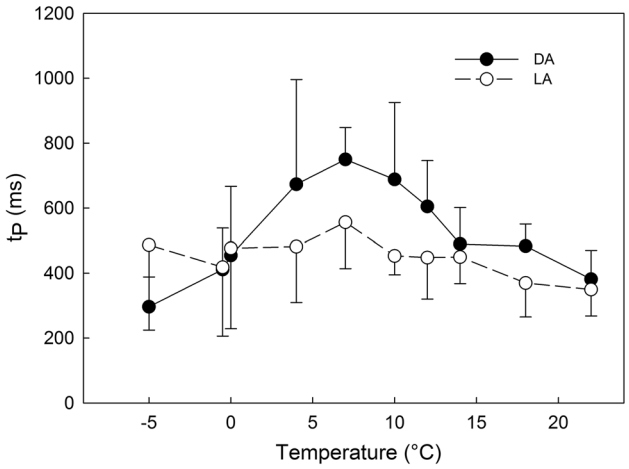

Fig. 2 Time of reaching peak P (t_P) in fast chlorophyll fluorescence curves measured in particular temperature. *DA* dark-adapted samples, *LA* light-adapted samples

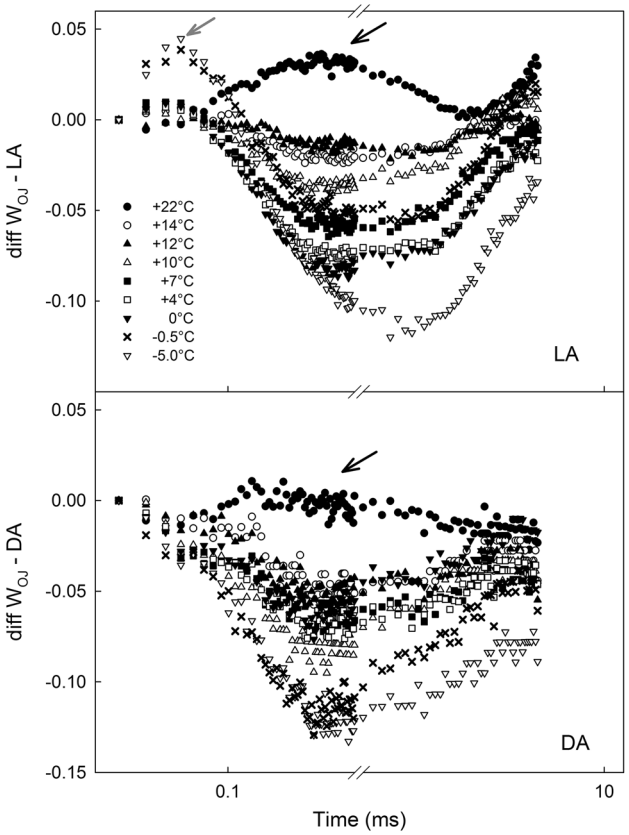

Fig. 3 Chlorophyll *a* fluorescence normalized between O and J, expressed as a difference $\Delta W_{OJ} = W_{OJ(Ti)} - W_{OJ\ (Tcontrol)}$, where $W_{OJ} = (F_t - F_0) / (F_J - F_0)$. Black arrows show position of the K-steps. *Upper panel LA* light-adapted samples, L-step apparent at − 0.5 and − 5 °C (0.08 ms, gray arrow), K-step at + 22 °C (0.4 ms), *Lower panel DA* dark-adapted samples, K-step detectable at + 22 °C (0.22 ms)

samples. When peak P (t_P) was attained, polyphasic change with decreasing temperature was recorded, and the highest t_P occurred at + 7 °C in both LA and DA treatments (see Fig. 2). Change of t_P with decreasing temperature was more apparent with DA samples than LA samples. On the OJIPs, H and G peaks were not distinguishable in the range of 800–1600 ms, since the chlorophyll fluorescence signal showed a linear decrease after the P peak (see inset in Fig. 1).

Fast chlorophyll fluorescence analysis: K-step, L-step

The K-step appeared both in DA and LA samples of *D. polyphyllizum* (see Fig. 3). In LA samples, the K-step was found at + 22 °C at 0.21 ms. In addition, the highest

values of V_K/V_J were detected at + 22 °C and decreased with temperature fall both in LA and DA samples (Fig. 4, upper panel). When plotted against F_V/F_M, V_K/V_J displayed a different slope for LA and DA samples (see Fig. 4, lower panel). It increased with F_V/F_M in LA samples, while a decreasing trend was exhibited among DA samples. In our data, the L-step was not identified in above-zero temperature (+ 22 to + 4 °C). Positive L-step resulted from a temperature decrease to subzero values (− 0.5 and − 5 °C) in LA samples, although with DA samples the L-step was hardly detectable (see Fig. 3).

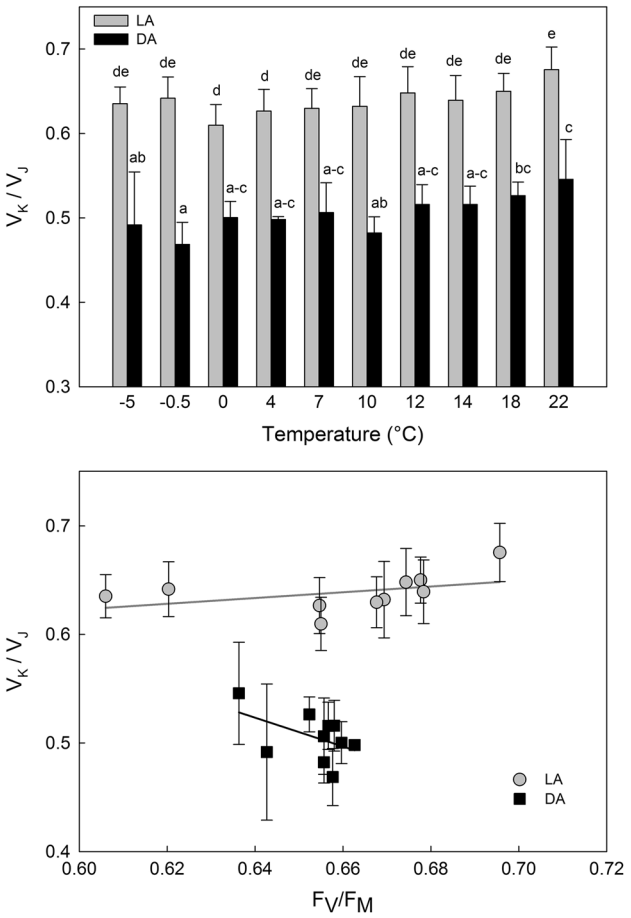

Fig. 4 V_K/V_J ratio of variable fluorescence at the time of K-step (peak in 0.4 ms for *LA* light-adapted samples and 0.22 ms for *DA* dark-adapted samples) to variable fluorescence at the time of J-step (2 ms). *Upper panel* V_K/V_J ratio at different temperatures for LA and DA samples. Statistical testing of temperature-induced changes in V_K/V_J ratio tested by one-way ANOVA. The significance of differences was evaluated by Fisher's LSD test at $p < 0.05$. *Lower panel* V_K/V_J plotted against F_V/F_M (maximum quantum efficiency of photosystem II). For statistical testing we used Spearman's rank order correlation. Both LA and DA variants exhibited significant changes (LA: $\rho = 0.5216$; DA: $\rho = -0.4276$ at $p < 0.05$)

Fast chlorophyll fluorescence-derived parameters

Relative values of major OJIP-derived parameters and signals are shown in a radar plot (Fig. 5). Decrease in thallus temperature produced a decrease in particular chlorophyll fluorescence signals, i.e., F_0, F_J, F_I, F_M and

