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Photosynthetic traits of freshwater lichens are consistent with the submersion conditions of their habitat

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Abstract – In this study, we compared the photosynthetic performance of epilithic freshwater lichens on siliceous stream rock submerged for: more than 9 (hyper-), 6–9 (meso-) or 3–6 months (sub-hydrophilic lichens). In the dry state, neither variable fluorescence nor respiration activity could be detected. In the wet state, rates of dark respiration (O_2 uptake and CO_2 production for immerged and in-air samples) were in the lower range of that reported for non-aquatic lichens. With 200 (under water) or 500 $\mu mol.m^{-2}.s^{-1}$ photosyntheticallly active photon flux density (PPFD) (aerial), photosynthesis was positive but rates were lower than that published for non-aquatic species. Under intense PPFD (2000 $\mu mol.m^{-2}.s^{-1}$, aerial), photosynthesis increased in sub- but became negative in hyper-hydrophilic species. After hydration, dry samples increased photosystem II (PSII) efficiency, which reached near steady state in < 6–7 min. Hyper-hydrophilic lichen took longer than sub-hydrophilic species. A long period of desiccation (4 months) had a negative effect on subsequent PSII photochemistry of hyper- but not of sub-hydrophilic hydrated lichens. When thalli were allowed to dehydrate, all types of lichens lost PSII activity after about 15–20 min. Deactivation was faster in the hyper- than in the sub-hydrophilic species. The metabolic traits presented here are thus consistent with the ecological amplitude of the freshwater lichens studied.

Key words: Lichens / freshwater / ergosterol / photosynthetic pigments / photosystem II activity

Introduction

Lichens, with a thallus, formed by the symbiotic association of a fungus and an alga and/or cyanobacteria, are desiccation-tolerant (poikilohydric), and their water content varies drastically with that of their environment (Richardson, 1993). Lichens colonize a remarkable range of habitats with great differences in the supply and abundance of water. Only a small number of freshwater lichens live permanently submerged so they are adapted to wetting and drying cycles as experienced during diurnal and seasonal fluctuations in water availability. Their response to drying and rewetting is a key feature for survival in their habitat (Richardson, 1993). In Europe, only a few papers have been devoted to freshwater lichens (Nascimbene and Nimis, 2006). According to Santesson (1939) and Aptroop and Seaward (2003), freshwater lichens are amphibious organisms, most being actually submerged only during a part of the year. Not only the rock substrate but also the duration of thallus flooding affects the structure of aquatic lichen groups (zonal distribution, Santesson, 1939; Coste, 2010; Thüs *et al.*, 2014). Gilbert (1996) showed that aquatic lichen species in England are found in the form of overlapping streaks connected with the duration and altitude of flooding. Four zones are distinguished for rivers: (1) submerged zone, (2) fluvial mesic zone, (3) fluvial xeric zone and (4) fluvial terrestrial zone (Gilbert and Giavarini, 1997). Coste (2010), examining the zonal distribution of epilithic freshwater lichens in France, distinguished three types: (1) hyper-hydrophilic lichens, (2) meso-hydrophilic lichens and (3) sub-hydrophilic lichens defined according to the duration of submersion, more than 9 months, between 6 and 9 months, and between 3 and 6 months, respectively. Except for permanently submerged species, freshwater lichens are exposed to fluctuations in light intensity and water availability, which can be extreme in time and space. Poikilohydric organisms, such as lichens, regularly dry out and gas

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exchange changes dramatically with thallus water content (Green *et al.*, 1993). Thus, lichens can be exposed to high solar radiation in a desiccated state suggesting that their photosynthetic apparatus is exposed to damage. Exposure to (strong) light in the desiccated state has been shown to cause reduction in quantum efficiency (Gauslaa and Solhaug, 1996). In previous studies, it has often been assumed that wet and illuminated lichens are fully photosynthetically active and that photosynthetic activation in lichens occurs instantaneously (Coxson, 1988; Lange *et al.*, 1989; Palmqvist and Sundberg, 2000; Dahlman and Palmqvist, 2003; Liden *et al.*, 2010). However, a few publications report a lag time of several hours with suboptimal photosynthetic activity following liquid hydration of dry thalli (Lange *et al.*, 1986). Compared with desiccation in darkness, desiccation in light aggravated the drying damage in chlorolichens, it prolonged the activation time lag, and reduced quantum efficiency (Gauslaa *et al.*, 2012). For these lichens, activity does not accurately coincide with wet time and the chances for positive net production will decrease if hydration is broken into short events (Jonsson-Cabrajic *et al.*, 2010). Persistence and growth of lichens are however dependent on positive net photosynthesis. Can this explain the confinement of freshwater species to habitats that provide sufficiently long hydration periods?

