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

Anamika Mishra[1], Josef Hájek[2], Tereza Tuháčková[2], Miloš Barták[2], Kumud Bandhu Mishra[1*]

[1]*Laboratory of Ecological Plant Physiology, Global Change Research Centre ASCR vvi, Bělidla 986/4a, 603 00 Brno, Czech Republic*
[2]*Department of Experimental Biology, Laboratory of Photosynthetic Processes, Faculty of Science, Masaryk University, University Campus – Bohunice, Kamenice 5, 625 00 Brno, Czech Republic*

Abstract

Chlorophyll fluorescence is an effective tool for investigating characteristics of any photosynthesizing organisms and its responses due to different stressors. Here, we have studied a short-term temperature response on three Antarctic green algal lichen species: *Umbilicaria antarctica, Xanthoria elegans*, and *Rhizoplaca melanophtalma*. We measured slow chlorophyll fluorescence transients in these Antarctic lichen species during slowely cooling of thallus temperature from 20°C to 5, 0 and -5°C with 20 minute acclimation at each temperature. The measurements were supplemented with saturation pulses for the analysis of chlorophyll fluorescence parameters: maximum yield of PS II photochemistry (F_V/F_M), effective quantum yield of PS II photochemistry (Φ_{PSII}) and quenching parameters. In response to decreasing thallus temperature, we observed species-specific changes in chlorophyll fluorescence parameters as well as in the shape of the chlorophyll fluorescence transients. We propose that species-specific changes in the slow phase of chlorophyll fluorescence transients can be potentially used as indicators of freezing stress in photosynthetic apparatus of lichen algal photobionts.

Key words: *Rhizoplaca melanophtalma, Umbilicaria antarctica, Xanthoria elegans*, temperature stress

Symbols and abbreviations: ChlF - Chlorophyll *a* fluorescence

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[*]Corresponding author: Kumud B. Mishra <mishra.k@czechglobe.cz>
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Introduction

Among chlorophyll fluorescence emission based techniques used in plant stress physiology, chlorophyll *a* fluorescence (ChlF) induction, *i.e.* a curve exhibiting polyphasic changes in chlorophyll fluorescence transients measured for several minutes, when predarkened plant material is exposed to continuous light, is widely used for non-invasive investigation of photosynthetic mechanism and responses of biotic/abiotic stresses. This fluorescence induction is also termed as the Kautsky kinetics, after it is discovered by Kautsky (Kautsky et Hirsch 1931). Depending on time resolution, the easy available methods for monitoring of ChlF induction kinetics can be classified into two groups: (1) Fast chlorophyll fluorescence induction (OJIP curve, where O is minimal fluorescence when reaction centres are fully oxidised after dark adaptation, J and I are inflections, and P is the peak under prevailling light intensity, Strasser et al. 1995) and (2) slow chlorophyll fluorescence kinetics (OPSMT, time resolution ~20-40 ms; where S is a semi steady state, M is secondary maxima and T is terminal steady state; Papageorgiou et al. 2007). The fast OJIP curve is typically recorded within the first two seconds of exposition to light, while measurement of slow chlorophyll fluorescence transient requires tens of seconds to several minutes.

In OJIP phase, fluorescence rise from minimal value (F_0, reaction centers are fully oxidised) to peak F_P with gradual reduction of plastoquinones (Q_A and Q_B); it is related to the electron transportation representing redox state of the plastoquinone pool connecting the PS II and PS I, and divided into fast photochemical phase (O-J, in 2 ms) and much slower photothermal (temperature sensitive) phase (J-I and I-P, in ~1 s), *see* Strasser et al. (1995) and Stirbet et Govindjee (2011). It is accepted that O-J is due to reduction of electron Q_A to Q_A^{-1}; while J-I and I-P are due to reduction of PQ pool. For thermally – affected samples, another point K was reported by *e. g.* Xue et al. (2011). The shape of the OJIP phase can be affected by several factors *e.g.*, efficiency of excitation energy transfer among PS II units (connectivity), the state of the oxygen evolving complex, and various processes involved in transformation of absorbed light energy in photosynthetic apparatus (see *e.g.* Tyystjärvi et al. 1999 for review). General pattern of OJIP curve is almost similar in all plant species. Currently, several commercial instruments are available for quick diagnosis of photosynthetic apparatus by measuring OJIP curve and associated derived parameters (Strasser et al. 1995). The OJIP test is highly useful and it is being potentially applied in multidisciplinary fields for detection of various stress effects such as heavy metals, UV-B effects *etc.* (Sayed 2003, Cuchiara et al. 2013, Wang et al. 2008). ChlF parameters derived from OJIP curve was reported to be useful for measuring freezing tolerance in wheat cultivars as it senses the early metabolic changes in PS II that are initiated following exposure to low and freezing temperatures (Rapacz 2007, Rapacz et al. 2007). In lichens, OJIP is used to evaluate responses of thalli to rehydration at different temperature (Oukarroum et al. 2012).