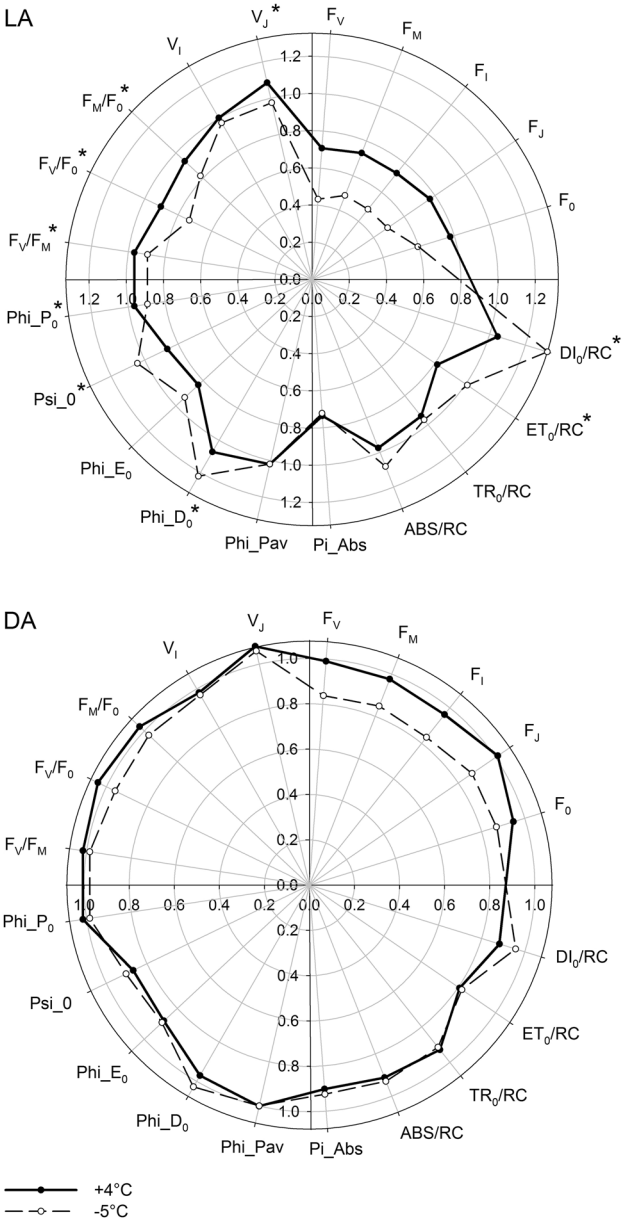

Fig. 5 Radar Plot of fast chlorophyll fluorescence-derived parameters measured at + 4 and − 5 °C compared to parameters at control temperature (+ 18 °C). *Upper panel LA* light-adapted samples. *Lower panel DA* dark-adapted samples. For the parameters abbreviation and definition see Table 1. Statisticaly significant differences of parameters are noted by asterisks (one-way ANOVA, Fishers's LSD test at $p < 0.05$)

F_V. Decreases were more apparent at − 5 °C than + 4 °C. Primary photochemical processes decreased both at + 4 and − 5 °C, as indicated by a decrease in Performance Index (Pi_ABS), a parameter sensitive to stress that integrates three components of absorbed energy flow through PSII (see Introduction). In contrast with DA samples, positive responses of some parameters (Phi_D_0-energy dissipation quantum yield at $t = 0$, ABS/RC-absorption flux of antenna chlorophylls per RC, and DI_0/RC-the dissipated excitation energy flux at time 0) were found in LA samples when compared to a reference temperature of + 18 °C. For − 5 °C, a positive change was detected for Phi_D_0, ABS/RC, and DI_0/RC. In photosynthesizing organisms, Phi_D_0 increased with severity stress, since parameter was related to non-photochemical quenching that increased with stress strength. Similarly, absorption per reaction center (ABS/RC) was also a sensitive stress indicator in the photosynthetic apparatus, given that it reflects total absorption of PSII antenna chlorophylls per active RC. DI_0/RC increase indicated an increase in the total dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs (Force et al. 2003). These authors also reported the loss of connectivity between PSII heterogeneous units as a cause of increased DI_0/RC. In our experiment, we expected low-temperature-induced increase in the number of inactive RCs that were unable to trap the photon, and, consequently, the untrapped amount of photons increased. From active RCs, dissipation occurred as heat, fluorescence, and energy transferred to other systems.

Again, at − 5 °C, the most pronounced decreases were found for Pi_ABS (Performance Index for energy conservation), Phi_E_0 (quantum yield for electron transport), and Psi_0 (probability that a trapped exciton is used for electron transport beyond quinone A). When a decrease in thallus temperature was combined with light, the negative effect on Phi_E_0 and Psi_0 was seen only at + 4 but not − 5 °C in LA samples. Irrespective of the low-temperature, decreases were higher in LA than DA samples. When temperature effect was studied in DA samples, resulting parameters showed the most substantial negative change: Phi_E_0 (quantum yield for electron transport), Psi_E_0 (efficiency of electron transport), and ET_0/RC (electron transport flux further than Q_A per reaction center). At − 5 °C, dissipation increased which was reflected in DI_0/RC, Phi_D_0 values.

Temperature response curves of the OJIP-derived parameters (see Fig. 6) indicated either decrease with temperature (F_V/F_M, Pi_ABS) or no apparent change (Phi_Pav, Psi_0, Phi_E_0 or ET_0/RC). The effect of light treatment (LA, DA) was recorded for the following ChlF parameters at high temperature (+ 22 and + 18 °C): F_V/F_M (maximum quantum efficiency of PSII photochemistry), DI_0/RC (the flux of dissipated excitation energy at time 0), and Pi_ABS (Performance Index for energy conservation). The decrease of thallus temperature to − 5 °C induced a decrease in maximum quantum efficiency of PSII photochemistry (F_V/F_M).

Slow chlorophyll fluorescence

The shape of slow Kautsky kinetics was temperature dependent. With thallus temperature decrease, F_P decreased as well as F_S (see Fig. 7, lower panel). The time at which constant F_S was reached (t_{FS}) was prolonged with temperature decrease. While t_{FS} was 180 s at + 22 °C, it increased to 240 s at 0 °C. At + 22 °C, P peak (see Fig. 7, upper panel) was attained at 9.94 s (t_P) after the actinic light was switched on (i.e. 30.96 s after the beginning of measurement). With temperature decrease, t_P was higher (10.76 s for + 10 °C, 11.32 s for 0 °C), i.e. the P peak was reached later in lower temperatures. While during high temperature, peak M was distinguishable at 98.04 s and 112.02 s for + 22 and + 18 °C, respectively, it was not evident in temperatures below + 10 °C. The decline of ChlF in the P to M part of Kautsky kinetics (60–200 s) was temperature dependent, indicating faster changes in electron carriers redox state at + 22 °C (slope − 0.132) then with temperatures near zero (slope of − 0.030 at 0 °C, and − 0.036 for − 2.5 °C, respectively).

Analyzed derived ChlF parameters (F_V/F_M, Φ_{PSII}, NPQ and qP) decreased with the temperature (see Fig. 8). Maximum quantum yield (F_V/F_M) and effective quantum yield of PSII photochemistry (Φ_{PSII}) decreased gradually with the falling temperatures. Photochemical quenching (qP) exhibited no change until + 12 °C and then sharply decreased. Non-photochemical quenching also decreased with the temperature, registering a small peak at + 7 °C.

Linear cooling and chlorophyll fluorescence

Chlorophyll fluorescence parameters in *D. polyphyllizum* measured during linear cooling from + 22 °C to − 40 °C decreased exponentially with falling temperature (Fig. 9). Critical subzero temperature for Φ_{PSII} was detected at − 12 °C (Fig. 9). At this temperature, Φ_{PSII} reached minimum value close to 0, indicating full limitation of primary photosynthetic process, particularly linear electron transport. Comparably to Φ_{PSII}, steady-state chlorophyll fluorescence (F_S) also declined with temperature reduction (Fig. 9). In this method, temperature decreased rapidly without any acclimation time, which is why Φ_{PSII} generally reached lower values than Φ_{PSII} measured by slow Kautsky kinetics.

