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ORIGINAL ARTICLE

Physiological consequences of desiccation in the aquatic bryophyte *Fontinalis antipyretica*

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Abstract The moss *Fontinalis antipyretica*, an aquatic bryophyte previously described as desiccation-intolerant, is known to survive intermittent desiccation events in Mediterranean rivers. To better understand the mechanisms of desiccation tolerance in this species and to reconcile the apparently conflicting evidence between desiccation tolerance classifications and field observations, gross photosynthesis and chlorophyll *a* fluorescence were measured in field-desiccated bryophyte tips and in bryophyte tips subjected in the laboratory to slow, fast, and very fast drying followed by either a short (30 min) or prolonged (5 days) recovery. Our results show, for the first time, that the metabolic response of *F. antipyretica* to desiccation, both under field and laboratory conditions, is consistent with a desiccation-tolerance pattern; however, drying must proceed slowly for the bryophyte to regain its pre-desiccation state following rehydration. In addition, the extent of dehydration was found to influence metabolism whereas the drying rate determined the degree of recovery. Photosystem II (PSII) regulation and structural maintenance may be part of the induced desiccation tolerance mechanism allowing this moss to recover from slow drying. The decrease in the photochemical quenching coefficient (qP) immediately following rehydration may serve to alleviate the effects of excess energy on photosystem I (PSI), while low-level non-photochemical quenching (NPQ) would allow an energy shift enabling recovery subsequent to extended periods of desiccation. The findings were confirmed in field-desiccated samples, whose behavior was similar to that of samples slowly dried in the laboratory.

Keywords Aquatic bryophytes · Bryophyta · Chlorophyll *a* fluorescence · Desiccation tolerance · *Fontinalis* · Recovery

Abbreviations

ANOVA	Analysis of variance
CO_2	Carbon dioxide
DT	Desiccation tolerance
FDT	Full desiccation tolerance
F_v/F_m	Maximum quantum efficiency of photosystem II
MDT	Modified desiccation tolerance
NPQ	Non-photochemical quenching
O_2	Oxygen
PAR	Photosynthetic active radiation
PSI	Photosystem I
PSII	Photosystem II
qP	Photochemical quenching coefficient
RH	Relative humidity
RWC	Relative water content
WC	Water content

Introduction

Vegetative desiccation tolerance (DT) can be divided into two forms (Toldi et al. 2009): (1) full desiccation tolerance

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(FDT), which allows plants to survive rapid drying due to constitutive tolerance, and (2) modified desiccation tolerance (MDT), in which plants possess inducible tolerance and are able to survive slow drying. In general, the more desiccation-tolerant bryophytes belong to the first group (Oliver and Bewley 1997).

Genetic and evolutionary evidence indicates that DT is a primitive character lost in those lineages that evolved mechanisms to resist desiccation or that adapted to into habitats where they were not subject to desiccation (Oliver et al. 2005; Alpert 2006). Present-day evidence of these tolerance mechanisms in bryophytes comes from intensive cellular and molecular studies in *Tortula ruralis* and *Physcomitrella patens*, both of which are adapted to desert or semi-arid habitats (Oliver et al. 2005; Wood 2007). However, different degrees of DT are also found in bryophyte species exhibiting other habitat preferences (Davey 1997), including fully aquatic environments. Despite the apparent advantage conferred by DT, these species are poor competitors and there is a tendency for their replacement by desiccation-sensitive species along gradients of increasing water availability, probably due to faster growth and reproduction by the latter (Alpert 2006).

Desiccation-tolerant bryophytes are characterized by their ability to survive desiccation, recovering their metabolic activity upon rehydration (Bewley 1979; Crowe et al. 1998; Alpert and Oliver 2002; Proctor et al. 2007b). A more quantitative definition establishes as desiccation-sensitive those species unable to survive drying to 20% water content (WC) and as DT those surviving drying to 10% WC or less (Alpert 2006). However, "recovery" is less clear and, depending on the context, has been defined as the return to a normal rate of carbon fixation (positive net photosynthesis) or the full restoration of all metabolic systems (Proctor and Pence 2002).

Desiccation time, rate of water loss, and relative water content (RWC) have been examined in numerous desiccation-tolerant/-sensitive species. However, since the conditions of those experiments ranged from several years of uncontrolled desiccation to 30 min, with different rates, temperatures, and light regimes (see review in Proctor and Pence 2002), it is difficult to compare the results and thus to fully understand DT. In general, the extent and rate of water loss (Krochko et al. 1978; Schonbeck and Bewley 1981) as well as the desiccation time (Hinshiri and Proctor 1971; Proctor 2001) have been shown to be important factors controlling the impact of desiccation on moss physiology, including that of DT species (Glime 2007).

