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Analysis of K- and L-band appearance in OJIPs in Antarctic lichens in low and high temperature

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Abstract

In this study, we evaluated the effect of temperature on the fast chlorophyll fluorescence (ChlF) transient (OJIP) and OJIP-derived parameters in Antarctic lichens *Xanthoria elegans*, *Usnea antarctica*, and *Dermatocarpon polyphyllizum*. Samples were exposed to a range of temperatures (−5 to +45°C) and measured after 15-min equilibration. High temperature (+45°C) caused a decrease of ChlF, an increased J-step, and shortened time to reach peak ChlF (F_p). Temperature below +5°C caused the increase of ChlF and J-step. The K-band was identified in *X. elegans* (above +20°C), *U. antarctica* (+35°C), and *D. polyphyllizum* (+45°C). L-band was well distinguishable in *X. elegans* (+45°C). As indicated by the OJIP-derived parameters, high temperature inhibited photosystem II function. The inhibition was apparent as less effective energetic connectivity. The OJIP transients and auxiliary measurement of ChlF temperature curves suggested that *X. elegans* had the lowest thermostability among the experimental species.

Additional key words: conductivity; photosynthesis; photosystem II; thermal stability; transient.

Introduction

Measurements of chlorophyll fluorescence (ChlF) using fast ChlF transient (induction kinetics, OJIP) is very often used to access photosynthetic function. It is well established that the O–J phase is photochemical phase of the transient since its height depends on intensity of used excitation light (Delosme 1967, Neubauer and Schreiber 1987, Strasser *et al.* 1995). The photochemical phase is related to the balance between reduction of photosystem II (PSII) primary electron acceptor (Q_A) and its reoxidation by Q_B (e.g., Boisvert *et al.* 2006). On the other hand, the J–I–P phase is the thermal phase of the transient since its shape depends on temperature of measurement (within physiological range) (Delosme 1967, Neubauer and Schreiber 1987). The P-step (peak) is in some types

of samples split into G- and H-steps (peaks) (Tsimilli-Michael *et al.* 1998, Ilík *et al.* 2006). For the further insight in processes associated with the photochemical and thermal phases, such as e.g., electric voltage across thylakoid membranes, recombinations between PSII electron acceptors and donors, electron transport reactions after PSII, see review by Lazár (2006). In the transient of samples stressed by high temperature, K-step (peak) appears at about 0.3 ms (between O and J). The presence of the K-step reflects an inhibition of oxygen-evolving complexes and diverse energy distribution as well as a progressive decrease in photochemical processes rates (Guissé *et al.* 1995, Srivastava *et al.* 1997, Martinazzo *et al.* 2012, Gururani *et al.* 2015). It was also reported (see Brestič and Živčák 2013) that K-step is indicative of serious high temperature-induced disorganization of PSII

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Abbreviations: ChlF – chlorophyll fluorescence; CTC – conductivity temperature curve; FTC – chlorophyll fluorescence temperature curve; OEC – oxygen-evolving complex; OJIP – fast chlorophyll fluorescence; PQ – plastoquinone; RC – reaction center.

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structure and thylakoid membrane components.

In the samples stressed by high temperatures, the K-step is usually distinguishable as a clear peak directly in the ChlF transient curve. However, if the high-temperature stress is not so severe, processes related to the K-step can be revealed by subtraction of relative variable fluorescence of reference sample (not stressed by high temperature) from the relative variable fluorescence of the high temperature-stressed samples (*see* ‘Materials and methods’). The difference is manifested as a peak found at about 0.3 ms, which is then called K-band. Similarly, L-band, appearing typically at about 0.08 ms is sometimes attributed to the limitation in the energetic connectivity, *see* Yusuf *et al.* (2010).

The OJIP ChlF transients have been commonly used for studies of plant responses to a great variety of abiotic stressors (for reviews, *see* Kalaji *et al.* 2016, Stirbet *et al.* 2018). The studies include *e.g.*, high light stress (Oukarroum and Strasser 2004), nitrogen deficiency (Cetner *et al.* 2017, Kalaji *et al.* 2017, Samborska *et al.* 2018), salinity (Dąbrowski *et al.* 2016), water deficit, drought stress (Kiani *et al.* 2008, Czyczyło-Mysza *et al.* 2011, Goltsev *et al.* 2012, Wang *et al.* 2012, Jedmowski *et al.* 2014, Rapacz *et al.* 2019), action of herbicides (Lázár *et al.* 1997a, 1998), and waterlogging (Bertholdsson *et al.* 2015). The studies focused on temperature effects on OJIP shape and derived parameters have addressed both low (*e.g.*, Fracheboud *et al.* 1999, Rapacz *et al.* 2015, Husičková *et al.* 2019) and high (Pastenes and Horton 1996, Azam *et al.* 2015) temperature.

The OJIP transient has been often used for study of high temperature effects in plants since the 90s (*see e.g.*, Srivastava *et al.* 1997, Strasser 1997), however, non-vascular plants and lichens, have been studied much less frequently. In lichens, ChlF parameters have been exploited mainly in the studies focused on low temperature effects (Hájek *et al.* 2001, 2006; Hájek 2009) because lichens (except of tropical lichen species – Pardow *et al.* 2010) are hydrated and thus photosynthetically active rather in low (typically below +20°C) than high thallus temperature (over +25°C). In high temperature, majority of lichen species desiccate rapidly and become photosynthetically inactive in tens of minutes. Therefore, previous studies on the tolerance of lichens to temperature stress were focused on a response of the OJIP-derived parameters to low temperature (Marečková *et al.* 2019). In previous study (Marečková *et al.* 2019), we found that *D. polyphyllizum* treated at –5°C showed an increase in several parameters: ABS/RC, ϕ_{D0} , and DI_0/RC , which reflect an increase in apparent antenna size of PSII and increase in dissipation of absorbed energy by nonphotochemical processes.