In this study, we wish to understand whether potential photosynthetic limitation related to activation/inactivation time lags following liquid hydration/air dehydration could affect the zonal distribution of epilithic freshwater lichens. Moreover, the basic characteristics of freshwater lichen gas exchanges and photosystem II (PSII) activity were to be determined. To achieve this, laboratory O_2 and CO_2 gas exchanges in dry or water immerged or aerial fully hydrated thalli were analyzed in freshwater hyper, meso and sub-hydrophilic lichen species (Coste, 2010). In addition, analysis of chlorophyll (Chl) fluorescence, which gives information about the efficiency of PSII, was used to characterize species-specific patterns in photosynthesis activation after hydration and inactivation during desiccation (Lange *et al.*, 1989; Green *et al.*, 1993).

Materials and methods

Lichen sampling and measurement of their dry and wet weight

Freshwater lichens on siliceous rock were collected from their specific habitats (sun-exposed watercourses) in the south of France in August 2011. Half of the lichens were collected in the Massif Central, around the "Montagne Noire" (Mazamet, Gorges du Banquet, long. 2°28′39″E, lat. 43°30′49″N). The other half were collected in the sub-Mediterranean region "Languedoc" (Rosis, Rieutord, long. 2°58′11″E, lat. 43°35′38″N). Both regions are located in the hills stage (between 520 and 700 m a.s.l. with mean annual temperature of 10–11 °C) and in a damp locality (precipitations are around 1000 and 1400 mm.year^{-1}). Rocks in both places are composed of "eyed" gneiss.

Freshwater lichens were distinguished according to three annual durations of submersion in the stream: hyper, meso and sub-hydrophilic lichens undergo more than 9 months, between 6 and 9 months, and between 3 and 6 months submersion, respectively (duration recorded over several years based on regular, *i.e.,* at least monthly field visits, personal observations, Coste, 2010). The most common and abundant species in the different zones were sampled for subsequent laboratory experiments. The species selected for the present study were chlorolichens with a crustaceous thallus: *Verrucaria funckii* (Spreng.) Zahlbr. (noted VF), a hyperhydrophilous lichen covering 78% of the zone; *Ionaspis lacustris* (With.) Lutz. (noted IL) and *Porpidia hydrophila* (Fr.) Hertel and Schwab (noted PH), two mesohydrophilous lichens covering 65–89% of the zone; and *Verrucaria praetermissa* (Trevis.) Anzi (noted VP), a subhydrophilous lichen covering 85% of the zone. At the time of collection, all lichens were exposed to air (dry) and full sunlight. Living colonies were taken from stones with hammer and graver (lichens attached to the stones), placed in paper bags and transported in a glass box to the laboratory where they were stored in ambient air (20 ± 2 °C and 50 ± 5% relative humidity *RH*) in the dark. IL and PH mesohydrophilous lichens were not distinguished because they were generally found in the form of overlapping streaks. We thus consistently used mixed samples of these two species.

In the laboratory, the so-called "dry thalli" contained less than 5% water. Each sample corresponded to one piece of rock with intact lichen cover. The time course of water gain or loss was assessed by weighing the thalli (with the substratum) at 2 min intervals for rehydration in water from their watercourse (excess water was removed by gentle shaking before measurements) and at 5 min intervals to determine the rate of desiccation in ambient air in the dark (20 °C and 50% *RH*). At the end of the experiments, the thalli were dried for 48 h in an oven at 80 °C and weighed. Then, the thalli were transformed into ash for 5 h at 450 °C. The ash was removed from the rock with a brush and weighed. Data allowed the dry, fresh and water saturated mass of the lichens to be calculated. The relative water content (*RWC*) on a dry mass basis was calculated as ((fresh mass – dry mass)/(dry mass)) × 100.

Pigment analysis and ergosterol determination

The samples were collected on August 14 and 15, 2011 between 10:00 and 13:00 h (temperature about 20 °C, humidity lower than 20% and photosyntheticallly active photon flux density (PPFD) about 1500 μmol.m^{-2}.s^{-1}) and transported in liquid nitrogen before storage in the laboratory at − 80 °C. All samples showed less than 10% hydration. The identification and quantification of the photosynthetic pigments were carried out according to the method described by Barlow *et al.* (1997). The samples

were harvested by scraping lichen thalli from the substratum and were then lyophilized. The pigments were extracted with methanol before analysis by high-performance liquid chromatography (HPLC).