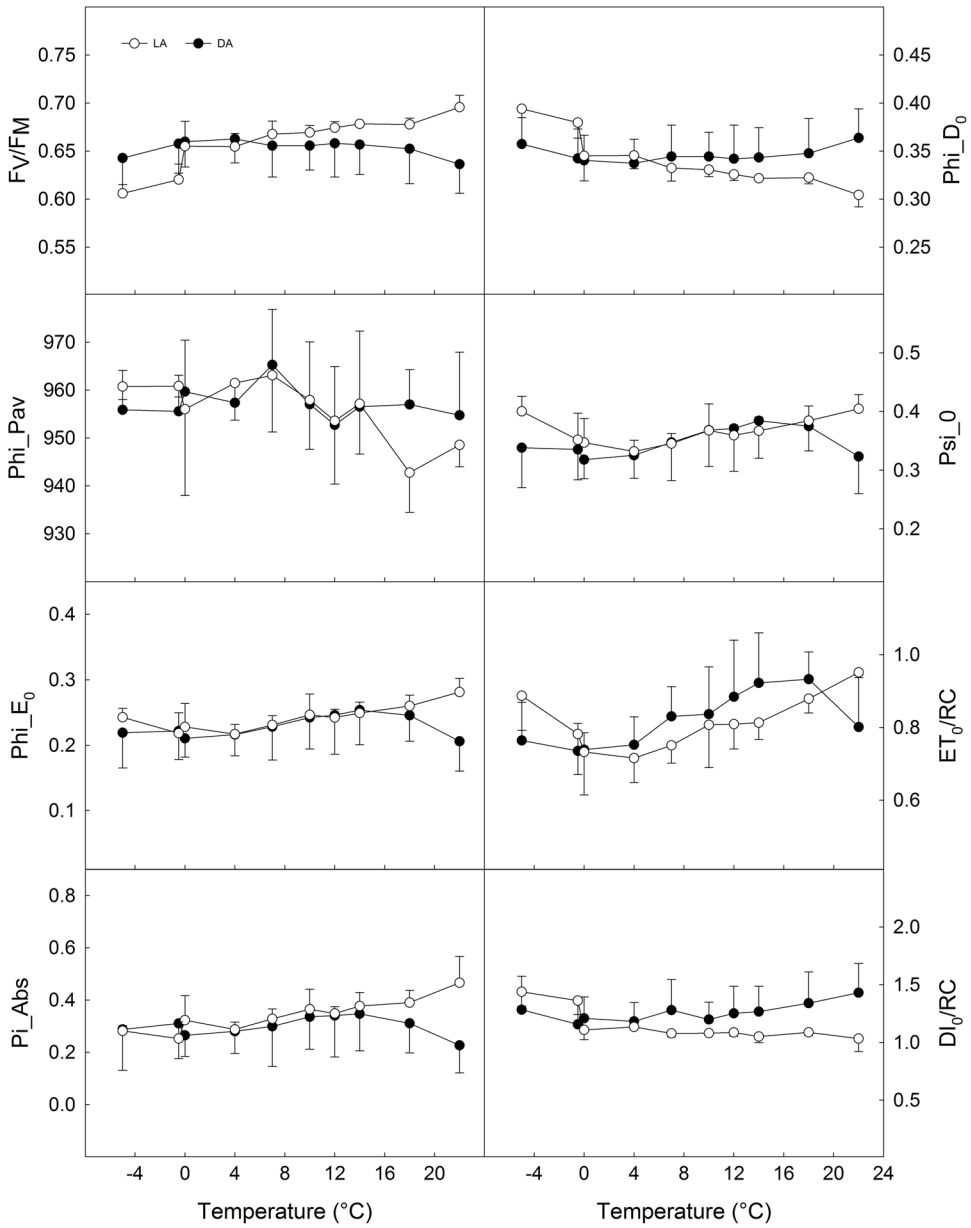

Fig. 6 Changes of selected fast chlorophyll fluorescence-derived parameters with decreasing temperature from +22 to −5 °C (*LA* light-adapted, *DA* dark-adapted samples): F_V/F_M maximum quantum efficiency of PSII photochemistry, *Phi_D*$_0$ quantum yield of energy dissipation at $t=0$, *Phi_Pav* time to reach maximal chlorophyll fluorescence, *Psi_0* probability that a trapped exciton is used for electron transport beyond Q_A, *Phi_E*$_0$ quantum yield for electron transport, *ET*$_0$*/RC* electron transport flux further than Q_A per reaction center, *Pi_ABS* Performance Index for energy conservation, *DI*$_0$*/RC* the flux of dissipated excitation energy at time 0. For statistical testing see Table 2

Discussion

Fast chlorophyll fluorescence (OJIP) curve shape

Low- and subzero temperature-induced decrease in chlorophyll fluorescence signal causing a 'flattening' of OJIP curves is a well-known phenomenon in plants documented for e.g. crops (Rapacz et al. 2015). It is assumed that the time of reaching peak P (t_P) reflects plant stress-resistance (Kalaji et al. 2012; Żurek et al. 2014). The shorter t_P is, the more stressed is the plant. In our study, however, t_P increased when temperatures decreased from +22 °C to +7 °C. With further reduction to subzero temperature, the increase stopped, and t_P remained at the same level in LA samples and decreased in DA samples (−0.5 and −5 °C, see Fig. 2).

Table 2 Statistical testing of temperature-induced changes in OJIP-derived prameters tested by one-way ANOVA

T (°C)	F_V/F_M		Psi_0		Phi_Eo		Phi_Do		Phi_Pav		Pi_Abs		ETo/RC		DIo/RC	
	LA	DA	LA	DA	LA	DA	LA	DA	LA	DA	LA	DA	LA	DA	LA	DA
22	e	a–c	c	a	b	a	a	c–e	ab	ab	b	a	e	a–e	a	d
18	de	b–d	a–c	a–c	ab	ab	ab	b–d	a	ab	ab	ab	b–e	de	a–e	b–d
14	de	b–e	a–c	a–c	ab	ab	ab	a–d	ab	ab	ab	ab	a—e	c–e	a	a–d
12	c–e	b–e	a–c	a–c	ab	ab	a–c	a–d	ab	ab	ab	ab	a–e	b–e	ab	a–d
10	c–e	b–d	a–c	a–c	ab	ab	a–c	b–d	ab	ab	ab	ab	a–e	a–e	a–c	a–d
7	c–e	b–d	a–c	a–c	ab	ab	a–c	b–d	b	b	ab	a	ab	a–e	ab	a–d
4	b–d	c–e	a–c	ab	a	a	b–d	a–c	b	ab	a	a	a	ab	a–c	a–d
0	b–d	b–e	a–c	a	ab	a	b–d	a–d	ab	ab	ab	a	ab	ab	a–c	a–d
−0.5	ab	b–e	a–c	a–c	a	ab	de	a–d	b	ab	a	ab	a–d	ab	cd	a–d
−5	a	a–d	bc	a–c	ab	a	e	b–e	b	ab	a	a	b–e	a–c	d	a–d

The significance of differences was evaluated by Fisher's LSD test

LA light-adapted samples, *DA* dark-adapted samples, F_V/F_M maximum quantum efficiency of PSII photochemistry, Phi_D_0 quantum yield of energy dissipation at $t=0$, *Phi_Pav* time to reach maximal chlorophyll fluorescence, *Psi_0* probability that a trapped exciton is used for electron transport beyond Q_A, Phi_E_0 quantum yield for electron transport, ET_0/RC electron transport flux further than Q_A per reaction center, *Pi_ABS* Performance Index for energy conservation, DI_0/RC the flux of dissipated excitation energy at time 0

In *D. polyphyllizum* OJIPs, no apparent H and G peaks on OJIP kinetics were seen (see Fig. 1). This was surprising, since H and G peaks are typical for lichens with symbiotic alga *Trebouxia* sp. (Ilík et al. 2006). H and G peaks result from very fast PSI reoxidation and are attributed to an alternative electron flow from PSI. The electron flow was originally connected with a fast activation of ferredoxin-$NADP^+$ oxidoreductase (Ilík et al. 2006) or Mehler reaction (Franck and Houyoux 2008). Recently, this phenomenon was investigated by dual-modulated fluorometers (Ilík et al. 2017) and attributed to a photoreduction of O_2 to water mediated by flavodiiron proteins (FDPs). In *Trebouxia*-possessing lichens, G and H peaks were evident at 1.6–1.8 s (e.g. *Umbilicaria antarctica*-Medina et Avalos-Chacon 2015). The lack of G, H peaks in our data indicates that, in contrast with chlorolichens possessing *Trebouxia* sp. as a photobiont, the above-noted mechanisms are likely not involved in *D. polyphyllizum.* Another explanation could involve an aspect of species specificity. In *D. polyphyllizum*, green alga *Diplosphaera* sp. was reported as the likely photobiont (Thüs et al. 2011; Fontaine et al. 2012). Since photosynthetic properties of the algal species are mostly unknown, the species itself could not be excluded as a possible reason for the absence of G, H peaks (Marečková and Barták 2017). Moreover, preliminary experiments with *D. polyphyllizum* (Ilík et al., unpublished) focused on PSI functioning did not reveal any correlation between fast chlorophyll fluorescence transient and measured P700 (photosystem I primary donor) reoxidation.

The differences in OJIP shape and chlorophyll fluorescence values might be attributed to the physiological properties of DA- and LA-treated lichens samples, and the effect of long dark-adaptation in DA samples which may alter the rate of photosynthetic electron flow from PSII to PS I. Such explanation might be supported by the fact that J step is relatively higher to the peak P in DA than LA-treated samples. This could be attributed to the reduction of the rate of electron transfer from Q_A and Q_B, which would lead to a greater reduction in Q_A in DA than in LA samples.