The relationship between the OJIP-derived parameters and Φ_{PSII} (efficiency of PSII) is still unclear in lichens, especially in relation to temperature. Moreover, the appearance of K- and L-bands in OJIPs in subzero and high temperatures has not been studied in lichens in details. There is a gap of knowledge on high temperature effect on K- and L-bands appearance in lichens. In our study, we hypothesized that K- and L-bands would be induced by high temperature in lichens. Our aim was to describe

and analyse K- and L-bands appearance at the temperature above +35°C. Moreover, we tried to verify the hypothesis that the temperature-treated lichens respond similarly in low and high temperature in order to protect core of PSII in photosynthesizing symbiont (alga) by alternations in LHC complexes (energetic connectivity). In order to evaluate interspecific differences in the response of the OJIP-derived parameters, we measured three different chlorolichens with different morphology and habitat requirements (*see* ‘Materials and methods’). Therefore, we focused on the presence/absence of K-, and L-band in low (–5°C) and high (+45°C) temperature. Since the lichens have never witnessed such a high temperature in physiologically active state in the field, the measurements taken at +45°C were focused rather on thermostability of photosynthetic apparatus in wet state than on photosynthetic performance. To analyse the effects of high temperature, we combined the OJIPs measurements with thermostability measurements. For the first time in the case of lichens, we applied a newly emerging method (Ilik *et al.* 2018) based on ion leakage technique combined with ChlF measurements during linear heating.

Materials and methods

Sample collection and handling: Thalli of the lichen species *Xanthoria elegans*, *Usnea antarctica*, and *Dermatocarpon polyphyllizum* were collected in January and February 2019 in the James Ross Island (Antarctica) near Mendel Polar Station of the Czech Republic. *X. elegans*, *D. polyphyllizum*, and *U. antarctica* are major representatives of Antarctic vegetation. They are abundant at the James Ross Island. The three species were chosen because of different thallus structure and ecological niches they thrive in.

The air temperature on the James Ross Island ranges from +2.5°C (January) to –15°C (July). The surface air temperature changes from –11 to +15°C (Láska *et al.* 2011). Samples were dried under natural conditions [+10°C, PAR dim light of 10 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$], stored dry at –20°C for a few weeks and then transported to Czech Republic. After the transportation, lichens were stored in the freezer (–20°C) for two months. Prior to the experiments, all samples of dry thalli were rinsed with demineralised water and placed on Petri dishes for 24 h to reach optimally-hydrated state [+10°C, 10 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$]. *D. polyphyllizum* upper cortex was removed in order to obtain higher signals for the ChlF measurements.

Xanthoria elegans is a yellowish-orangish through orange to reddish, crustose to foliose lichen. It has a broad circumpolar and alpine distribution. The lichen thallus forms rosettes tightly bound to rock/stone surfaces. It possesses green unicellular alga *Trebouxia* sp. as photosynthesizing photobiont. In the James Ross Island, it preferably occupies habitats with availability of nitrogen and phosphorus. It is known as a nitrophilous and ornithocoprophilous species. Typically, it grows on rock/stone surfaces with water availability, frequently on margins of shallow depressions and along the stony creeks.

Usnea antarctica is a lichen with fruticose thallus

morphology. It forms clusters of erected branched shrub-like structures covering the upper surfaces of stones and rocks. The thallus is pale yellow or yellowish-green, sometimes variegated, with black apical parts of individual branches. *U. antarctica* possesses unicellular alga *Trebouxia* sp. as a photosynthesizing partner that forms an o-ring structure (photobiont layer) under the upper cortex. In the James Ross Island, the species grows mainly on stone fields and the plateaus of table mountains at the altitude of 350 m a.s.l.

Dermatocarpon polyphyllizum is a foliose lichen species forming a complex thallus composed of clustered individual lobes. Thallus is dark brown in a wet state, grey in the dry state. It possesses the green alga *Diplosphaera* sp. as the symbiotic photosynthesizing partner. Foliose thalli of *D. polyphyllizum* grow on rocks/stones in high altitudes or in polar regions. In the James Ross Island, it occurs in similar habitats as *X. elegans*, *i.e.*, margins of shallow depressions and/or creeks with availability of melt water.

Temperature treatment: At least six samples of each species were placed on measuring supports with a small piece of square-shaped filter paper under the lichen to provide a full hydrated state of the samples during the whole experiment. Lichen samples were placed into Petri dishes with a small amount of demineralised water. They were acclimated at room temperature (+20°C) for 25 min before the first measurements. This was the temperature selected as reference or control temperature. Then, a heating unit (*UNE 200, Memmert, Germany*) and a cooling unit were used to increase or decrease samples temperature (15–20 min) and then acclimate for 15 min at each target temperature. This procedure was repeated at ten different temperatures: +25°C, +30°C, +35°C, +40°C, +45°C (by using the heater); and +15°C, +10°C, +5°C, 0°C, and –5°C (by using the cooler unit). Two sets of three equal lichen samples were used: one set for low temperature and another set for high temperature measurements. As a result, measured samples were not affected by previous high or low temperature treatment. Temperature was measured during the whole experiment by a hairy Cu-Co thermocouple (0.5 mm) in 30-s interval and stored into a data logger (*EdgeBox, Environmental Measuring Systems, Czech Republic*).