The scientific community frequently uses physiologically significant parameters such as F_0, F_M, F_V/F_M, NPQ *etc.*, derived from slow ChlF transients as indicators of stress effects in photosynthetic apparatus (Brestič et Zivčák 2013). Although the shape of the slow ChlF transient is highly informative and represents regulation of several biophysical processes, it is seldom used in plant stress physiology. The slow decline of ChlF from peak P (F_P) to S is more complex to interpret because several processes begin to involve such as non-photochemical quenching, ATP synthesis, Calvin–Benson cycle and state transition among others. The appearance of second-

ary SMT fluorescence phase is very common in cyanobacteria and in green alga (Kaňa et al. 2012, Kodru et al. 2015) but also found in higher plants under specific conditions (Mishra et al. 2011). Indeed the mechanisms of S-M rise and M-T decline is highly complex; it was demonstrated that state transition is the main phenomenon behind the S-M rise in green alga; the S-M rise is due to transition of low fluorescence state 2 to higher fluorescence state 1 since a mutant locked in state change did not show SM rise (Kodru et al. 2015, Kaňa et al. 2012).

In lichens, slow ChlF transient was used to characterize sensitivity to photoinhibition (*e.g.* Conti et al. 2014). In this investigation, we hypothesized that, similar to higher plants (Tyystjärvi et al. 1999), species-specific differences in shape of slow ChlF transients would be large in lichens as well. Further, we expect that exposing lichen species to short term stress may amplify the species-specific differences. Therefore, to evaluate species-specific sensitivity of photosynthetic processes to low temperature in three contrasting lichen species, we selected several ChlF parameters derived from slow ChlF transient.

Material and Methods

Species characteristics

Three of Antarctica based lichen species: *Rhizoplaca melanophthalma* (Ram.), *Umbilicaria antarctica* and *Xanthoria elegans* were used in this investigation. The lichen *Rhizoplaca melanophthalma* (Ram.) Leuck. and Poelt is a common species particularly found in coastal deglaciated areas of Antarctica (Øvstedal et Lewis Smith 2001). It is commonly distributed in non-polar regions and being utilized for biomonitoring (*e.g.* Dillman 1996). In Antarctica, *R. melanophtalma* is frequently found growing in large populations on rocky substrates close to a seashore or nestling sites of birds, pinguin colonies (*e.g.* Olech 1994, Olech et Singh 2010). Some authors reported about *R. melanopthalma* from Schimacher oases as well (Rai et al. 2011, Olech et Singh 2010). Physiological studies on the species are rather rare focusing specific aspects *e.g.* content of usnic acid (Duman et al. 2008).

Umbilicaria antarctica is a foliose macrolichen forming typical umbilicate thallus, generally 6-7 cm, but exceptionally 15 cm in diameter (Barták 2014). This lichen species is attached to rock or stony substrate by a central holdfast (umbilicus). The upper surface of the thallus has pale, gray or brown colour. Inside the thallus, *U. antarctica* preliminary has an unicellular green alga (*Trebouxia* sp.) as photosynthesizing photobiont. This lichen species is commonly distributed in maritime Antarctica (see *e.g.* Øvstedal et Lewis Smith 2001, Lee et al. 2008), and used for both field (responses to hydration – Barták et al. 2005) and laboratory-based (responses to low and freezing temperature – Barták et al. 2007) ecophysiological studies of photosynthesis. Recently, this species has got attention because presence of rich variety of secondary metabolites in its thallus, such as phenolics, usnic acid, atranorin (Quilot 1998 – *see* Other sources), lecanorin (Luo et al. 2009).