Ergosterol, a membrane component largely restricted to eumycotic fungi, was used as a surrogate for mycelial biomass (Gessner and Chauvet, 1993). Extraction and analysis followed the procedure indicated in Gessner (2005). Ergosterol was extracted from dried portions of thalli (38 ± 2 mg) in 5 mL of KOH/methanol (8 g.L^{-1}) for 30 min at 80 °C. The extract was then purified by solid phase extraction (Waters Oasis HLB 3 cc cartridges; Waters Corp, Milford, MA) and quantified by HPLC pump 422, HPLC detector 432, HPLC autosampler 360 (Kontron Instruments, Neufahrn, Germany) by measuring absorbance at 282 nm. The HPLC system was equipped with a FLT 0.5 µm A-316 precolumn (Upchurch Scientific, Oak Harbour, WA) and a LispRP 18–5 250 × 4.6-mm column (Thermo-Hypersil Keystone, Bellefonte, PA) maintained at 33 °C. The mobile phase was 100% methanol, and the flow rate was set to 1.4 mL.min^{-1}.

Respiration and photosynthesis

Respiration and photosynthesis were measured either via O_2 (under water) or CO_2 (aerial) exchanges as stated in the section Results. These activities were measured on about 1 cm^2 of the thalli with the substratum a few days after collection from the watercourse. Before measurements, samples were fully hydrated by immersion for 24 h in the stream water at 20 °C and under a PPFD of 100 µmol.m^{-2}.s^{-1}.

O_2 exchange was measured on under-water samples enclosed in Plexiglas chambers with stirring, placed in a water bath at 15 °C (Liden *et al.*, 2010). Rates of O_2 exchange were measured using oxygen microprobes in hermetically sealed chambers (Strath-Kelvin 928 System, North Lanarkshire, UK) and a PPFD of 0.80 and 200 µmol.m^{-2}.s^{-1} at the lichen level (type lamp: FHO 24W/T5 550 Osram Sylvania Ltd, Canada). Thalli were immerged in 5 mL filtered stream water (0.2 µm sterile cellulose membrane, Whatman International Ltd., Maidstone, UK) containing 0.07 g.L^{-1} K_2CO_3, pH 6.8.

CO_2 exchanges were measured on aerial samples enclosed in 50 mL 'Venoject' tubes hermetically sealed and placed in the dark or under PPFD of 500 µmol.m^{-2}.s^{-1}(type lamp: FHO 24W/T5 550 Osram Sylvania Ltd, Canada) or 2000 µmol.m^{-2}.s^{-1} (natural sunlight). Every 60 min, 1 mL of the air in the tube was taken using a syringe and injected into a Licor 830 gas analyzer (Li-Cor, USA) for determination of CO_2 concentration.

Photosynthetic activity was also measured as the maximal (dark-adapted) quantum efficiency of PSII "F_v/F_m" which is the ratio of the variable ($F_v = F_m - F_o$) to the maximal (F_m) Chl*a* fluorescence measured by a Licor 6400. This activity was investigated in 24-h dark hydrated thalli a few days after collection of the lichens from their watercourse or after 4 months storage at room temperature in the dark. To monitor lichen activation/deactivation patterns for the different species, photosynthetic activity (PSII) was recorded following dry thallus rehydration by liquid water and then following desiccation of 24 h fully hydrated thalli in-air with a water potential of − 100 MPa (50% *RH* and 20 °C).

Statistical analysis

F_v/F_m was obtained from an average of 5 pulses applied for each of 10 different thalli. All the results were variance-tested and submitted to Tukey tests (with significant differences) with Statgraphics software Centurion XV.II. Different homogeneous groups are identified with letters on graphs: a, b, c, d ($P < 0.001$). Error bars show the standard error of replicates.

Results

Biomass characteristics and pigment composition

VP (Sub) and VF (Hyper) species displayed similar low (8 mg dry weight (*DW*).cm^{-2}) specific thallus mass (STM), whereas IL and PH (Meso) species had a 2-fold higher biomass. The pools of ergosterol and of the major photosynthetic pigments are described in Table 1 for the four species. VP (Sub) had the highest ergosterol concentration (1.4 mg.g^{-1} *DW*), while species IL and PH (Meso) and VF (Hyper) showed significantly lower concentrations (0.45 mg.g^{-1} *DW*). The levels of chlorophyll *a* and *b*, and pheophytin*a* were the highest in lichen VP (Sub) as was ergosterol. Chl*a* concentrations followed a similar pattern to ergosterol for the four lichen species but Chl*b* and pheophytin*a* displayed different profiles. Pheophytin*a* represented at most 7% of the amount of chlorophyll*a* + *b*. In all species, caroten, lutein and zeaxanthin were the most abundant carotenoids in the tissues. Violaxanthin was present in very small amounts compared with zeaxanthin (less than 1%).