Chlorophyll fluorescence: The effects of temperature treatments on primary photosynthetic processes of the lichens were evaluated using OJIP curves and ChlF parameters measured with the portable fluorometer *FluorPen (Photon Systems Instrument, Drásov, Czech Republic)*. The samples were kept in darkness before initial measurement for at least 15 min and then kept in dark during the acclimation period to allow RC of PSII reach fully open (oxidized) state and obtain the real fluorescence maximum after the saturation pulse application (Lichtenthaler 1988). OJIP curves were measured for each sample and at each temperature. The transients were induced by red light [630 nm; 3,000 $\mu\text{mol}(\text{photon})\text{m}^{-2}\text{s}^{-1}$] lasting 2 s. Then, they were double normalised in order to

compare species-specific response to the modification in thallus temperature.

From the OJIP curves, ChlF parameters were derived as follows: ABS/RC is the absorption flux per RC, TR_0/RC is the trapped energy flux per RC, ET_0/RC is the electron transport flux per RC, and DI_0/RC is the flux of dissipated excitation energy, all for dark-adapted state, and PI_{ABS} (the so-called performance index) is the potential for energy conservation from exciton to the reduction of plastoquinone pool, whereas $\text{PI}_{\text{ABS}\text{total}}$ is the potential for energy conservation from exciton to the reduction of final acceptors of PSI (for reviews see Strasser *et al.* 2004, Stirbet *et al.* 2018). For visualisation of K-band, the data of ChlF transients were double normalised between F_0 and F_J (2 ms), expressed as $W_{\text{OJ}} = (F_t - F_0)/(F_J - F_0)$ (Tsimilli-Michael and Strasser 2008, Marečková *et al.* 2017). For the L-band analysis, the data were double normalised as $W_{\text{OK}} = (F_t - F_0)/(F_K - F_0)$, where F_K represents the ChlF value at 0.3 ms. For L- and K-bands, the data used were the difference between the temperature-stressed samples and control lichens measured at +20°C. The differences were plotted as a function of time, *i.e.*, the time of light exposure. Statistically significant interspecific differences in the OJIP-derived parameters were tested by one-way analysis of variance (ANOVA) (*Statistica, StatSoft*), Fisher's LSD test ($P < 0.05$).

Thermostability measurements: Thermostability of lichen thalli was measured by a *PlanTherm PT 100* instrument (*Photon Systems Instruments, Drásov, Czech Republic*) that allows estimation of heat stability of samples. The instrument combines the measurement of ion leakage-dependent changes in electrical conductivity of heated sample with ChlF signal measurement (Ilík *et al.* 2018). The instrument consists of four main units: thermoregulation unit, conductivity probe unit, ChlF unit, and a mini-PC. Lichen thalli segments were fixed in a stainless steel holder and immersed in the cuvette with the deionized water bath (thermoregulation unit). The sample was heated (from +25 to +70°C) using a constant rate of heating $2^\circ\text{C}\text{min}^{-1}$ and conductivity increase was measured by the conductivity probe (fixed to the holder) with two platinum electrodes. The measured values of conductivity of the bath were logged together with temperature record and stored by a controlling software in a PC. Simultaneously with conductivity measurements, ChlF signal was measured by integrated *AquaPen-AP-P-100* fluorometer (*Photon Systems Instruments, Drásov, Czech Republic*). ChlF from the lichen segments (immersed in the water bath) was excited by 0.03-ms blue flashes (LEDs with 8-mm light guide, 470 nm) spaced by a 100-ms dark period. The emitted ChlF was transmitted *via* a light guide on a set of filters (spectral range of 690 to 770 nm). Then, the detected signal was digitized and logged together with the conductivity and temperature data. All data sets were plotted dependent on sample temperature (CTC – conductivity temperature curve, FTC – ChlF temperature curve). Critical temperatures T_{COND} and T_{F} were estimated as described by Ilík *et al.* (2018) and interspecific differences evaluated. In graph of the FTC,

the T_F roughly corresponds to temperature of intercept of a horizontal line at position of ChlF at room temperature with a line through a linear part of the ChlF increase to the first maximum of FTC (see e.g., Lazár and Ilík 1997).

Results

Fast chlorophyll fluorescence (OJIP) curves shape:

OJIP transients in several different temperatures (ranging from -5 to $+45^\circ\text{C}$) were measured and analysed. In order to compare the OJIPs from three lichen species, we double normalised data to F_0 and F_P (Fig. 1). There are clear differences distinguishable in ChlF signals and in the shape of OJIP transient. In all experimental species, the highest ChlF signals were measured at $+5^\circ\text{C}$ and the lowest signals at $+45^\circ\text{C}$. *U. antarctica* samples reached only half of *X. elegans* ChlF signals, the highest OJIPs measured at $+5^\circ\text{C}$. In *D. polyphyllizum*, ChlF was very low (up to 50, data not shown). In order to get higher ChlF signals, we carefully removed pigmented upper cortex from its samples. This uncovered the photobiont layer and allowed much higher ChlF signals (comparable with those of *X. elegans*, see Fig. 1C).

In all measured OJIPs, there were apparent changes in the shape of the OJIP transient. The most pronounced changes were in *X. elegans* and the most consistent shape

was in *D. polyphyllizum*. Samples showed higher ChlF signals in low temperature measurements (particularly at $+5^\circ\text{C}$). The J-step increased with increasing level of stress (either high or low temperature). In high temperature-stressed samples, the shape of the OJIP transient was flattened with more pronounced decrease of fluorescence after reaching the peak P. The time of reaching the peak P proved to be also very sensitive to the temperature changes. Along with increasing temperature, the peak P was reached sooner than that at the temperature optimum [$+20^\circ\text{C}$, the temperature optimum was evaluated for *D. polyphyllizum* from Antarctica in the earlier study of Marečková *et al.* (2019)] and it was reached later with decreasing temperature.