Xanthoria elegans is a bipolar lichen species abundantly found in alpine, Arctic and Antarctic habitats. It is a foliose lichen forming a round-shaped foliose yellow to orange thallus with marginal lobes. The species form a large rosettes up to 6 cm in diameter. In Antarctica, the species exhibit low growing rates (Armstrong et Bradwell 2011) and is

used for dating age of lithological items using a lichenometry approach (McCarty 1997). Several studies have been done both *in situ* (in Antarctica) and under semi-laboratory conditions addressing *e.g.* decline of photosynthetic processes in response to dehydration (Barták et al. 2005) and low temperature (Barták et al. 2007). Since *X. elegans* is considered as one of the model lichen species for ecophysiological and extreme environments studies, a great attention has been devoted to investigate its capacity for photoprotective secondary metabolites production (*e.g.* parietin, Nybakken et al. 2004), resistance of the species to extraterrestrial environments (Brandt et al. 2014). Its population genetics is studied (*e.g.* Murtagh et al. 2002) as well.

Collection and storage of lichens

Rhizoplaca melanophtalma was collected in February 2015 from individual stones and small boulders located 500 m W from the Czech Antarctic station (J. G. Mendel, 63° 48′ 17′′ S, 57° 55′ 14′′ E). The site of collection was situated close to a seashore (50 m) at the altitude of 15–20 m. The lichen thalli were collected in semi-dry state and dried out naturally at a laboratory at the station at 20°C. Then they were stored in a refrigerator at 5°C and transported in a portable freezing box to Brno (Czech Republic).

Thalli of *Umbilicaria antarctica,* were collected at Galindez Island (65° 15′ 00′′ S, 64° 15′ 00′′ W), Antarctica, in 2003. The site of collection was a N-facing rock wall of the Woozle Hill. The thalli were dehydrated in natural (outside) conditions. When dried, they were stored at 5°C and transported at such temperature to a laboratory in Brno (Czech Republic). Before experiments, thalli were stored in dry state in dark inside a refrigerator.

Xanthoria elegans was collected from several sites located on the foothill of the Berry Hill mesa (63° 48′ 18′′ S, 57° 49′ 38′′ E, James Ross Island). The site of collection was 5-10 degrees inclined, facing N. Individuals and clusters of *X. elegans* formed irregularly dense and wide margins of temporal freshwater streams fed by a snowfield and/or permafrost. The lichen thalli were collected in semi-dry state and dried out naturally at a laboratory at the station at 20°C. Then they were stored in a refrigerator at 5°C and transported in a portable freezing box to Brno (Czech Republic).

Experimental set up

Lichen thalli were wetted in Petri dishes by a demineralized water for 48 h before experiments. During rehydration, they were kept under dim light (5 μmol m^{-2} s^{-1} of photosynthetically active radiation) and at low temperature (5°C). When rehydrated, re-establishment of primary photosynthetic processes of photosynthesis was tested on 10 min. dark-adapted material by images of F_V/F_M. For experiment, those thalli exhibiting high F_V/F_M values over large lichen thallus area were selected for investigations. Measurements of ChlF transients was supplemented with quenching analysis by using a pulse-modulated fluorometer (PAM 2000, Heinz Walz, Germany) connected with a probe which was inserted inside a temperature-controlled box (URAS 4 cooling unit, Hartmann and Braun, Germany) through a predarkening clip placed over experimental lichen thallus. Measurements of slow ChlF transients (*see* Fig. 1) was started at 25°C. After the record of individual curve, temperature in the box was gradually lowered to 5, 0, and -5°C, with an equilibration time of 20 min. at each temperature. ChlF transient

was recorded at each temperature. During the measurements, lichen thalli were exposed to several saturation pulses so that ChlF parameters (*see* below and Table 1) could be evaluated.

Chlorophyll fluorescence measurements

Individual measurements used kinetic mode of the fluorometer with 0.1 ms time resolution for recording of ChlF emission. The measurements started on pre-darkadapted material with exposition of lichen sample to measuring light (ML=5, damping 4) for 60 s in order to determine F_0. Then, a saturation pulse (0.8 s, 5 000 μmol m^{-2} s^{-1}) was given in order to induce and record maximum ChlF (F_M). Following 10 s of dark adaptation, the thalli were exposed to actinic light (AL= 4) for 300 s and a polyphasic time course of ChlF emission was recorded. Then, a saturation pulse was given to induce F_M' level of ChlF, *i.e.* maximum value in light-adapted material. After switching of actinic light, background ChlF (F_0') was recorded for 20 s. Then, another saturation pulse was given in order to induce F_M'' value.