Gas exchange

Rates of steady-state dark respiration in freshwater lichens were measured through O_2 uptake by thalli immersed in water or through CO_2 production on fully hydrated thalli in-air. Both methods gave similar values in the range of 2–5 nmol.g^{-1} *DW*.s^{-1} (Fig. 1) corresponding to 0.2–0.5 µmol.m^{-2}.s^{-1}. Sub-hydrophilic lichen (VP) displayed significantly higher dark respiration than hyper-hydrophilic (VF) species, while meso-hydrophilic (IL and PH) species showed intermediate values. Dark respiration on dehydrated lichens in-air was undetectable (not shown).

For all species, photosynthetic activity estimated through the rate of O_2 production by underwater thalli

Table 1. Pigment and ergosterol concentrations in freshwater lichens distinguished according to their durations of submersion in streams. Lichens were collected in the dry state in full sunlight.

	VP sub		IL and PH meso		VF hyper	
Units			$\mu g.g^{-1}$			
Ergosterol	a	1394 ± 54	b	431 ± 53	b	467 ± 17
chlorophyll*a*	a	842 ± 129.5	b	244 ± 60	b	324 ± 21.7
chlorophyll*b*	a	91 ± 15	b	29 ± 7	b	56 ± 41
Pheophytin*a*	a	80 ± 1.58	b	18 ± 2	b	39 ± 2.84
β caroten	a	411 ± 61	b	92 ± 37	b	157 ± 10.8
Lutein	a	145 ± 18.9	b	44 ± 10	b	64 ± 45
Zeaxanthin	a	53.9 ± 1.1	b	14.9 ± 0.5	b	12.6 ± 0.85
Violaxanthin	a	0.226 ± 0.003	b	0.086 ± 0.009	b	0.103 ± 0.007

VP sub, *Verrucaria praetermissa*, subhydrophilic; IL and PH meso, *Ionaspis lacustris* and *Porpidia hydrophila*, mesohydrophilic; VF hyper, *Verrucaria funckii*, hyperhydrophilic.
Values are means ± SE; $n = 10$. The different letters correspond to statistically different groups ($P < 0.001$).

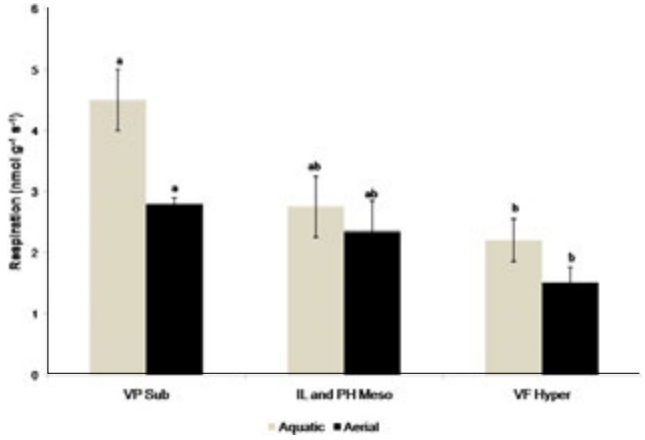


Fig. 1. Dry mass-related dark respiration of wet thalli of freshwater lichens. Respiration of immerged thalli (O_2 uptake) or aerial fully hydrated thalli (CO_2 evolution) was measured on selected species. Experiments were performed a few days after collection of dry lichens from sun-exposed watercourses. Values are means ± SE; $n = 30$, the different letters correspond to statistically different groups ($P < 0.001$). Statistical analyses were performed separately for immerged or aerial thalli. VP sub, *Verrucaria praetermissa* subhydrophilic; IL and PH meso, *Ionaspis lacustris* and *Porpidia hydrophila* mesohydrophilic; VF hyper, *Verrucaria funckii* hyperhydrophilic.