OJIP analysis – K-band, L-band: The K-band was revealed in high temperature measurements in all three lichen species (Fig. 2). In *X. elegans* samples, the K-band occurred at all temperatures higher than optimum $+20^\circ\text{C}$, i.e., at $+25$, $+30$, $+35$, and $+45^\circ\text{C}$ (selected data shown in Fig. 2). Compared to that, *U. antarctica* and *D. polyphyllizum* were less sensitive to high temperature stress. In both lichens, the K-band was found at $+30$, $+35$, $+40$, and $+45^\circ\text{C}$, although it was much lower in *D. polyphyllizum*. The highest K-band in *X. elegans* was found at the highest temperature ($+45^\circ\text{C}$). In the other

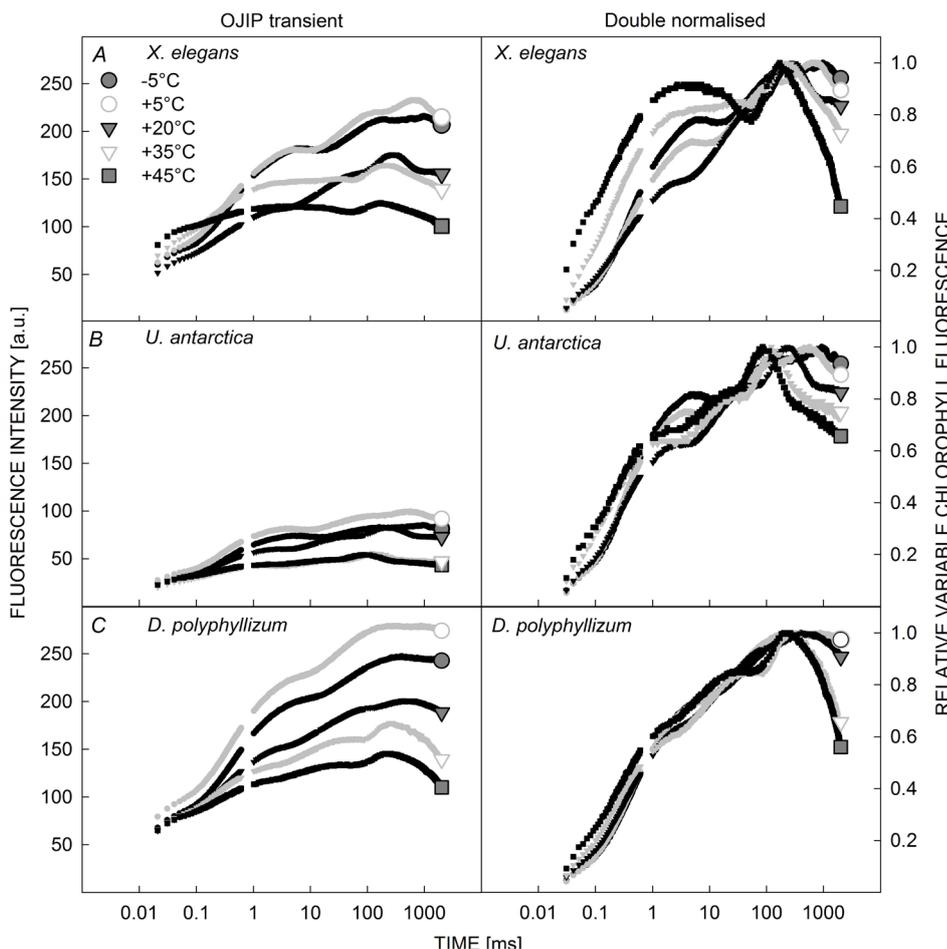


Fig. 1. Fast ChlF transients (OJIPs) measured in lichen species *Xanthoria elegans* (A – right), *Usnea antarctica* (B – right), and *Dermatocarpon polyphyllizum* (C – right) in different temperatures from -5 to $+45^\circ\text{C}$ upon $3,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ (saturation pulse). Samples of *D. polyphyllizum* (C) had upper cortex removed. The transients represent mean ChlF values of 3–4 measured samples at each temperature. In order to compare species-specific differences, these transients were double normalised as $V = (F_t - F_0)/(F_M - F_0)$ (A–C – left).