Fast chlorophyll fluorescence analysis: K-step, L-step

Recent studies (Guéra et al. 2016) reported K-step in cultures of *Trebouxia* sp. measured on cellulose-acetate disks. In heat-treated lichens, K-step is reported for chlorolichen *Parmelina tiliacea* (Oukarroum et al. 2012). Since there is *Diplosphaera* sp. in *D. polyphyllizum,* K-step would be reported for the first time in lichen with such photobiont. In LA samples, K-step (found at + 22 °C at 0.21 ms) was attributed to high temperature effects on photosynthetic apparatus (Kalaji et al. 2016). This might be supported by the highest values of V_K/V_J recorded at + 22 °C that decreased with temperature decrease in both LA and DA samples (Fig. 4, upper panel). LA samples revealed an increase in V_K/V_J values in all experimental temperatures (V_K/V_J range of 0.61–0.67). Such increase might be resulting from additional stress to PSII functioning caused by light applied before OJIPs measurements during the acclimation time (see Material and methods). V_K/V_J increase might be induced by several stressors, Kalaji et al. (2017) report the effect of temperature above physiological value leading to an increase in V_K/V_J (values of about 1.4). During temperature decrease (from + 22 to − 5 °C), the K-step is gradually diminished (see Fig. 3). In our data, the K-step

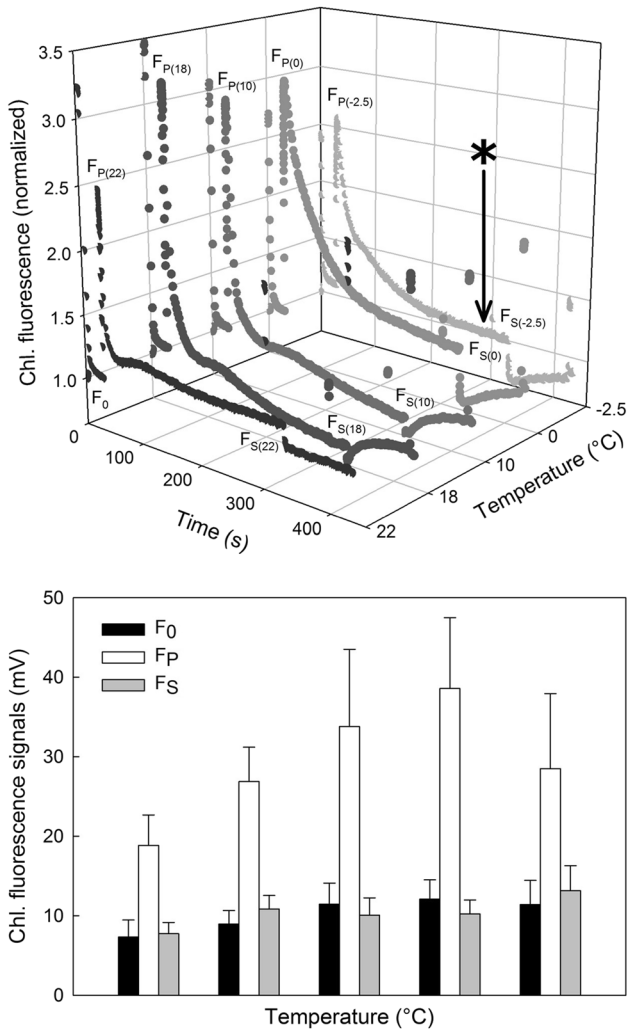

Fig. 7 *Upper panel* Slow Kautsky kinetics measured in *Dermatocarpon polyphyllizum* at varied temperature (from + 22 °C to − 2.5 °C). Kinetics are normalized to F_0. The arrow indicated by an asterisk points out high variable chlorophyll fluorescence signal with uncompleted F_S equilibrium after 300 s of the exposition to the actinic light at low temperature (− 2.5 °C). *Lower panel* Temperature-induced changes in selected parameters of Kautsky kinetics. Initial fluorescence (F_0), fluorescence of the peak P (F_P) and steady-state fluorescence (F_S)

reflects a temperature-induced imbalance between the electron flow leaving the RC towards the acceptor side and the electron flow coming to the RC from the donor side. Thus, K-step presence is caused by an inhibition of the OEC (see e.g. Guissé et al. 1995; Strasser et al. 1995, Strasser et al. 2007). In this context, the amplitude of step K is frequently used as a specific indicator of damage to the PSII donor side (see e.g. Hendrickson et al. 2003), especially in plants exposed to heat stress. In lichens, the K-step was studied by Oukarroum et al. (2012) who identified a pronounced K-step in a heat-treated chlorolichen *Parmelina tiliacea* in wet state. Similar to this study, our data indicated that donor side inhibition of PSII may occur in Antarctic lichen *D. polyphyllizum* at a temperature higher than + 20 °C. With a decrease in thallus temperature to near zero temperatures, the K-step is not detectable, suggesting that no PSII donor side inhibition occurs in symbiotic alga of *D. polyphyllizum.*

L-step presence at the range from 0.10–0.15 ms is typically associated with changes in connectivity between LHCs and RCs (Yusuf et al. 2010). Lack of L-step in our data (+ 22 to + 4 °C, see Fig. 3), suggests that treatment at above-zero temperature did not facilitate any change to the energetic connectivity (grouping) of PSII units (sensu Strasser and Stirbet 1998). However, further decrease to subzero temperature produced a positive L-step, most perceivably at − 0.5 and − 5 °C, indicating excitation energy exchange lower cooperativity between PSII units relative to the reference sample (T = + 18 °C). L-step presence was a direct effect of low-temperature stress that leads to a decrease of energetic connectivity. According to Strasser et al. (2004), and Yusuf et al. (2010), low connectivity results in less efficient consumption of excitation energy and lower system thermal stability. Moreover, our data indicated an increased proportion of inactive PSII RCs in samples measured at 0 and − 5 °C (V_T/B_T curves according to Guéra et al. 2016-not shown). In lichens from polar regions, however, LHC and RC thermal stability of a lichenized photosynthetic partner might be high and probably unchanged at the range from 0 to − 5 °C. Species-specific, yet high resistance to freezing temperature has been reported for several chlorolichens (Sadowsky and Ott 2012) and isolated *Trebouxia* sp., a common algal photobiont present in a vast majority of chlorolichens, exposed to shock freezing (Hájek et al. 2012). Moreover, lichen capability to perform photosynthetic processes even during below-zero temperature has been reported for several lichen species and can be detected by gas exchange measurements (Kappen et al. 1998) and chlorophyll fluorescence (Barták et al. 2007). Therefore, connectivity changes recorded in our study would not necessarily lead to a decrease of PS II thermal stability and photosynthetic performance at subzero temperatures ranging from 0 to − 10 °C.

Fast chlorophyll fluorescence-derived parameters

Regarding parameters that exhibited the most substantial change with experimental temperature decrease to + 4 and − 5 °C, respectively, thallus temperature reduction generally brought a decrease in specific chlorophyll fluorescence signals. The most apparent negative change was recorded at these DA sample parameters: Phi_E_0, Psi_0, and ET_0/RC. These parameters were, therefore, the most sensitive to temperature reduction similarly to those of