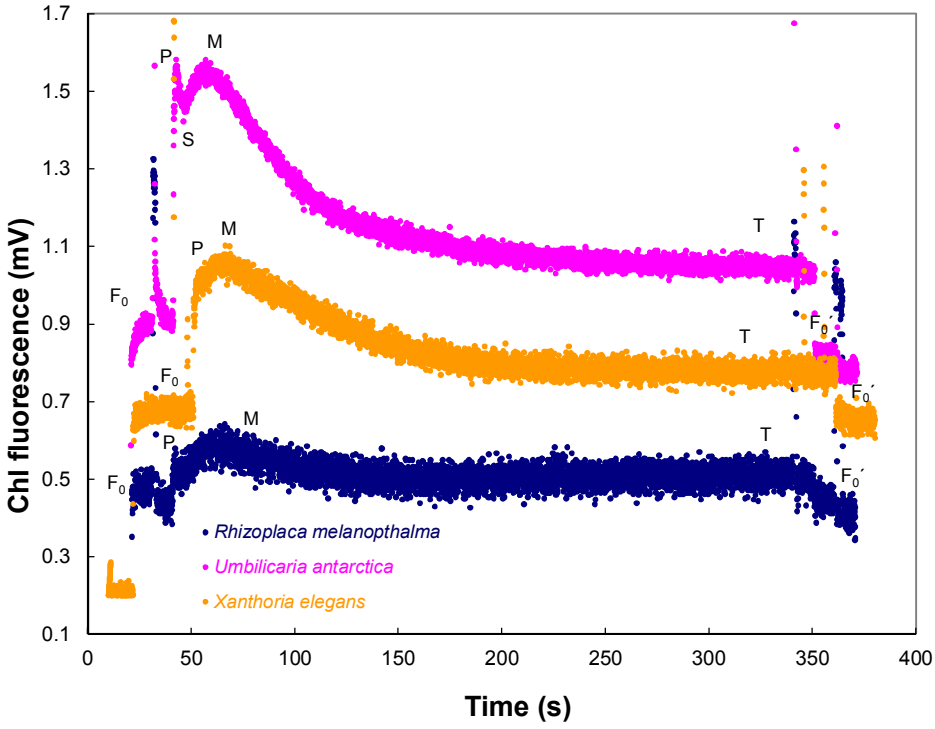

Fig. 1. Typical slow chlorophyll fluorescence (ChlF) transients recorded for *Rhizoplaca melanopthalma*, *Umbilicaria antarctica*, and *Xanthoria elegans* at 25°C. ChlF fluorescence levels P, S, M, T are clearly distinguishable in *U. antarctica*. In *X. elegans*, P and M is much less apparent. With decrease in temperature, P, M and S could be distinguished with difficulties – *see* Fig. 2.

Using the below-specified equations, the following ChlF parameters were calculated from the particular ChlF signals depicted form a record. Temperature-induced changes in the shapes of slow ChlF transients as well as ChlF parameters were evaluated.

$F_V/F_M = (F_M - F_0) / F_M$	
$\Phi_{PSII} = (F_M' - F_S) / F_M'$	
$Rfd = (F_P - F_S) / F_S$	(*Lichtenthaller et al. 2005*)
$qP = (F_M' - F_S) / (F_M' - F_0')$	
$qN = (F_M - F_0) - (F_M' - F_0') / (F_M - F_0)$	
$NPQ = (F_M - F_M')/ F_M'$	
$QCN = (F_M - F_M')/ F_M$ (*Roháček 2002*), $qCN = (F_M - F_M') / F_M$ (*Roháček et Barták 1999*)	
$qF_0 = (F_0 - F_0') / F_0$	

Table 1. Overview of chlorophyll fluorescence (ChlF) parameters used in this study.

Results and Discussion

At room temperature, all experimental lichen species showed typical slow ChlF transient during the exposition to continuous light (Fig. 1). The shape was species specific, however, typical points (P, M, S, T) were distinguished similarly to other lichen species (*see* Conti et al. 2014). Polyphasic time course of variable ChlF from P to T levels is caused by several reasons. Among them, redox state of electron acceptors (Q_A in particular), changes in proportion between absorbed light energy and emission of ChlF should be mentioned. The latter one is associated with the formation of transthylakoidal gradient (delta pH) and consequent involvement of xanthophyll cycle pigments into quenching of absorbed light energy. Changes in structure (antenna size) and function of light-harvesting complexes of PS II, as well as state transition are also involved (for review see *e.g.* Kalaji et al. 2014). At T point, variable ChlF was constant (steady state ChlF) in all experimental species, indicating that photosynthetic electron transport processes was fully coupled to biochemical reactions in the carbon reduction cycle.