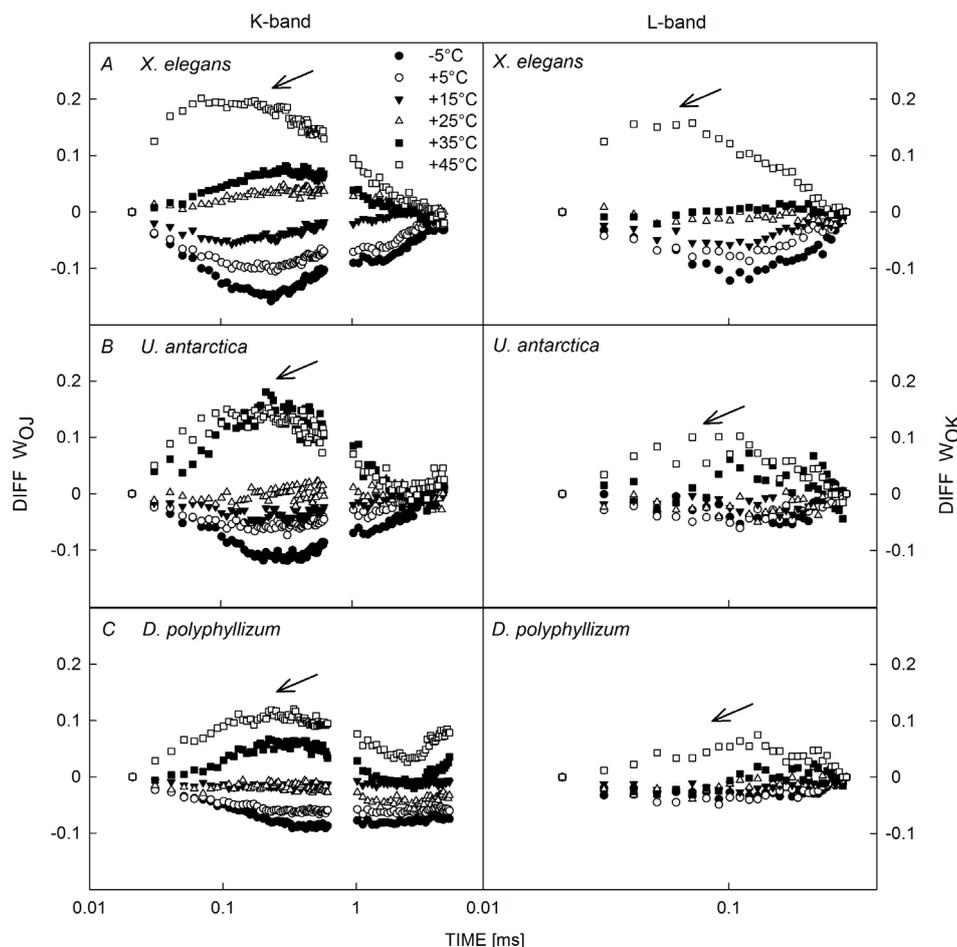


Fig. 2. Visualization of the K-band in *Xanthoria elegans* (A – left), *Usnea antarctica* (B – left), and *Dermatocarpon polyphyllizum* (C – left): OJIP transients normalised between O and J, expressed as difference $W_{OJ} = W_{OJ(T_i)} - W_{OJ(T_{control})}$, where $W_{OJ} = (F_t - F_0)/(F_J - F_0)$. Visualization of the L-band in *X. elegans* (A – right), *U. antarctica* (B – right), and *D. polyphyllizum* (C – right): OJIP transients normalised between O and K, expressed as difference $W_{OK} = W_{OK(T_i)} - W_{OK(T_{control})}$, where $W_{OK} = (F_t - F_0)/(F_{0.3ms} - F_0)$. Control temperature was +20°C. Arrows indicate position of the K-band (L-band, respectively).

lichen species, the K-band was on the same level in +40°C and +45°C (*D. polyphyllizum*) or it was higher at +35 and +40°C (*U. antarctica*, selected data shown in Fig. 2). None of lichen samples showed a visible K-band at low temperature.

The L-band was revealed in *X. elegans* as well as in *D. polyphyllizum* and *U. antarctica* samples. In *X. elegans*, the L-band was clearly apparent at +45°C, but not at lower temperatures. A broad maximum around the L-band also appeared at the same temperature in *U. antarctica* samples (Fig. 2). At +45°C, a hint of the L-band appeared also in *D. polyphyllizum*. Low temperature measurements did not show any L-band.

OJIP-derived parameters: Values of selected OJIP-derived parameters were calculated (Fig. 3). Most of the derived parameters (e.g., maximum quantum yield PSII of photochemistry in dark-adapted state – F_v/F_m , dissipated excitation energy flux per RC – DI_0/RC or absorption flux per RC – ABS/RC) were not affected by low temperature (below +10°C). In high temperature measurements, typically at the temperature above +20°C, the changes of OJIP-derived parameters were more apparent. ABS/RC increased exponentially until +45°C, the highest values were recorded for *X. elegans* samples. Trapped energy flux per RC (TR_0/RC) raised as well and reached the highest

values at +40 to +45°C. Maximal quantum yield of PSII photochemistry in dark-adapted state (F_v/F_m) decreased gradually with higher thallus temperature. Performance index (PI_{ABS}) increased until temperature optimum was reached (+20°C) in *X. elegans* and *U. antarctica* samples and did not change in *D. polyphyllizum* (Fig. 3B). High temperature stress at the temperature above +20°C caused a rapid decrease of PI_{ABS} in all experimental lichen species. The lowest values were measured in *X. elegans* samples at +45°C. Total performance index ($PI_{ABS_{total}}$) peaked at 0°C and then decreased with increasing temperature. Electron transport flux per RC (ET_0/RC) raised linearly until +20°C, then decreased in *X. elegans* samples (Fig. 3F). In *U. antarctica* and *D. polyphyllizum* ET_0/RC increased until +40°C. Statistically significant interspecific differences were analysed and marked by asterisks (see Fig. 3 for ET_0/RC , TR_0/RC , ψ_{E0} , ABS/RC , and $PI_{ABS_{total}}$).

Thermostability: The temperature dependence of conductivity (CTC) reflecting ion-leakage from lichen samples into deionized water during linear heating showed certain differences between the lichen species. In the presented CTCs (Fig. 4A), the critical temperature T_{COND} seems to be lower (of about +60°C) in *X. elegans* in comparison to *U. antarctica* and *D. polyphyllizum*, but the difference was not statistically significant (Fig. 4A insert).