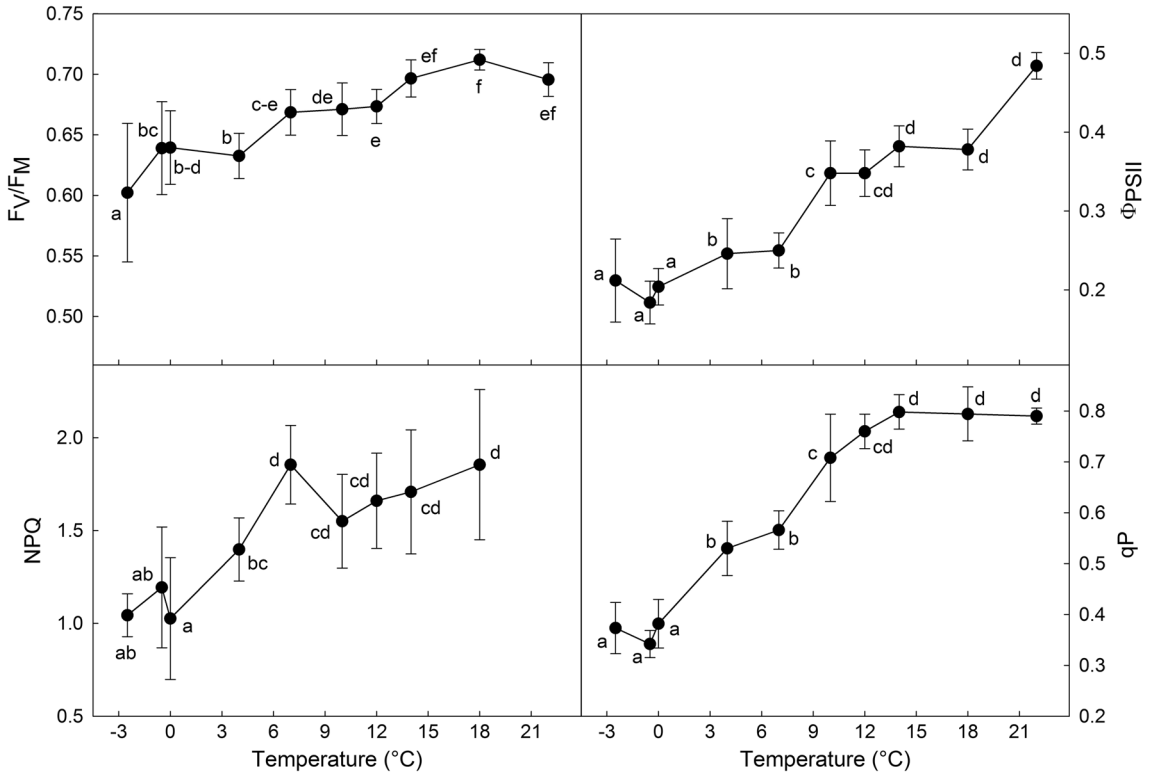

Fig. 8 Changes of selected chlorophyll fluorescence parameters derived from slow Kautsky kinetics with decreasing temperature from + 22 °C to − 2.5 °C. Selected parameters are maximum quantum efficiency of photosystem II (F_V/F_M), non-photochemical quenching (NPQ), photochemical quenching indicating the openness of PSII centers for absorbed energy flow (qP) and effective quantum yield of photosystem II (Φ_{PSII}). Statistical testing by one-way ANOVA, the significance of differences evaluated by Fisher's LSD test

plants exposed to short-term chilling stress (Zushi et al. 2012). Our results suggest that a part of RCs became inactive at −5 °C and total absorption of chlorophyll

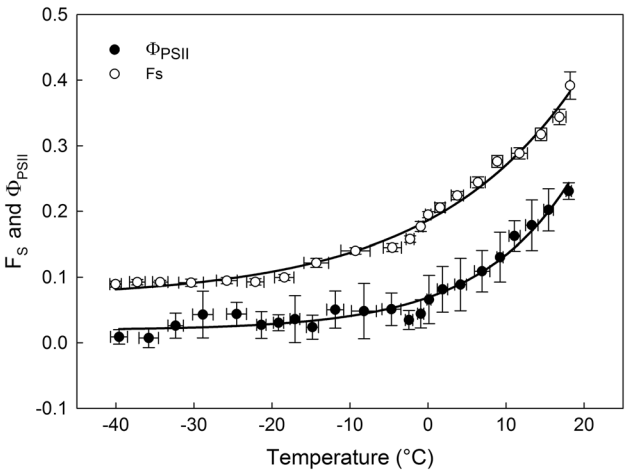

Fig. 9 Exponential decrease of steady-state chlorophyll fluorescence (F_S, $R^2 = 0{,}9942$) and effective quantum yield (Φ_{PSII}, $R^2 = 0{,}9828$) recorded during linear cooling from + 23 °C to − 40 °C at the rate of cooling 2 °C min^{-1}. Vertical and horizontal error bars represent standard deviations of F_S, Φ_{PSII} and thallus temperature, respectively

molecules in light-harvesting complexes of active PSII, RCs in particular, increased. At − 5 °C, heat dissipation increased which was reflected in DI_0/RC, Phi_D_0 values. Generally, DI_0/RC represents the ratio of the total dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs (Mathur et al. 2013). Hence, dissipation in *D. polyphyllizum* was influenced by the ratio of active/inactive RCs. In this context, the number of inactive centers increased, and the inactive centers were unable to trap the photon from incident radiation in LA samples. In crops, DI_0/RC increased with heat stress (from + 25 to + 45 °C, Mathur et al. 2011). The same response was exhibited in desert plants (+ 38 to + 44 °C, Li et al. 2014). Low temperature effect on DI_0/RC was reported only for red alga (*Kappaphycus alvarezii*-Li et al. 2016). In lichens, the increase in DI_0/RC at subzero temperature is reported in our study for the first time. It might be associated with low-temperature-induced changes in PSII of symbiotic alga and specifically the initial phase of inhibition/damage of PSII. Under such conditions, PSII can not transfer the energy from PSII to the photosynthetic linear electron transport chain and energy dissipation (DI_0/RC or DI_0/CS-dissipation per cross section), therefore, increases. As a consequence,

low-temperature damage of thylakoid membrane components may occur. Rapacz et al. (2015) reported negative correlations between DI_0/CS and the parameters derived from OJIPs that may be considered as indirect indicators of freezing tolerance and winter hardiness. Specifically for F_V/F_M, a decrease was observed at − 5 °C in LA treatment. Typically, F_V/F_M value is more or less constant at low temperature, because photosynthetic process capacity is not affected in a majority of plants. In optimally hydrated lichens, however, both no change (e.g. Hájek et al. 2001) and a decrease in F_V/F_M (Barták et al. 2007; Mishra et al. 2015) is reported with temperature decrease from + 20 to − 5 °C. Performance index decline (Pi_ABS) in both + 4 and − 5 °C indicates a primary photochemical process abatement. For follow-up field and laboratory studies focused on lichen physiology at subzero temperature, the following OJIP-derived parameters could be recommended: DI_0/RC, ABS/RC, and Phi_D_0, as these responded most sensitively to the temperature decrease (Fig. 5). These parameters represent two different aspects of short-term response to subzero temperature: (1) thermal dissipation (DI_0/RC) and its quantum yield (Phi_D_0), and (2) absorbed energy utilized in photosynthetic processes (ABS/RC). With high temperature (+ 22 and + 18 °C), LA/DA treatment effect was seen in F_V/F_M, DI_0/RC, and Pi_ABS parameters. The difference found in these ChlF parameters suggest that light intensity used during temperature treatment was an additional stressor that led to the initial phase of high-temperature photoinhibition.

LA treatment effect during the acclimation period of particular temperatures was seen in certain measured parameters: F_V/F_M, Phi_D_0, ET_0/RC, and DI_0/RC. In such cases, light represented a co-acting factor with temperature change that might e.g. promote PSII functioning at low temperature. In Phi_D_0, Phi_Pav, Psi_o, and ET_0/RC, LA treatment had a positive effect on parameters at − 5 °C. (see Fig. 6, $T = -0.5, -5$ °C). A possible explanation is that ATP and NADPH utilization during photosynthesis biochemical processes happening with LA treatment during a temperature acclimatory period promotes PSII effectivity during standard OJIP measurements. At a high temperature (+ 22 °C), LA treatment induced a decrease in Phi_D_0 and DI_0/RC. Explanation of such response is not easy since our earlier study (Marečková and Barták 2017) produced inconsistent and sometimes contradictory results. However, the decrease in the two parameters might be associated with lower thermal dissipation in LA than DA samples. To elucidate the effect of LA treatment on the OJIP-derived parameters, more detailed studies focusing on a wider temperature range from supraoptimal (about + 30 °C) to freezing temperatures at which primary photosynthetic processes are heavily limited (about − 25 °C) are needed.

Slow Kautsky kinetics

D. polyphyllizum exhibited typically slow ChlF kinetics during exposition to continuous light (Fig. 7, upper panel). A polyphasic time course of variable ChlF from F_P to F_S level was determined temperature-dependent similarly to the other Antarctic species—*Rhizoplaca melanophthalma* and *Umbilicaria antarctica* (Mishra et al. 2015). These authors recorded an increase in F_P and ChlF signal at + 5 °C (compared to + 25 °C), followed by a decrease at subzero temperature (c.f. Figure 7, lower panel). For *D. polyphyllizum*, changes in the shape of slow ChlF kinetics might be attributed to temperature-induced changes in electron acceptor redox state (Q_A in particular) and transthylakoidal gradient formation (Δ pH) resulting in zeaxanthin production. The latter process, however, only plays an important role at subzero temperature (Mishra et al. 2015). In our data, no NPQ increase was observed at − 2.5 °C. Therefore, the zeaxanthin formation effect might be overlooked. At 0 and − 2.5 °C, variable ChlF signal (F_S) was determined to be high and not constant after 300 s of actinic light exposure (i.e. steady-state chlorophyll fluorescence was not attained, see arrow in Fig. 7, upper panel), which could be explained as a consequence of imbalance between ATP, NADPH synthesis rates and CO_2 fixation. In this situation, the Calvin–Benson cycle utilizes less ATP, NADPH for CO_2 fixation than that produced by the primary photochemical photosynthesis processes. Increased F_S is attributed to low-temperature effects on the photosynthetic apparatus of grass species (Rapacz et al. 2004), trees (Soukupová et al. 2008) and lichens (Marečková and Barták 2017).