With decreasing thallus temperature, shape of the slow ChlF transient is changes in the following aspects: (a) variable ChlF is increased compared to the initial record at 20°C, (b) proportion between P and M ChlF level was changed and, in some cases, P was not recognizable (*see* Fig. 2), and (c) the time taken to reach from peak P to steady state level T is increased. Decline of thallus temperature to 0 and -5°C, led to an overall decrease in variable ChlF throughout whole slow ChlF transient. At lowest thallus temperature, variable ChlF was not constant after ~5 min. exposition (during the Kautsky effect) to actinic light, it means that the ratio between ATP, NADPH synthesis and CO_2 fixation is not in equilibrium. At such situation, proton concentration in the thylakoid lumen is increased and consequently this may increase the non-photochemical quenching (Krause et Weiss 1991, Dewez et al. 2007) – *see* below.

Time courses of ChlF parametres were species-specific; however, followed a general trends in Antarctic lichens. Decline of F_V/F_M with lowering thallus temperatures is most apparent in *R. melanopthalma* (*see* Fig. 3). Such behavious is well comparable to experimental evidence (Barták et al. 2007). Similarly, Φ_{PSII} decreased with low-

ering temperature until 0°C, but slight increase in its value was observed at -5°C. Values of photochemical quenching (qP) showed somewhat different trends in particular for species *R. melanopthalma*: qP was more or less constant until 0°C followed by a dramatic increase at freezing temperature (-5°C). For other species qP declined with lowering thallus temperature followed by either no change (*U. antarctica*), or a slight increase (*X. elegans*) at -5°C.

Lowering of thallus temperature to close-to- and below-zero temperature led to a decrease followed by an increase in non-photochemical quenching in *R. melanophalma* (*see* NPQ in Fig. 3 and non-photochemical quenching parameters in Table 1). For other two species NPQ decreased with temperature decline. The courses differed among species reflecting mainly species-specific differences in optimum thallus temperature for primary photosynthetic processes.

However, QCN, as well as qN showed different trends when compared to NPQ. QCN and qN were high at room (25°C) temperature and during initial phases of thallus temperature decrease. At close-to-zero and freezing temperature, QCN and qN showed low and more or less constant values and lacking rise in its value at freezing temperature (-5°C). A reason for such response is unknown. Further investigation of different ChlF parameters related to non-photochemical quenching is required in lichens to find a parameter reflecting involvement of protective mechanisms in lichen photosynthetic apparatus during low and freezing temperature treatments.

Fluorescence decline ratio (R_{FD}) decreases with lowering temperature in all three species; however, some values fluctuating from a general trend were apparent as well. For *U. antarctica,* an increase in R_{FD} was apparent at physiological temperature during initial cooling (25 to 5°C). In all three experimental species, R_{FD} values were found comparable to earlier investigations on *Cladonia* sp. (Tuba et al. 2008) measured at physiological temperature. The authors reported range of R_{FD} values between 0.5-1.5 which correspond to data recorded at 25 and 5°C (*see* Table 2). Freezing temperature (-5°C) caused decrease in R_{FD} value to 62, 64 and 50% as compared to its value measured at 25°C for *R. melanophalma*, *U. antarctica* and *X. elegans*, respectively. Quenching of background chlorophyll fluorescence (qF_0) did not show any clear trend with decline of thallus temperature indicating that there is no direct effect of low temperature *per se* on functioning of light harvesting complexes of PS II in lichen algal photobionts. In lichens, qF_0 has not been investigated in much detail. Studies form higher plants, however, indicated that qF_0 is sensitive to day-night cycles (*Picea abies* – Roháček et al. 2008), and a variety of stress factors. Therefore, we propose future studies in lichens to be focused on detailed analysis of qF_0 photosynthetic responses to *e.g.* high light, thallus dehydration to reveal if it dependents on particular stress factors.