Most studies on the effects of desiccation and rehydration in bryophytes have involved terrestrial mosses (recently reviewed in Oliver et al. 2005; Proctor et al. 2007b) as it is assumed that all aquatic bryophytes, due to their habitat preferences, are intolerant of desiccation (Kimmerer and Allen 1982; Seel et al. 1992; Franks and Bergstrom 2000). However, that conclusion does not have substantial support from physiological experimental data (see review in Glime and Vitt 1984) except in some cases (Lee and Stewart 1971; Brown and Buck 1979; Šinžar-Sekulić et al. 2005). For example, after 90 min rehydration, *Fontinalis antipyretica* and *Brachythecium rivulare* photosynthesis was completely inhibited when these species were previously dried under zero relative humidity (RH). When submitted to 50 and 98% RH, *Fontinalis antipyretica* showed, respectively, 50 and 100% photosynthesis compared to a control sample (Lee and Stewart 1971). However, Proctor (2000) found that the primary metabolic response to desiccation by tolerant and intolerant bryophytes was similar, such that it was not possible to draw any conclusions regarding the nature of DT based only on this parameter.

The aquatic bryophyte *Fontinalis antipyretica* L. ex Hedw. has long branches with many ramifications, with free distal extremities and rhizoids that attach to the substrate (Glime 1980). It is commonly used as biomonitor for heavy-metal pollution (Sérgio et al. 1992; Martins et al. 2004) but little is known about its physiology, especially its ability to tolerate desiccation. This bryophyte was classified as desiccation-intolerant, due to intracellular potassium leakage in response to desiccation (Brown and Buck 1979). This was the only criterion used by these authors to classify bryophytes as tolerant or intolerant to desiccation (Brown and Buck 1979). By contrast, Irmscher (1912) showed that *Fontinalis* can survive 3 weeks of desiccation, although survival was due to new growth originating in the highly protected apical bud.

The term "rheophytic" applies to species that mainly, but not exclusively, inhabit the flooded areas of rivers and streams (Akiyama 1995). In Portugal, the Iberian Peninsula, and generally in the Mediterranean region, the species *F. antipyretica* is periodically exposed to desiccation, in intermittent streams that lose their water during the dry season (Vieira 2008). However, uncertainty remains concerning the ability of *F. antipyretica* to withstand the seasonal desiccation imposed by its habitat. Therefore, based on field observations suggesting desiccation tolerance by *F. antipyretica*, the main objectives of this work were: (1) to examine metabolic indicators in order to establish DT by *F. antipyretica* and (2) to test whether the recovery of this species is mainly determined by the extent or rate of dehydration, or by both. Our starting hypothesis was that *Fontinalis antipyretica* had some degree of desiccation tolerance and that recovery would be affected by both parameters. Specifically, we studied photosynthesis in *F. antipyretica* by monitoring the oxygen production rate, a very sensitive indicator of desiccation stress effects (Tuba et al. 1996). In addition, we measured chlorophyll *a*

fluorescence, as a non-invasive technique to follow photosynthesis in organisms subjected to stress conditions (Maxwell and Johnson 2000).

Materials and methods

Plant material and culture conditions

Submerged and emerged *Fontinalis antipyretica* L. ex Hedw. samples were collected from Serra de S. Mamede (central Portugal; 39°16′N, 7°19′W) and then transported under cool conditions (about 5°C) to the laboratory, where they were rinsed in distilled water, transferred to culture medium (Traubenberg and Ah-Peng 2004), and grown under controlled conditions (17°C day/13°C night, 20–30 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR), and a 16-h photoperiod). Emerged and dry samples of *F. antipyretica* were collected on 4 April 2009 and kept dry between several sheets of paper. Although we were unable to determine when these mosses had emerged, the cumulative precipitation between 1 January 2009 and the time of collection, as recorded at the Alegrete climatic station (São Mamede, Portugal), was 5.2 mm, with no measurable precipitation in March. Moreover, the maximum daily precipitation during this period was 0.8 mm (National Information System of Water Resources 2010). Accordingly, these samples had probably emerged more than 1 month prior to the collection date. These so-called field-desiccated samples were cleaned in the laboratory using a flux of N_2 gas before being used in the studies described below.

Ten shoot tips of 1 cm each were selected for the three to six replicates used for each measurement. Relative water content (RWC) was calculated according to Deltoro et al. (1998). Full turgor weight was determined before drying treatment and after blotting any external water away from the tips. Preliminary data of pressure–volume curves confirmed the removal of external water after blotting (data not shown). Fresh weight (stress weight) was determined at the end of the stress period and before oxygen-electrode measurements. Dry weight was determined at the end of the assays by placing the samples at 80°C for 48 h. In field-desiccated samples, the weight after rehydration was defined as the full turgor weight in RWC determinations. According to this method, the RWC for the field-desiccated samples was 15%.