Temperature response curves of F_V/F_M and Φ_{PSII}, respectively, were comparable to the evidence for *Xanthoria elegans* and *Umbilicaria antarctica* (Barták et al. 2007). While F_V/F_M, only decreased to a limited extent by − 2.5 °C, Φ_{PSII} diminished to less than 50% of initial value recorded found at + 22 °C. Similarly, qP, which indicates relative openness of PSII centers for absorbed energy flow, decreased by more than 50% but still retained value of about 0.35 at − 2.5 °C. This indicates that in spite of limitation caused by subzero temperature, actual PSII photochemical processes in *D. polyphyllizum* still have reasonably high capacity to maintain photosynthesis. Indeed, relatively high rates of photosynthetic CO_2 fixation are reported for the Antarctic macrolichens at temperatures of − 5 °C (0.1 mg CO_2 mg Chl^{-1} h^{-1}, Kappen et al. 1998).

Non-photochemical quenching (NPQ) decreased with thallus temperature decrease, and no sign of NPQ increase was found at − 2.5 °C as reported in earlier studies with temperature below − 5 °C (Hájek et al. 2001; Barták et al. 2007). This corresponds well with the fact that isolated

lichenized algae exhibit NPQ increase with temperature decrease as reported by Sadowsky and Ott (2015). At subzero temperatures of about − 5 °C or lower, ice nucleation starts in symbiotic algae of chlorolichens. As a consequence, photosynthesis photochemical processes decrease and protective mechanisms including NPQ increase. Haranczyk et al. (2003) determined that − 5 °C is an edge temperature for water freezing in several lichens with contrasting anatomy. The algae are generally considered as poor ice nucleators and their freezing temperature usually ranges from − 5 to − 20°C. Our results confirm these observations (Kvíderová et al. 2013).

Linear cooling and chlorophyll fluorescence

Chlorophyll fluorescence parameters in *D. polyphyllizum* decreased exponentially during linear cooling from + 22 °C to − 40 °C (Fig. 9). Minimal value of Φ_{PSII} was attained at a temperature of − 12 °C (Fig. 9), indicating full inhibition of primary photosynthetic processes, especially linear electron transport. This is quite comparable to Φ_{PSII} data determined by the same method in other lichen species from polar regions by Hájek et al. (2016): *Usnea antarctica* (− 11.2 °C), *Usnea aurantiaco-atra* (− 9.7 °C), and *Umbilicaria cylindrica* (− 13.6 °C). In *D. polyphyllizum*, there was a similar exponential decline of temperature response curve of Φ_{PSII} as reported for *Usnea auraniaco-atra* (Hájek et al. 2016). Inhibition of Φ_{PSII} at critical subzero temperature is associated with cellular ice nucleation found in lichens (Moffett et al. 2015), and mosses (Buchner et Neuner 2010). In lichen symbiotic alga *Trebouxia* sp., a temperature range from − 12 to − 16 °C has been reported for the ice nucleation (Kvíderová et al. 2013).

Steady-state chlorophyll fluorescence (F_S) was reduced with temperature decrease (Fig. 9). The phenomenon of F_S decrease with thallus temperature reduction is well documented for polar lichen species and attributed to an overall decrease in chlorophyll fluorescence signal throughout whole slow ChlF transient recorded at low and subzero temperature (Mishra et al. 2015).

Concluding remarks

With this study, we focused on low-temperature-induced changes in primary photosynthetic processes in an Antarctic chlorolichen sensed by several chlorophyll fluorescence methods. Parameters derived from fast chlorophyll fluorescence transients (OJIPs) indicated that they might be used for detection of early events in low-temperature-affected lichens, e.g., thermal dissipation of absorbed light energy (DI_0/RC and Phi_D_0 were determined most sensitive to low temperature, see Figs. 5 and 6). Parameter use would mainly be beneficial with laboratory studies of thermal tolerance of lichens exposed to repeated freezing/thawing cycles (e.g. Wang et al. 2014), desiccation at low temperature (Veerman et al. 2007), and low-temperature photoinhibition (Barták et al. 2012). On the other hand, electron transport rate estimated from OJIP may be used as a proxy of photosynthesis to only a limited extent. Our data, however, recognize a strong positive corelation (linear relation, not shown, $R^2 = 0.763$) between ET_0/RC (related exclusively to PSII) and Φ_{PSII} (related to energy flow from PSII). It is well established that Φ_{PSII} relates to (1) the processes associated with PSII (2) the photosynthetic linear electron transport chain, and (3) utilization of ATP, NADPH in biochemical part of photosynthesis. OJIP-derived parameter ET_0/RC, however, does not include the (2) and (3) processes. Therefore, while this could be used as a proxy of primary photosynthetic processes, simultaneous measurement using another method is still highly recommended. Results gained by a linear cooling approach indicate that steady-state chlorophyll fluorescence and Φ_{PSII} were well correlated (linear regression, $R^2 = 0.933$ for the F_S and Φ_{PSII} data presented in Fig. 9). Therefore, F_S might be used as a raw estimator of primary photosynthetic processes in poikilohydric polar lichens and could be only recommended for experiments taken in optimally hydrated samples or with experiments carried out under controlled conditions. For field study, F_S is not recommended because it may overestimate photosynthetic processes (Φ_{PSII} in particular), which may specifically happen immediately after rewetting of dry samples. Under such circumstances, F_S signal is restored thanks to early activity of rehydrated chlorophyll molecules, however, Φ_{PSII} remains close to zero because of the severely limited biochemical phase of photosynthesis. Among ChF parameters derived from slow Kautsky kinetics, Φ_{PSII} and qP were most sensitive to thallus temperature of *D. polyphyllizum* (see Fig. 8). Therefore, in accordance with existing data on low and subzero temperature effects on lichens (Solhaug et al. 2018), these parameters should be used in follow-up studies as estimators of temperature effects on photosynthetic processes in lichens.

Acknowledgements The authors are grateful to the CzechPolar-2 infrastructure (LM2015078) that enabled sample collection and handling. The experimental part of our work was undertaken in the EEL laboratory (CzechPolar infrastructure) and supported by the ECOPOLARIS Project (CZ0.02.1.01/0.0/0.0/16_013/0001708). The authors are also very grateful to Peter Váczi (Masaryk University, Brno), who greatly assisted us with the statistical testing of our results and to Richard Alan Zimmerman (Masaryk University, Brno), who provided the language correction.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

References

Bao H, Zhang C, Ren Y, Zhao J (2010) Low-temperature electron transfer suggests two types of QA in intact photosystem II. Biochim Biophys Acta (BBA)-Bioenerg 1797:339–346.

Barták M, Váczi P, Hájek J, Smykla J (2007) Low-temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*. Polar Biol 31:47–51. https://doi.org/10.1007/s00300-007-0331-x

Barták M, Hájek J, Očenášková 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 Rep 2:42–51. https://doi.org/10.5817/CPR2012-1-5

Barták M, Váczi P, Stachoň Z, 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 Rep 5:75–87. https://doi.org/10.5817/CPR2015-1-8

Boisvert S, Joly D, Carpentie R (2006) Quantitative analysis of the experimental O-J-I-P chlorophyll fluorescence induction kinetics. FEBS J 273:4770–4777. https://doi.org/10.1111/j.1742-4658.2006.05475.x

Brestic M, Zivcak M, Olsovska K, Repkova J (2013) Involvement of chlorophyll a fluorescence analyses for identification of sensitiveness of the photosynthetic apparatus to high temperature in selected wheat genotypes. In: Kuang T, Lu C, Zhang L (eds) Photosynthesis research for food, fuel and the future. Advanced Topics in Science and Technology in China, Springer, Berlin, Heidelberg, pp 510–513

Buchner O, Neuner G (2010) Freezing cytorrhysis and critical temperature thresholds for photosystem II in the peat moss *Sphagnum capillifolium*. Protoplasma 243:63–71. https://doi.org/10.1007/s00709-009-0053-8

Fontaine KM, Beck A, Stocker-Wörgötter E, Piercey-Normore MD (2012) Photobiont relationships and phylogenetic history of *Dermatocarpon luridum* var. *luridum* and related *Dermatocarpon* species. Plants 1:39–60. https://doi.org/10.3390/plants1020039

Force L, Critchley C, van Rensen JJS (2003) New fluorescence parameters for monitoring photosynthesis in plants 1. The effect of illumination on the fluorescence parameters of the JIP-test. Photosynth Res 78:17–33. https://doi.org/10.1023/A:1026012116709