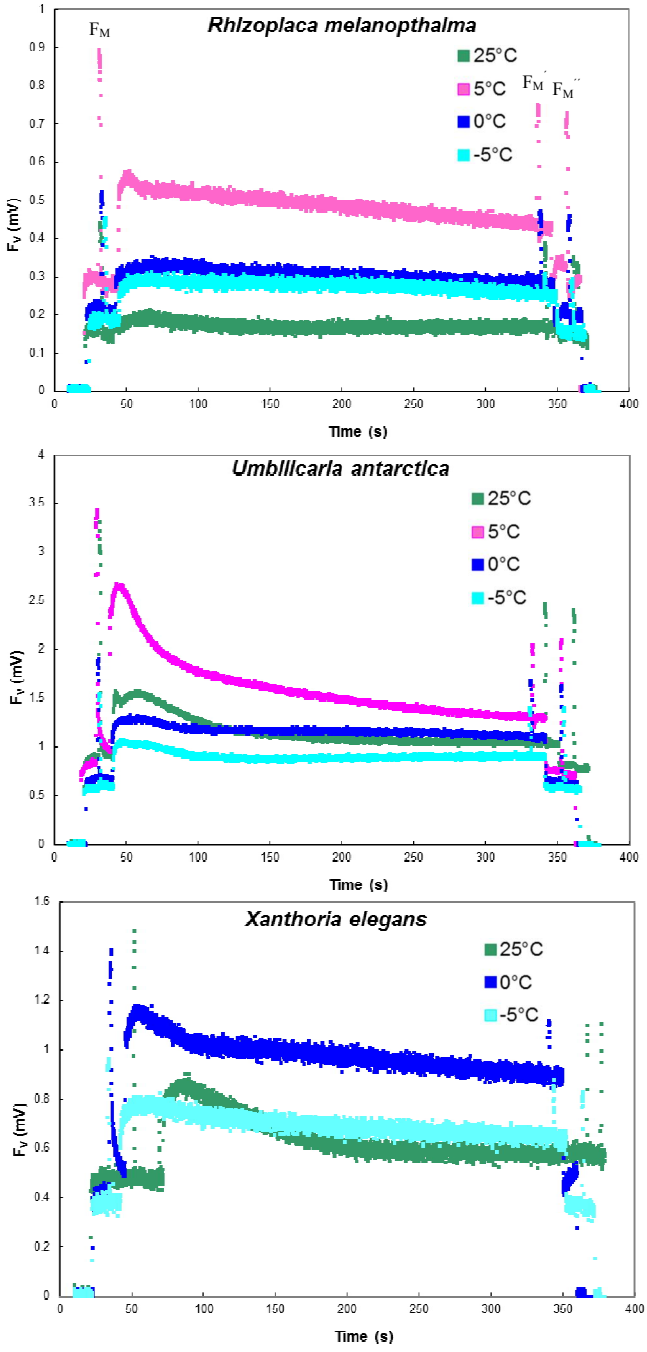

Fig. 2. Slow chlorophyll fluorescence (ChlF) transients supplemented with saturation pulses for *Rhizoplaca melanopthalma* (upper panel), *Umbilicaria antarctica* (middle panel), and *Xanthoria elegans* (lower panel), measured at decreasing thallus temperature: 25°C, 5°C, 0°C, -5°C.