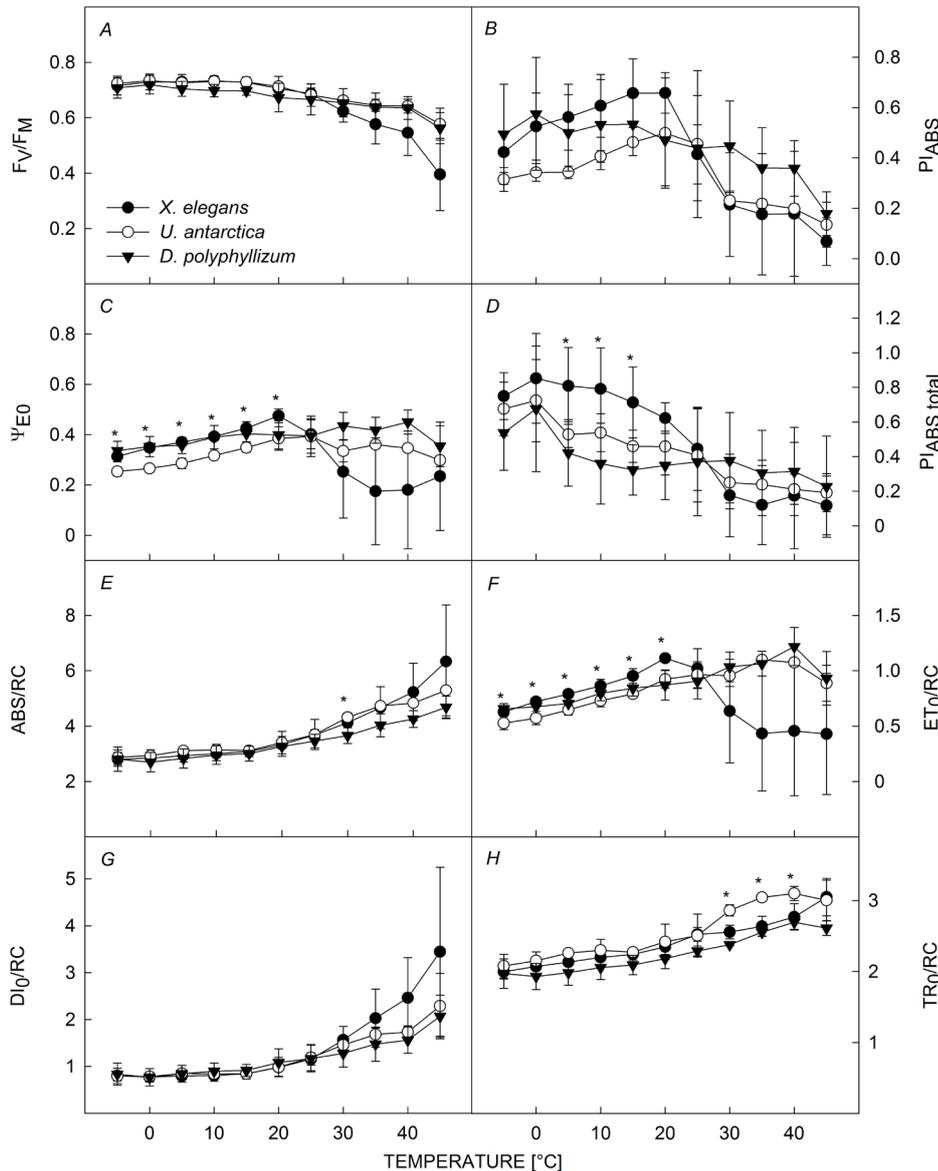


Fig. 3. OJIP-derived parameters measured at different temperatures in *Xanthoria elegans*, *Usnea antarctica*, and *Dermatocarpon polyphyllizum*: F_v/F_m – maximal quantum yield of PSII (A), PI_{ABS} – performance index on absorption basis (B), ψ_{E0} – probability (efficiency) that an electron moves further than Q_A (C), $PI_{ABS\text{total}}$ – total performance index on absorption basis (D), ABS/RC – absorption flux of antenna chlorophyll per RC (E), ET_0/RC – electron transport flux per RC (F), DI_0/RC – flux of dissipated excitation energy per RC (G), and TR_0/RC – trapped energy flux per RC (H). The means \pm SD ($n = 3$) are shown. Statistically significant interspecific differences ($P < 0.05$) are marked with asterisks.

A comparison of fluorescence temperature curves (FTCs) of the three lichen species confirmed the lower thermostability of *X. elegans* indicated by the results of OJIP measurements. The critical temperature T_F , which is considered as an indicator of heat stability of thylakoid membranes (Ilík *et al.* 2018), was in *X. elegans* about $+40.5^\circ\text{C}$, whereas T_F in the other two lichens was significantly higher [of about $+42.5$ and $+44^\circ\text{C}$ for *U. antarctica* and *D. polyphyllizum*, respectively (Fig. 4B insert)].

Discussion

Fast chlorophyll fluorescence (OJIP) curves shape: High temperature-induced decrease in ChlF signal caused a ‘flattening’ of the OJIP curves which is well-known phenomenon in plants (Lazár and Ilík 1997, Chen *et al.* 2016) and, more recently, reported for lichens (Oukarroum *et al.* 2016b). Except of the OJIPs recorded for *X. elegans*

after the $+45^\circ\text{C}$ treatment, the OJIPs did not show a decrease of the ChlF signal after J-step and following increase to F_P as reported for plants treated by temperature over $+40^\circ\text{C}$ (Guissé *et al.* 1995). However, *X. elegans* exhibited a decrease of ChlF signal between J and P while the other two species did not. It might indicate reopening of RCs of PSII by electron transfer from Q_A to Q_B (Strasser 1997). Alternatively, as stress by high temperature causes also inhibition of electron transport from Q_A to Q_B (e.g., Pospíšil and Tyystjärvi 1999), accumulated Q_A^- and P_{680}^+ can recombine, which causes a decrease of ChlF after the K-step (Lazár *et al.* 1999). Therefore, the presence/absence of the decrease of ChlF signal between K (J) and P seems to be related to the severity and/or duration of high temperature stress. On the other hand, the K-step appearance was reported even for high temperature-acclimated barley exposed to high temperature (Lazár *et al.* 1997b, Lazár and Ilík 1997) which is consistent with our data (K-peak at $+35$, $+40$, $+45^\circ\text{C}$). More severe or