Franck F, Houyoux P-A (2008) The Mehler reaction in Chlamydomonas during photosynthetic induction and steady-state photosynthesis in wild-type and a mitochondrial mutant. In: Gantt E, Golbeck JH, Osmond B (eds) Allen JF. Springer, Photosynthesis. Energy from the sun. Dordrecht, pp 581–584

Genty B, Briantais J-M, Baker RN (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta (BBA)-Subj 990:87–92. https://doi.org/10.1016/S0304-4165(89)80016-9

Guéra A, Gasulla F, Barreno E (2016) Formation of photosystem II reaction centers that work as energy sinks in lichen symbiotic Trebouxiophyceae microalgae. Photosynth Res 128:15–33. https://doi.org/10.1007/s11120-015-0196-8

Guissé B, Srivastava A, Strasser RJ (1995) The polyphasic rise of the chlorophyll-a fluorescence (O-K-J-I-P) in heat-stressed leaves. Arch Sci 48:147–160

Gururani MA, Upadhyaya ChP, Strasser RJ, Woong YJ, Park SW (2012) Physiological and biochemical responses of transgenic potato plants with altered expression of PSII manganese stabilizing protein. Plant Physiol Biochem 58:182–194. https://doi.org/10.1016/j.plaphy.2012.07.003

Hájek J, Barták M, Gloser J (2001) Effects of thallus temperature and hydration on photosynthetic parameters of *Cetraria islandica* from contrasting habitats. Photosynthetica 39:427–435. https://doi.org/10.1023/A:1015194713480

Hájek J, Váczi P, Barták M, Jahnová L (2012) Interspecific differences in cryoresistance of lichen symbiotic algae of genus *Trebouxia* assessed by cell viability and chlorophyll fluorescence. Cryobiology 64:215–222. https://doi.org/10.1016/j.cryobiol.2012.02.002

Hájek J, Barták M, Hazdrová J, Forbelská M (2016) Sensitivity of photosynthetic processes to freezing temperature in extremophilic lichens evaluated by linear cooling and chlorophyll fluorescence. Cryobiology 73:329–334. https://doi.org/10.1016/j.cryobiol.2016.10.002

Haranczyk H, Grandjean J, Olech M (2003) Freezing of water bound in lichen thallus as observed by H-1 NMR. II. Freezing protection mechanisms in a cosmopolitan lichen Cladonia mitis and in Antarctic lichen species at different hydration levels. Colloid Surf B 28:251–260. https://doi.org/10.1016/S0927-7765(02)00150-9

Hendrickson L, Ball MC, Osmond CB, Furbank RT, Chow WS (2003) Assessment of photoprotection mechanisms of grapevines at low temperature. Funct Plant Biol 30:631–642. https://doi.org/10.1071/FP02114

Ilík P, Pavlovič A, Kouril R, Alboresi A, Morosinotto T, Allahverdieyeva Y, Aro E-M, Yamamoto H, Shikanai T (2017) Alternative electron transport mediated by flavodiiron proteins is operational in organisms from cyanobacteria up to gymnosperms. New Phytol 214:967–972. https://doi.org/10.1111/nph.14536

Ilík P, Schansker G, Kotabová E, Váczi P, Strasser RJ, Barták M (2006) A dip in the chlorophyll fluorescence induction at 0.2–2 s in *Trebouxia*-possessing lichens reflects a fast reoxidation of photosystem I. A comparison with higher plants. Biochim Biophys Acta (BBA)-Bioenerg 1757:12–20. https://doi.org/10.1016/j.bbabio.2005.11.008

Kalaji HM, Goltsev V, Bosa K, Allakhverdiev SI, Strasser RJ, Govindjee (2012) Experimental in vivo measurements of light emission in plants: a perspective dedicated to David Walker. Photosynth Res 114:69–96. https://doi.org/10.1007/s11120-012-9780-3

Kalaji HM, Jajoo A, Oukarroum A, Brestic M, Zivcak M, Samborska IA, Cetner MD, Lukasik I, Golstev V, Ladle RJ (2016) Chlorophyll *a* fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. Acta Physiol Plant 38:102. https://doi.org/10.1007/s11738-016-2113-y

Kalaji HM, Schansker G, Brestic M, Bussotti F, Calatayud A, Ferroni L, Goltsev V, Guidi L, Jajoo A, Li P, Losciale P, Mishra VK, Misra AN, Nebauer SG, Pancaldi S, Penella C, Pollastrini M, Suresh K, Tambussi E, Yanniccari M, Zivcak M, Cetner MD, Samborska IA, Stirbet A, Olsovska K, Kunderlikova K, Shelonzek H, Rusinowski S, Bąba W (2017) Frequently asked questions about chlorophyll fluorescence, the sequel. Photosynth Res 132:13–66. https://doi.org/10.1007/s11120-016-0318-y

Kappen L, Schroeter B (1997) Activity of lichens under the influence of snow and ice. Proc NIPR Symp Polar Biol 10:163–168

Kappen L, Schroeter B, Seppelt RD, Green TGA (1998) Chlorophyll *a* fluorescence and CO_2 exchange of *Umbilicaria aprina* under extreme light stress in the cold. Oecologia 113:325–331. https://doi.org/10.1007/s004420050383

Kvíderová J, Hájek J, Worland RM (2013) The ice nucleation activity of the extremophilic algae. CryoLetters 34:137–148

Lange OL (2002) Photosynthetic productivity of the epilithic lichen Lecanora muralis: Long-term field monitoring of CO_2 exchange and its physiological interpretation. I. Dependence of photosynthesis on water content, light, temperature, and CO_2 concentration from laboratory measurements. Flora-Morphol Distrib Funct Ecol Plant 197:233–249. https://doi.org/10.1078/0367-2530-00038

Láska K, Barták M, Hájek J, Prošek P, Bohuslavová O (2011) Climatic and ecological characteristics of deglaciated area of James Ross Island, Antarctica, with a special respect to vegetation cover. Czech Polar Rep 1:49–62

Lazár D (2009) Modelling of light-induced chlorophyll *a* fluorescence rise (O-J-I-P transient) and changes in 820 nm-transmittance signal of photosynthesis. Photosynthetica 47:483–498. https://doi.org/10.1007/s11099-009-0074-8

Li L, Li XY, Xu XW, Lin LS, Zeng FJ (2014) Effects of high temperature on the chlorophyll *a* fluorescence of *Alhagi sparsifolia* at the southern Taklamakan Desert. Acta Physiol Plant 36:243–249. https://doi.org/10.1007/s11738-013-1405-8

Li H, Liu J, Zhang L, Pang T (2016) Effects of low temperature stress on the antioxidant system and photosynthetic apparatus of *Kappaphycus alvarezii* (Rhodophyta, Solieriaceae). Mar Biol Res 12:1064–1077. https://doi.org/10.1080/17451000.2016.1232827

Lichtenthaler HK, Buschmann C, Knapp M (2005) How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll fluorescence decrease ratio RFd of leaves with the PAM fluorometer. Photosynthetica 43:379–393. https://doi.org/10.1007/s11099-005-0062-6

MacKenzie TD, MacDonald TM, Dubois LA, Campbell DA (2001) Seasonal changes in temperature and light drive acclimation of photosynthetic physiology and macromolecular content in *Lobaria pulmonaria*. Planta 214:57–66. https://doi.org/10.1007/s004250100580

Marečková M, Barták M (2017) Short-term responses of primary processes in PS II to low temperature are sensitively indicated by fast chlorophyll fluorescence kinetics in Antarctic lichen *Dermatocarpon polyphyllizum*. Czech Polar Rep 7:74–82. https://doi.org/10.5817/CPR2017-1-8

Martinazzo EG, Ramm A, Bacarin MA (2012) The chlorophyll *a* fluorescence as an indicator of the temperature stress in the leaves of *Prunus persica*. Braz J Plant Physiol 24:237–246. https://doi.org/10.1590/S1677-04202013005000001

Mathur S, Mehta P, Jajoo A (2013) Effects of dual stress (high salt and high temperature) on the photochemical efficiency of wheat leaves (*Triticum aestivum*). Physiol Mol Biol Plants 19:179–188. https://doi.org/10.1007/s12298-012-0151-5

Mathur S, Allakhverdiev SI, Jajoo A (2011) Analysis of high temperature stress on the dynamics of antenna size and reducing side heterogeneity of Photosystem II in wheat leaves (*Triticum aestivum*). Biochim Biophys Acta (BBA)-Bioenerg 1807:22–29. https://doi.org/10.1016/j.bbabio.2010.09.001

Medina MG, Avalos-Chacon R (2015) Physiological performance of a foliose macrolichen *Umbilicaria antarctica* as affected by supplemental UV-B treatment. Czech Polar Rep 5:222–229. https://doi.org/10.5817/CPR2015-2-19