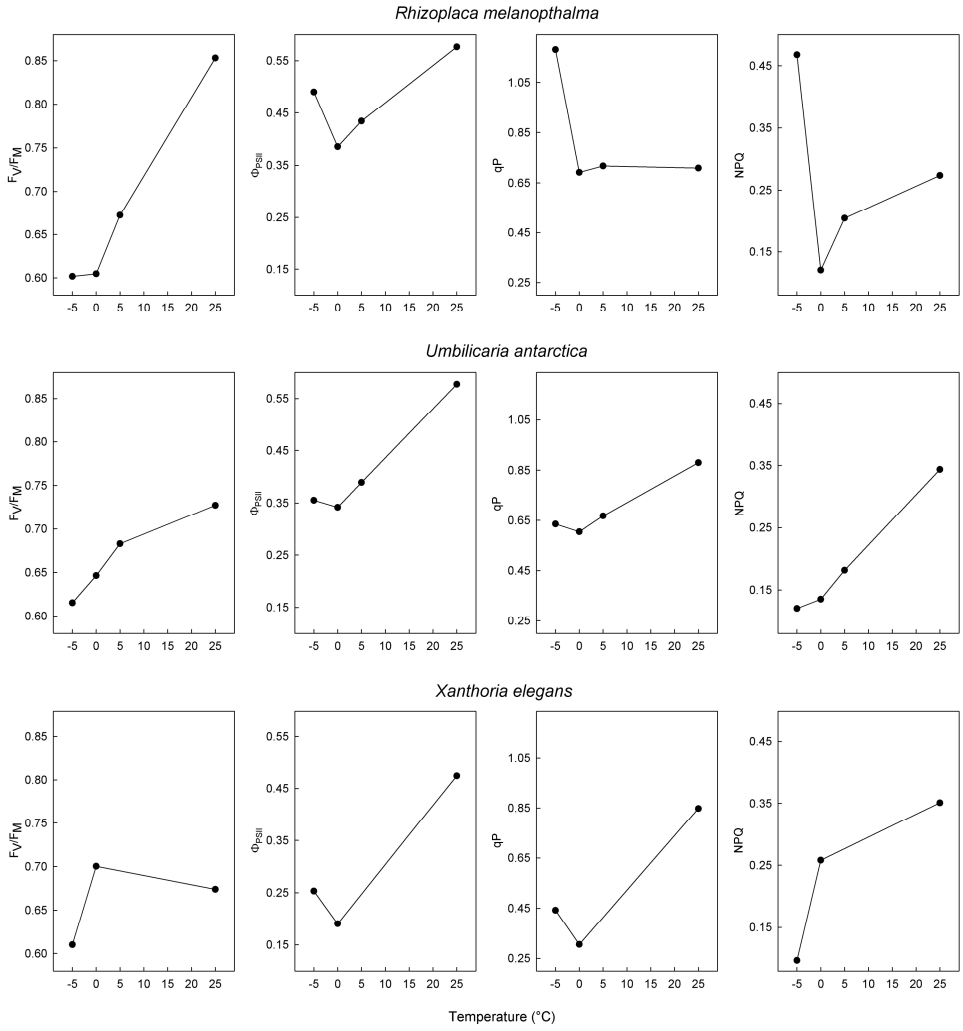

Fig. 3. Chlorophyll fluorescence (ChlF) parameters (from left to right: F_V/F_M, Φ_{PSII}, qP, NPQ) for *Rhizoplaca melanopthalma* (upper row), *Umbilicaria antarctica* (middle row), and *Xanthoria elegans* (lower row) plotted with respect to thallus temperature.

Thallus temperature	*Rhizoplaca melanopthalma*			
	R_{FD}	qF_0	qN	QCN
25°C	0.510	0.094	0.283	0.215
5°C	0.306	0.099	0.195	0.163
0°C	0.238	0.058	0.140	0.107
-5°C	0.194	-0.005	0.533	0.319

Thallus temperature	*Umbilicaria antarctica*			
	R_{FD}	qF_0	qN	QCN
25°C	0.514	0.062	0.329	0.256
5°C	1.019	0.001	0.525	0.395
0°C	0.187	-0.083	0.230	0.119
-5°C	0.185	-0.024	0.189	0.108

Thallus temperature	*Xanthoria elegans*			
	R_{FD}	qF_0	qN	QCN
25°C	0.516	n.d.	0.385	0.260
0°C	0.297	n.d.	0.293	0.205
-5°C	0.258	n.d.	0.145	0.088

Table 2. Summary of different quenching parameters evaluated from ChlF transients measured at decreasing thallus temperature.

Concluding remarks

In this study, species-dependent differences in shape and time courses of slow ChlF transients were found between three lichen species. Since the lichens differed in their sensitivity to a short-term low temperature stress, species-specific differences were apparent, even general trends in response to low temperature were similar. We used ChlF technique to reveal some species-dependent photosynthetic responses. The results suggest that slow ChlF transient can, similarly to higher plants (Mishra et al. 2014), be used for evaluation of lichen photobiont short-term responses to low and freezing temperature. Similarly to mosses (Lovelock et al. 1995a, b) Antarctic lichens undergo numerous thawing-freezing cycles during austral summer season accompanied with changes in effectivity of photosynthetic apparatus. Therefore, the measurements of slow ChlF transients might be used in the evaluation of interspecific differences in lichen resistence to repetitive freezing-thawing cycles.

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