was positive under a PPFD of 200 $\mu mol.m^{-2}.s^{-1}$ (at lichen level, Fig. 2). Species VP (Sub) displayed the highest rate (3.5 nmol $O_2.g^{-1}.s^{-1}$), whereas VF (Hyper) species showed the lowest activity (0.1 nmol $O_2.g^{-1}.s^{-1}$). Under reduced PPFD (80 $\mu mol.m^{-2}.s^{-1}$), only VP (Sub) lichen species showed positive (although strongly decreased) O_2 evolution, while other species displayed slightly negative O_2 evolution. CO_2 uptake on dehydrated lichens in-air was undetectable (not shown). Aerial fully hydrated thalli exposed to PPFD of 500 $\mu mol.m^{-2}.s^{-1}$ revealed positive CO_2 assimilation for all species (Fig. 2). VP (Sub) lichen presented the highest rate of CO_2 assimilation as compared with other species. The order of magnitude of gas exchanges for immerged (O_2) or aerial (CO_2) samples was consistent. A very high PPFD (2000 $\mu mol.m^{-2}.s^{-1}$) inhibited CO_2 assimilation in fully hydrated aerial IL and PH (Meso) and VF (Hyper) lichens, which appeared to be negative. By contrast, VP (Sub) species showed enhanced rates of CO_2 assimilation under high light intensity.

PS II function of the hydrated and desiccated states, and after rehydration/dehydration cycle

The maximal stable quantum efficiency of PSII photochemistry was investigated on 24 h hydrated lichens the day after they were collected from their habitat or after they were air-dried at room temperature in the dark for 4 months (Table 2). The maximum F_v/F_m values differed among the species being the highest in VP (Sub) and the lowest in VF (Hyper). Four months spent in a desiccated state led to decreased F_v/F_m in hyper and mesohydrophilic lichens but did not affect fluorescence yield in sub-hydrophilic plants.

Lichens with a water content of about 5% of their *DW* (Fig. 3) did not show any variable fluorescence at room temperature (Fig. 4). When dry samples of all lichen species were rehydrated in the dark by wetting with liquid water, they became fully water saturated within 8 min (Fig. 3). More than 80% of the maximal *RWC* was already reached after 2 min. Maximal *DW* related water content was the highest (200%) in the hyper-hydrophilic lichen. An increase was noted in maximal variable fluorescence during this procedure (Fig. 4). All lichens regained near steady-state fluorescence in less than 6–7 min following immersion in liquid water (the value reached at $t = 9$ min was not enhanced by an additional 24 h hydration with water). However, slight differences between the species appeared in the recovery process. For VP (Sub), PSII activation was already 95% of the maximum value after the shortest interval tested, *i.e.,* 1 min. In contrast, VF (Hyper) and IL and PH (Meso) exhibited slower activation, with only around 30–35% of steady state F_v/F_m after 1 min and 95% after 3–4 min rehydration. Furthermore, the maximal F_v/F_m values reached at $t = 9$ min by the species displayed the same ranking as that reported in Table 2: VP (0.60), IL and PH (0.44) and VF (0.31), in decreasing order.

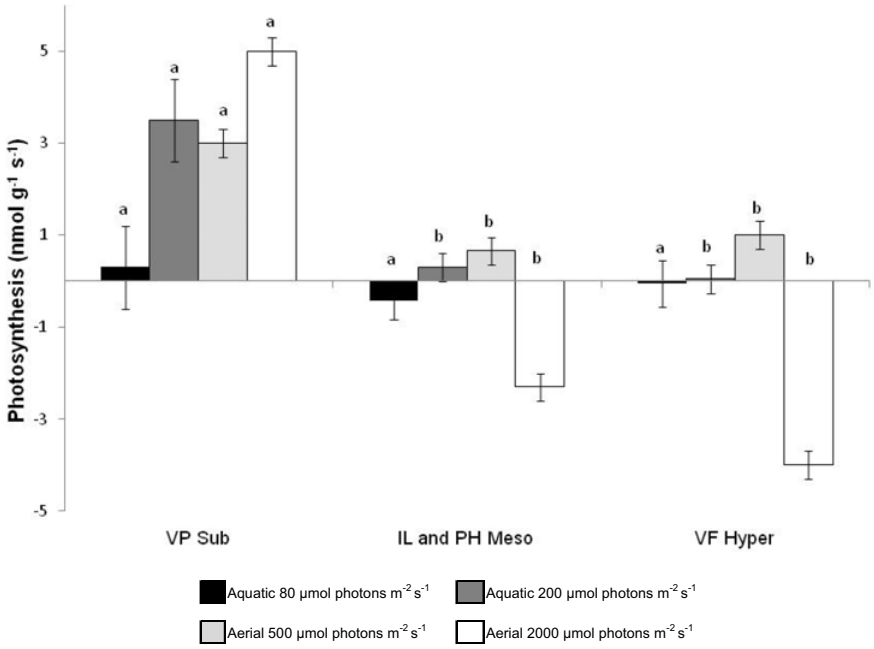


Fig. 2. Dry mass-related net photosynthesis of wet thalli of freshwater lichens. Photosynthesis of immerged thalli (O_2 evolution) or aerial fully hydrated thalli (CO_2 uptake) were measured on selected species. Light irradiance at thallus level was 80 µmol.m^{-2}.s^{-1} *PAR* (photosynthetically active radiation) or 200 µmol.m^{-2}.s^{-1} *PAR* for underwater measurements and 500 µmol.m^{-2}.s^{-1} *PAR* or 2000 µmol.m^{-2}.s^{-1} *PAR* for aerial measurements. Values are means ± SE; $n = 30$, the different letters correspond to statistically different groups ($P < 0.001$). Statistical analyses were performed separately for immerged or aerial thalli. Lichen species and sampling, see legend of Figure 1.