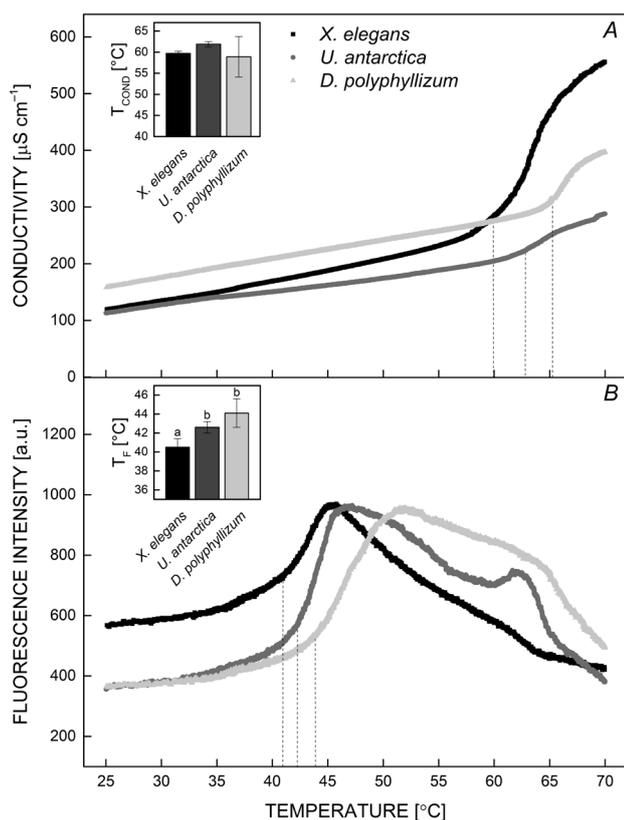


Fig. 4. Typical conductivity temperature curves (CTCs) (A) and fluorescence temperature curves (FTCs) (B) measured with thalli of *Xanthoria elegans*, *Usnea antarctica*, and *Dermatocarpon polyphyllizum*. Critical temperatures T_{COND} and T_F of the individual curves are indicated in CTCs and FTCs, respectively. The means \pm SD of T_{COND} and T_F are shown in bar graph ($n = 6$). Statistically significant differences are indicated by different letters ($P < 0.05$). Heating rate of 2°C min^{-1} was used in all measurements.

long-term stress (in terms of tens of minutes) leads to a decrease of ChlF signal between K/J and P as seen in the data reported by Guissé *et al.* (1995), Martinazzo *et al.* (2012), Li *et al.* (2014), Oukarroum *et al.* (2016a), and Stirbet *et al.* (2018).

High temperature-induced increase in F_0 (Fig. 1) and F_P decrease could be associated with a detachment of LHCs from RC PSII and inhibition of electron transport from OEC toward PQ pool (Yamane *et al.* 2000). In high temperature-treated lichens, more apparently in *X. elegans*, the peak P was reached earlier than in the samples treated at $+5^\circ\text{C}$. It is assumed, that the time of reaching peak P (t_P) reflects stress-resistance (Kalaji *et al.* 2012, Žurek *et al.* 2014). Shorter t_P indicates more stressed plants. High-temperature stress was also reflected in the increase of the ChlF signal at the J-point (relatively to peak P – see Fig. 1) which was apparent in *X. elegans* but not in *D. polyphyllizum*. A remarkable decrease in ChlF signal was found in all experimental species after the peak P (Fig. 1).

No apparent peaks H and G were seen in the OJIP

kinetics. It was surprising because the peaks H and G are typically attributed to lichens with symbiotic alga *Trebouxia* sp. (Medina and Avalos-Chacon 2015) measured at a wide range of excitation light intensities [$100\text{--}1,800 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ – Ilík *et al.* 2006]. The peaks H, G are caused by a very fast reoxidation of PSI and attributed to an alternative electron flow from PSI which was originally explained by a fast activation of ferredoxin-NADP⁺ oxidoreductase (Ilík *et al.* 2006) or Mehler reaction (Franck and Houyoux 2008). Recently, the phenomenon has been investigated (Ilík *et al.* 2017) and attributed to a photoreduction of O₂ mediated by flavodiiron proteins (FDPs). The lack of the peaks G and H in our data might be attributed either to the fact that the above-mentioned mechanisms were likely not involved in the three studied lichen species or by different light intensity used for excitation.

K-, L-bands: Generally, the K-step (distinguishable on the OJIP) and/or the K-band (visible in differential curve) are well documented in high temperature-treated crops (reported by *e.g.*, Lazár *et al.* 1997b, Brestič *et al.* 2008, Jain *et al.* 2018). K-step and/or K-band might be attributed to 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. The K-band is reported in cultures of lichen symbiotic alga *Trebouxia* sp. (Guéra *et al.* 2016) and *Diplosphaera* possessing *D. polyphyllizum* (Marečková *et al.* 2019). In heat-treated lichen *Parmelina tiliacea*, Oukarroum *et al.* (2012) found the K-step. The presence of the K-band is induced by an inhibition of the OEC (see *e.g.*, Strasser *et al.* 2004). Our data suggest that the K-band appearance in all experimental lichen species above $+35^\circ\text{C}$ might be attributed to negative high temperature effect causing donor side inhibition (see *e.g.*, Chen *et al.* 2016). This might be supported by the fact that the K-band was not detectable below $+20^\circ\text{C}$ in all three experimental lichen species.

The L-band was distinguished exclusively at the temperature of $+45^\circ\text{C}$ (Fig. 2). The lack of L-band at the other temperatures was consistent with an earlier study (Marečková *et al.* 2019) that reported no L-band within the temperature range of $+4$ to $+22^\circ\text{C}$ in *D. polyphyllizum*. The L-band was found in the range 0.10–0.15 ms and associated with the changes in the energetic connectivity, caused by lower thermal stability of the system (Yusuf *et al.* 2010). According to Strasser *et al.* (2004), low connectivity results in less efficient consumption of the excitation energy. In lichens from polar regions, however, the thermal stability at the temperature above $+40^\circ\text{C}$ might be affected by the fact that the lichens never experience such a high temperature in a wet state. Thanks to fast desiccation, they become dry even at lower temperature.