Míguez F, Fernández-Marín B, Becerril JM, García-Plazaola JI (2017) Diversity of winter photoinhibitory responses: a case study in co-occurring lichens, mosses, herbs and woody plants from subalpine environments. Physiol Plant 160:282–296. https://doi.org/10.1111/ppl.12551

Mishra A, Hájek J, Tuháčková T, Barták M, Mishra KB (2015) Features of chlorophyll fluorescence transients can be used to investigate low temperature induced effects on photosystem II of algal lichens from polar regions (Short Communication). Czech Polar Rep 5:99–111. https://doi.org/10.5817/CPR2015-1-10

Moffett B, Getti G, Henderson-Begg SK, Hill TCJ (2015) Ubiquity of ice nucleation in lichen—possible atmospheric implications. Lindbergia 38:39–43 https://doi.org/10.25227/linbg.01070

Oukarroum A, Madidi SE, Schansker G, Strasser RJ (2007) Probing the responses of barley cultivars (*Hordeum vulgare* L.) by chlorophyll a fluorescence OLKJIP under drought stress and rewatering. Environ Exp Bot 60:438–446. https://doi.org/10.1016/j.envexpbot.2007.01.002

Oukarroum A, Strasser RJ, Schansker G (2012) Heat stress and the photosynthetic electron transport chain of the lichen *Parmelina tiliacea* (Hoffm.) Ach. in the dry and the wet state: differences and similarities with the heat stress response of higher plants. Photosynth Res 111:303–314. https://doi.org/10.1007/s11120-012-9728-7

Piccotto M, Tretiach M (2010) Photosynthesis in chlorolichens: the influence of the habitat light regime. J Plant Res 123:763–775. https://doi.org/10.1007/s10265-010-0329-2

Rapacz M, Gasior D, Zwierzykowski Z, Lesniewska-Bocianowska A, Humphreys MW, Gay AP (2004) Changes in cold tolerance and the mechanisms of acclimation of photosystem II to cold hardening generated by anther culture of Festuca pratensis × Lolium multiflorum cultivars. New Phytol 162:105–114. https://doi.org/10.1111/j.1469-8137.2004.01024.x

Rapacz M, Sasal M, Kalaji HM, Kościelniak J (2015) Is the OJIP Test a Reliable Indicator of Winter Hardiness and Freezing Tolerance of Common Wheat and Triticale under Variable Winter Environments? PLoS ONE 10:e0134820. https://doi.org/10.1371/journal.pone.0134820

Roháček K (2010) Method for resolution and quantification of components of the non-photochemical quenching (q (N)). Photosynth Res 105:101–113. https://doi.org/10.1007/s11120-010-9564-6

Roháček K, Soukupová J, Barták M (2008) Chlorophyll fluorescence: A wonderful tool to study plant physiology and plant stress. In: Schoefs B (ed) Plant cell compartments-selected topics. Kerala-India: Research Signpost, 1st edition, pp 41–104. ISBN 978-81-308-0104-9

Sadowsky A, Ott S (2012) Photosynthetic symbionts in Antarctic terrestrial ecosystems: the physiological response of lichen photobionts to drought and cold. Symbiosis 58:81–90. https://doi.org/10.1007/s13199-012-0198-7

Sadowsky A, Ott S (2015) Symbiosis as a successful strategy in continental Antarctica: performance and protection of *Trebouxia* photosystem II in relation to lichen pigmentation. Polar Biol 39:139–151. https://doi.org/10.1007/s00300-015-1677-0

Schansker G, Tóth SZ, Strasser RJ (2005) Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the Chl *a* fluorescence rise OJIP. Biochim Biophys Acta (BBA)-Bioenerg 1706:250–261. https://doi.org/10.1016/j.bbabio.2004.11.006

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 Phytol 131:273–285. https://doi.org/10.1111/j.1469-8137.1995.tb05729.x

Solhaug KA, Chowdhury DP, Gauslaa Y (2018) Short- and long-term freezing effects in a coastal (*Lobaria virens*) versus a widespread lichen (*L.pulmonaria*). Cryobiology 82:124–129. https://doi.org/10.1016/j.cryobiol.2018.03.007

Soukupová J, Cséfalvay L, Urban O, Košvancová M, Marek MV, Rascher U, Nedbal L (2008) Annual variation of the steady-state chlorophyll fluorescence emission of evergreen plants in temperate zone. Funct Plant Biol 35:63–76. https://doi.org/10.1071/FP07158

Stirbett A, Riznichenko GY, Rubin AB, Govindjee (2014) Modeling chlorophyll a fluorescence transient: Relation to photosynthesis. Biochemistry (Moscow) 79:291–323. https://doi.org/10.1134/S0006297914040014

Strasser RJ, Stirbet AD (1998) Heterogeneity of Photosystem II probed by the numerically simulated chlorophyll *a* fluorescence rise (O-J-I-P). Math Comp Simul 48:3–9. https://doi.org/10.1016/S0378-4754(98)00150-5

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61:32–42. https://doi.org/10.1111/j.1751-1097.1995.tb09240.x

Strasser RJ, Tsimilli-Michael M, Dangre D, Rai M (2007) Biophysical phenomics reveals functional building blocks of plants systems biology: A case study for the evaluation of the impact

of mycorrhization with piriformospora indica. In: Varma A, Oelmüler R (eds) Advanced techniques in soil microbiology, soil biology. Springer, Berlin, pp 319–341

Strasser RJ, Srivastava A, Tsimilli-Michael M (2000) The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P (ed) Probing photosynthesis: mechanism, regulation and adaptation. Taylor and Francis, UK, pp 445–483.

Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the Chlorophyll *a* Fluorescence Transient. In: Papageorgiou GCh etGovindjee (ed) Chlorophyll *a* fluorescence. Advances in photosynthesis and respiration. vol 19. Springer, Dordrecht, pp 321-362

Thüs H, Muggia L, Pérez-Ortega S, Favero-Longo SE, Joneson S, O'Brien H, Nelsen MP, Duque-Thüs R, Grube M, Friedl T, Brodie J, Andrew CJ, Lücking R, Lutzoni F, Gueidan C (2011) Revisiting photobiont diversity in the lichen family Verrucariaceae (Ascomycota). Eur J Phycol 46:399–415. https://doi.org/10.1080/09670262.2011.629788

Tsimilli-Michael M, Pêcheux M, Strasser RJ (1999) Light and heat stress adaptation of the symbionts of temperate and coral reef foraminifers probed in hospite by the chlorophyll *a* fluorescence kinetics. Zeitschrift für Naturforschung 54C:671–680. https://doi.org/10.1515/znc-1999-9-1009

Veerman J, Vasilev S, Paton GD, Ramanauskas J, Bruce D (2007) Photoprotection in the lichen *Parmelia sulcata*: the origins of desiccation-induced fluorescence quenching. Plant Physiol 145:997–1005. https://doi.org/10.1104/pp.107.106872

Wang L, Liu H, Wu Q-F (2014) Responses of photosynthetic activities of two foliose lichens to freezing-thawing alternation under different thallus water conditions. Mycosystema 33:680–689. https://doi.org/10.13346/j.mycosystema.130118

Xue W, Li XY, Lin LS, Wang YJ, Li L (2011) Effects of elevated temperature on photosynthesis in desert plant *Alhagi sparsifolia* S. Photosynthetica 49:435–447. https://doi.org/10.1007/s11099-011-0054-7

Yusuf MA, Kumar D, Rajwanshi R, Strasser RJ, Tsimilli-Michael M, Govindjee Sarin NB (2010) Overexpression of gamma-tocopherol methyl transferase gene in transgenic Brassica juncea plants alleviates abiotic stress: physiological and chlorophyll a fluorescence measurements. Biochim Biophys Acta 1797:1428–1438. https://doi.org/10.1016/j.bbabio.2010.02.002

Zivcak M, Brestic M, Olsovska K, Slamka P (2008) Performance index as a sensitive indicator of water stress in *Triticum aestivum* L. Plant Soil Environ 54:133–139. https://doi.org/10.17221/392-PSE

Żurek G, Rybka K, Pogrzeba M, Krzyzak J, Prokopiuk K, Subramanyam R (2014) Chlorophyll *a* fluorescence in evaluation of the effect of heavy metal soil contamination on perennial grasses. PLoS ONE 9:e91475. https://doi.org/10.1371/journal.pone.0091475

Zushi T, Kajiwara S, Matsuzoe M (2012) Chlorophyll a fluorescence OJIP transient as a tool to characterize and evaluate response to heat and chilling stress in tomato leaf and fruit. Sci Hortic 148:39–46. https://doi.org/10.1016/j.scienta.2012.09.022