Table 2. Maximal quantum yield of PSII (F_v/F_m) in 24 h-hydrated freshwater lichens after 1 day or after 4 months air dry dark storage at room temperature following collection from the watercourse.

		Duration in the dry state after sampling before 24 h-rehydration	
		1 day	4 months
Verrucaria praetermissa (VP) Sub	a	0.64 ± 0.01	0.66 ± 0.01
Ionaspis lacustris and *Porpidia hydrophila* (IL and PH) Meso	ab*	0.59 ± 0.01	0.53 ± 0.02
Verrucaria funckii (VF) Hyper	b*	0.42 ± 0.03	0.33 ± 0.07

Means ± SE; n = 10. The different letters correspond to statistically different groups ($P < 0.001$). The * correspond to statistical tests performed on the same species at day 0 and 4 months ($P < 0.01$).

RWC and the fluorescence yield were then investigated during dehydration in-air (20 °C with 50 ± 5% *RH*) of the fully hydrated lichens. The species-specific desiccation time-series presented in Figure 3 revealed that lichens lost about 50% of their water every 5 min. F_v/F_m was also very sensitive to desiccation (Fig. 4). Cessation of photosystem activity, indicated by an F_v/F_m value below 0.1 (Liden *et al.*, 2010) was reached after 15 min in-air for VF (Hyper), whereas it took longer for IL and PH (Meso) or VP (Sub) species corresponding to a water content of about 20 and 15% of their *DW*, respectively.

Discussion

Ergosterol is the major sterol of the fungal plasma membrane and is used as an indicator of the relative proportions of metabolically active fungal cells in lichens (Sundberg *et al.*, 1999), while Chl*a* may serve as a marker of total photobiont cells (Descy and Metens, 1996; Palmqvist *et al.*, 1998). In freshwater lichens, the Chl*a* to ergosterol ratio was similar in the four species investigated (around 0.6, Table 1). Values for Chl*a*, *b* and ergosterol in freshwater lichens were in the range of those previously found for non-aquatic lichens (Demmig-Adams *et al.*, 1990; Sundberg *et al.*, 1999).

STM appeared small compared with most non-aquatic lichens (Palmqvist *et al.*, 1997, 1998, 2002) but some forest species have even lower values (Esseen *et al.*, 2015). Because changes in STM of freshwater lichens were associated with opposite and proportional changes in Chl*a* + ergosterol concentrations in the tissues, living STM (photobiont + mycobiont) was similar in the various freshwater lichens. Indeed, microscopic observations showed that dead biomass was more abundant in IL and PH (Meso) than in other species (not shown).

Rate of dark respiration in well watered freshwater lichens (2–5 nmol.g^{-1}.s^{-1} and 0.2–0.5 µmol.m^{-2}.s^{-1}) was