OJIP-derived parameters in response to temperature:

Our data showed the F_V/F_M and PI_{ABS} decrease (above $+20^\circ\text{C}$). Temperature response curves of F_V/F_M and PI_{ABS} (Fig. 3) were similar to those reported for poikilohydric autotrophs from polar and alpine ecosystems (Folgar-

Cameán and Barták 2018, Marečková *et al.* 2019). In recent studies (*e.g.*, Chen *et al.* 2016), PI_{ABS} is considered a standard for identification of heat sensitivity of plants. The parameter responds to various stress conditions (Strasser *et al.* 2004, Stirbet *et al.* 2018) since three independent components of RC functioning are used for calculation: (1) RC/ABS (the density of active RCs per chlorophyll absorption), (2) ϕ_{P0} (F_v/F_M , *see* Fig. 3), and (3) ψ_{E0} (for reviews, *see* Strasser *et al.* 2004, Stirbet *et al.* 2018). The $PI_{ABStotal}$ decrease with temperature rise reflects limitation of energy conservation from PSII RCs to the reduction of PSI end acceptors (Stefanov *et al.* 2011, Stirbet *et al.* 2018). Although Stefanov *et al.* (2011) reported an increase of $PI_{ABStotal}$ in response to high temperature stress in higher plants, our $PI_{ABStotal}$ data indicate a remarkable high temperature-induced decrease of functioning of PSII and PSI. The limitation includes also the PSII to PSI electron transport (above +20°C). Such temperature is reported optimum temperature for primary photosynthetic processes in lichens (*e.g.*, Marečková *et al.* 2019).

The dramatic decrease in ET_0/RC and increase of DI_0/RC found in *X. elegans* treated by the temperature above +25°C indicate severe stress of PSII. The other two lichens (*U. antarctica* and *D. polyphyllizum*) were much less affected by the heat treatment. It might be interpreted that for *X. elegans*, such temperature is above physiological limit since the species, due to relatively thin thallus, desiccates more rapidly than the other two species and hardly reaches the thallus temperature above +25°C in the wet state in the field. Therefore, it showed more pronounced inhibition of photosynthesis (decrease in F_v/F_M and ET_0/RC) and higher thermal dissipation of excess energy (DI_0/RC) than that of the other two species. This conclusion might be supported by the quantum yield of dissipated energy (ϕ_{D0}), increasing in all experimental species with an increase of thallus temperature, reached the highest values in *X. elegans* at the temperature over +30°C (data not shown).

The ABS/RC and TR_0/RC increase with the experimental temperature rise is comparable to the evidence reported for higher plants treated by high temperature (Martinazzo *et al.* 2012). The response might be attributed to high temperature-induced decrease in number of active RCs in PSII (Lawlor and Tezara 2009). The increase in the two parameters is also reported for other stressors affecting negatively number of active RCs and their functionality (salinity – Demetriou *et al.* 2007, salt and drought stress – Killi and Haworth 2017, high temperature and drought stress – Soni and Swarnkar 2016, waterlogging – An *et al.* 2016, water deficiency – Haworth *et al.* 2018). Our earlier study (Barták *et al.* 2018) proved that ABS/RC and TR_0/RC increased with dehydration of algal photobiont in *D. polyphyllizum*. In general, temperature-induced increase in ABS/RC and TR_0/RC is accompanied by the increase in the yield of energy dissipation. In our experiments, such responses were found in all lichen experimental species since the ϕ_{D0} values were well related to ABS/RC and TR_0/RC (data not shown).

Thermostability measurements: The FTC measurements

confirmed that the primary photosynthetic processes (PSII activity) in the wet state were more sensitive to high temperature in *X. elegans* than that in *U. antarctica* and *D. polyphyllizum*. The inhibition of the electron transport in *X. elegans* started at the lowest temperature (T_F ; Fig. 4B) which indicates the lowest heat stability of algal thylakoid membranes. The other two species showed slightly higher but still low thermostability. The similarly low value of T_F (of about +40.5°C) reflecting a relatively low heat stability of thylakoid membranes and primary photosynthetic processes has been found for some species of higher plants including *Arabidopsis thaliana* (Ilik *et al.* 2018). The measurement of conductivity changes during linear heating of the lichens did not provide convincing results. As the critical temperature T_{COND} did not differ significantly between the lichen species (Fig. 4A *insert*), heat stability of their cell membranes was probably similar. It seems that the thermostability of cell membranes need not correlate with the thermostability of primary photosynthetic processes. Interpretation of the data is complicated by the facts that lichens are symbiotic organisms composed of fungus and alga/cyanobacteria. It is likely that the thermostability of each symbiotic partner differs and contribute differently to T_{COND} .

Concluding remarks: Our study revealed that OJIP measurements provide data on PSII functioning sensitive enough to monitor temperature-induced changes in energy and electron transport of lichen photobionts. The OJIP-derived parameters used in our study evaluated species-specific differences in the response of three lichen species to high temperature stress. It might be concluded that OJIP is highly important tool to study photobionts of lichens, their responses to high temperature in particular. It is because of the initial phase of the transient (O–J) that responded to heat stress very sensitively. The OJIP measurements could be used in follow-up studies to study inter-specific differences in lichen thermotolerance. For ecophysiological field studies, performance index (PI_{ABS}) might be, because of its high sensitivity, recommended as general indicator of negative effects of temperature on overall biological activity in lichens. Thermal dissipation (DI_0/RC) represents another sensitive parameter indicating the involvement of PSII protective mechanisms activated during early stress in the photosynthetic apparatus of the algal partner in chlorolichens.

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