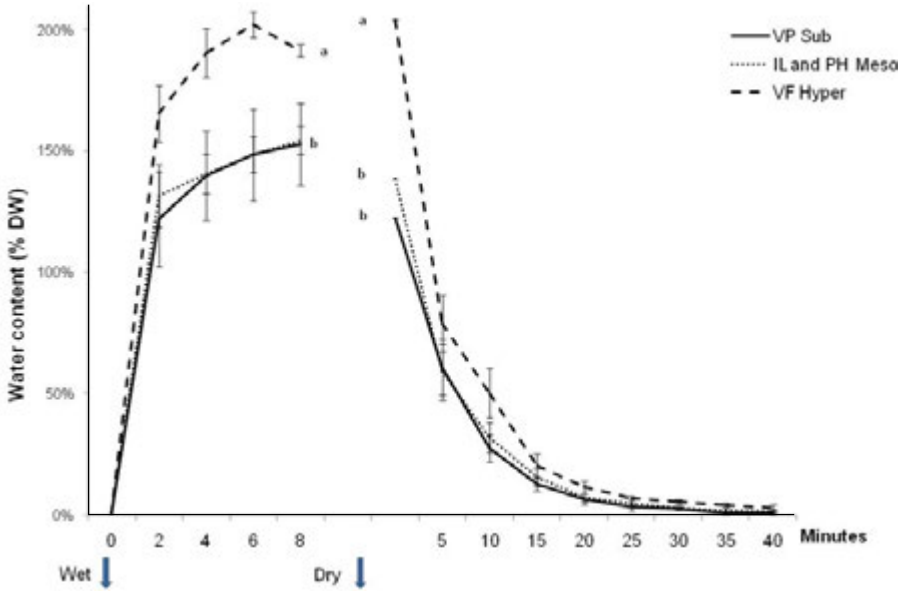


Fig. 3. Changes in lichen freshwater water content versus time after immersion in liquid water of dry thalli and then after placing the hydrated thalli in-air (at $T = 20\,^{\circ}C$ and $RH = 50\%$). Values are means $\pm$ SE; $n = 10$, the different letters correspond to statistically different groups ($P < 0.001$). Lichen species and sampling, see legend of Figure 1. RH, relative humidity.

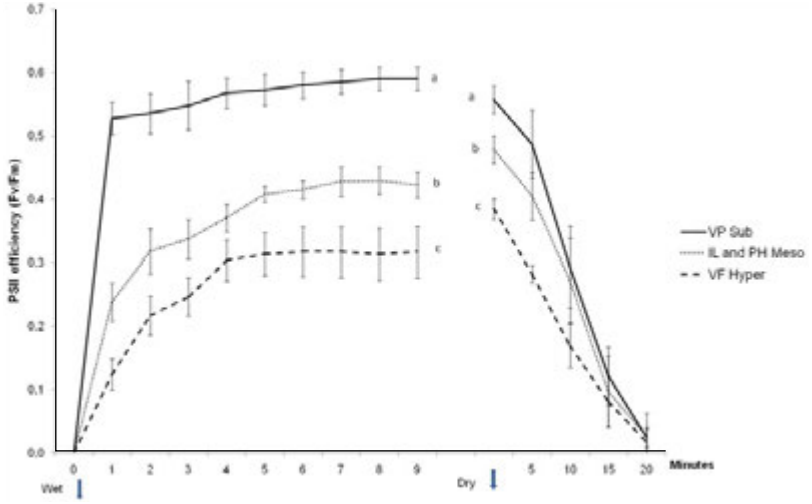


Fig. 4. Recovery of photosynthetic activity (F_v/F_m) after hydration of dry freshwater lichen thalli by liquid water and then cessation of activity of fully hydrated fragments during dessication in-air (at $T = 20\,^{\circ}C$ and $RH = 50\%$). Values are means $\pm$ SE; $n = 10$, the different letters correspond to statistically different groups ($P < 0.001$). Lichen species and sampling, see legend of Figure 1. RH, relative humidity.

in the range of that reported by Lange *et al.* (1993a, 1993b, 2006) and Sundberg *et al.* (1999), but an order of magnitude lower than that in other studies (Lange *et al.*, 1997) for non-aquatic lichens. Dark metabolic activity in sub-hydrophilic lichen was clearly higher than in hyper-hydrophilic species.

Under low PPFD (immerged samples, 80 $\mu mol.m^{-2}.s^{-1}$), net O_2 evolution was close to zero, indicating that this PPFD is near the light compensating point. In a survey of lichens covering a range of habitats in New Zealand, saturation PPFD was measured from 82 to 766 $\mu mol.m^{-2}.s^{-1}$ and light compensation point from 4 to 136 $\mu mol.m^{-2}.s^{-1}$ (Green *et al.*, 1997).

Under moderate PPFD (200 $\mu mol.m^{-2}.s^{-1}$ for immerged samples and 500 $\mu mol.m^{-2}.s^{-1}$ for wet lichens in-air), O_2 evolution and CO_2 fixation were positive for all lichen species. Maximal rates of photosynthetic O_2 evolution and CO_2 fixation (3–5 $nmol.g^{-1}.s^{-1}$ and 0.25–0.4 $\mu mol.m^{-2}.s^{-1}$) were in the low range of data for non-aquatic species reported by Demmig-Adams *et al.* (1990), Lange *et al.* (1993a, 1993b, 2006), but were much lower (more than 10-fold lower) than data of Lange *et al.* (1997, 2003), Lange (2003), and Green and Lange (1995). Freshwater lichens appeared to have a photosynthetic activity in the lower range of that of non-aquatic lichens. Many slow-growing lichens have low maximal

photosynthetic rates; this is especially the case when photosynthesis is related to thallus area (and not Chl content) because of their low biomass-to-area ratio.

Under high PPFD (2000 $\mu mol.m^{-2}.s^{-1}$, aerial thalli), CO_2 assimilation was notably increased in VP (Sub) lichens, whereas it became dramatically negative in VF (Hyper) and IL and PH (Meso) species. Hyper-hydrophilic lichens, growing closer to the stream, are less exposed to direct sunlight than sub-hydrophilic species. Shade-adapted plants can photosynthesize positively at low light levels, whereas they display lower rates of photosynthesis under high irradiances (Loach, 1967). The sensitivity of hyper-hydrophilic lichens to high PPFD is thus consistent with their less sunny microhabitat.

The thalli studied here underwent periods of desiccation under natural light in their habitat with potential photoinhibitory effects, which require a long period to relax (Gauslaa *et al*., 2012). Lichen performance was characterized on 24 h-hydrated thalli. This duration may not be sufficient to allow complete recovery from photoinhibition but our study reveals the metabolic abilities of freshwater lichens collected in summer when not submerged by the water of the stream.

In the dehydrated state, no photosynthetic CO_2 assimilation occurred and therefore no photosynthetic energy consumption occurred. Simultaneously, Chl fluorescence (photon yield of photosynthesis) was extremely low in all freshwater lichens. The decrease in the photon yield of photosynthesis could either be due to "damage" to the PSII or to a "protective" process in the form of harmless energy dissipation (Demmig-Adams *et al*., 1990). The absence of variable fluorescence in the dry freshwater lichens studied was caused by a dramatic reduction in F_m (not shown). This indicates a pronounced increase in harmless thermal dissipation as reported by Demmig-Adams *et al*. (1990) in green algal lichen. Dry freshwater lichens had the typical complement of carotenoids present in green algae and higher plants (β carotene, lutein, violaxanthin and zeaxanthin (Demmig-Adams *et al*., 1990). In the thalli studied (dry and sun-exposed, sampled in August around midday), a considerable portion of the violaxanthin + zeaxanthin pool appeared to be under the form of zeaxanthin. This finding is consistent with the functioning of the xanthophyll cycle in lichens but does not prove that zeaxanthin is necessary for photoprotective dissipation of excessive energy. In fact, zeaxanthin apparently did not contribute to photoprotection of desiccation in the light in the green algal lichen *Lobaria pulmonaria* (Heber *et al*., 2000).

The present study reports the photosynthetic behavior of freshwater lichens sampled around midday in a dry state in summer. When the species were rehydrated in the dark, they all exhibited rapid activation of PSII, which reached a near steady-state value in less than 7 min. This may allow freshwater lichens to exploit brief hydration events (Liden *et al*., 2010). Values of 0.4–0.6 for maximal efficiency of PSII following 24 h rehydration of the dry thalli (Table 1) are probably below the real maximal efficiency. Lichens may have been photoinhibited in the dry state under natural light and relaxation of photoinhibition when moistened can take longer than 24 h (Gauslaa *et al*., 2012). The lichens studied underwent unknown periods of aerial (drying) events in their watercourse before sampling. Light exposure during drying could be highly detrimental. However, chlorolichens do not need liquid water to restore photosynthesis (Lange *et al*., 1986). Hydration of in-air freshwater lichen thalli in their watercourse with cool and humid nights could allow regular photosynthetic activation in summer.

Comparing sub- and hyper-hydrophilic species, VF (hyper-hydrophilic) had the highest maximal *RWC* and the lowest metabolic (rates of dark respiration and photosynthesis) and photochemical performance in the wetted state, displayed negative photosynthesis under high irradiance, took more time for PSII activation to reach near steady-state values after rehydration but less time to deactivate following exposure to air, and displayed lowered PSII photochemistry following a long period of dehydration. These differences are consistent with the duration of submersion undergone by the lichen in its natural environment and may partly explain habitat distribution patterns of the sub- versus the hyper-hydrophilic species. The present differences between sub- and hyper-hydrophilic freshwater lichens were smaller than those of species-specific patterns of PSII activation time-lags and water-holding capacity which allowed Liden *et al*. (2010) to explain habitat restriction. However, following Jonsson-Cabrajic *et al*. (2010), we consider that *slightly* slower activation (6 min against 1 min) and higher sensitivity of PSII to desiccation may be important factors to explain the confinement of the most freshwater-related species to habitats that provide sufficiently long hydration periods. Indeed, small differences in activation/deactivation time-lag could strongly affect the lichen's long-term performance if hydration/desiccation events are brief and frequent.

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