Dehydration and recovery treatments

Dehydration was induced in the laboratory by placing the samples in small containers over saturated salt solutions of $KC_2H_3O_2$ (23% RH, −202 MPa), $Ca(NO_3)_2 \cdot 4H_2O$ (50% RH, −100 MPa), or K_2SO_4 (95% RH, −6 MPa, slow desiccation rate), which resulted in, very fast, fast, and slow drying rates, respectively. These samples were incubated at ambient temperature (20–23°C) at low PAR (2–5 μmol m^{-2} s^{-1}) for stress times of 0.5, 1, 2, 24, 168, 336, and 960 h for short-term recovery assays. Additionally, long-term recovery assays were carried out by incubating the fast dried for stress times of 0.5, 1, 2, and 3 h, and the slow dried samples for 2, 4, 12, 24, 48, 96, and 168 h. In all cases, rehydration was achieved by direct immersion of the samples in an oxygen-electrode solution (0.1 mM $KHCO_3$) at 17°C. In the long-term recovery assays, the bryophyte tips were placed in culture medium under the previously described conditions.

Gross photosynthesis and chlorophyll *a* fluorescence analysis

Oxygen consumption and production and chlorophyll *a* fluorescence were measured prior to desiccation in order to determine the control values, and then either 30 min or 5 days after rehydration for short-term and long-term recovery, respectively. All samples were rehydrated using a Clark-type liquid-phase oxygen electrode (DW2/2 electrode chamber, Hansatech Instruments Ltd., Norfolk, UK) coupled to a PAM 101 chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) connected to a PAM data acquisition system PDA 100 (Heinz Walz GmbH) adapted to the electrode chamber by fiber optics and controlled by the software WinControl v2.08 (2003) (Heinz Walz GmbH). Control and desiccated bryophyte samples were placed inside the electrode chamber containing 0.1 mM $KHCO_3$ solution for 10 min in the dark, allowing respiration (as oxygen consumption) to be measured in the absence of light (OxConsump1). Immediately, before the end of the dark period, a saturating light pulse (approximately 4,000 μmol m^{-2} s^{-1}) (KL 2500 LCD, Schott AG, Mainz, Germany) was applied over the measuring light to determine the maximum quantum efficiency of PSII (F_v/F_m), i.e., when all reaction centers are open (Baker and Oxborough 2005). Subsequently, a light source (KL 1500 LCD, Schott AG) was switched on for 10 min to determine net photosynthesis, measured as the oxygen production rate. Previous photosynthesis versus irradiation response curves indicated an optimum PAR of 46 μmol m^{-2} s^{-1}, at which oxygen (O_2) production is maximal (results not shown). Another saturating pulse was administered immediately prior to the end of the light period to determine the photochemical quenching coefficient (qP) (Schreiber et al. 1986) and non-photochemical quenching (NPQ) (Bilger and Björkman 1994). Subsequently, the light was switched off and respiration again measured for 5 min (OxConsump2). Photorespiration, resulting from the

Rubisco using O_2 instead of CO_2 as a substrate, with associated energy losses, was minimized by the addition of $KHCO_3$ (final concentration of 0.1 mM) as a non-limiting inorganic carbon source. Gross photosynthesis was calculated as $A + |R|$ (A = net photosynthesis; R = OxConsump2).

Statistical analysis

Relationships between variables/parameters and RWC and stress time were investigated by linear and non-linear regression analyses. Pearson correlation coefficients (r) and degrees of freedom (df) were used to determine the levels of significance (P) between observed and predicted data.

Gross photosynthesis values varied greatly, depending on the collection time, previous weather conditions (Vieira et al. 2009), time in the laboratory, etc. Accordingly, a pool of 174 replicate samples from the control population, corresponding to four different collection periods, were used to establish the control value for gross photosynthesis and chlorophyll *a* fluorescence. All previous values of all the assays were used to create box-and-whiskers plots. The horizontal line in those plots represents the median, boxes the 25 and 75% quartiles, and whiskers the 5 and 95% quantiles.

Whenever necessary, significant differences between groups were determined using ANOVA, with the Tukey post-test (significance level $\alpha = 0.05$).

All statistical analyses were performed with GraphPad Prism 5.02 for Windows (2008) (GraphPad Software, San Diego, CA, USA).

Results

Relative water content and relative water loss

Samples of bryophyte tips differed in their drying rates when submitted to the three different RHs, reaching the same RWC at different times and with half-desiccation times of about 35 min (23% RH), 1 h (50% RH), or 10 h (95%) (Fig. 1). Values below 15% RWC were discarded as they mainly reflected the effects of storage time at low RWC.

Effects of desiccation on the recovery of gross photosynthesis and chlorophyll *a* fluorescence

Short-term recovery

To evaluate the physiological performance of samples submitted to different drying rates (slow, fast, and very fast) in the laboratory, four photosynthetic indicators

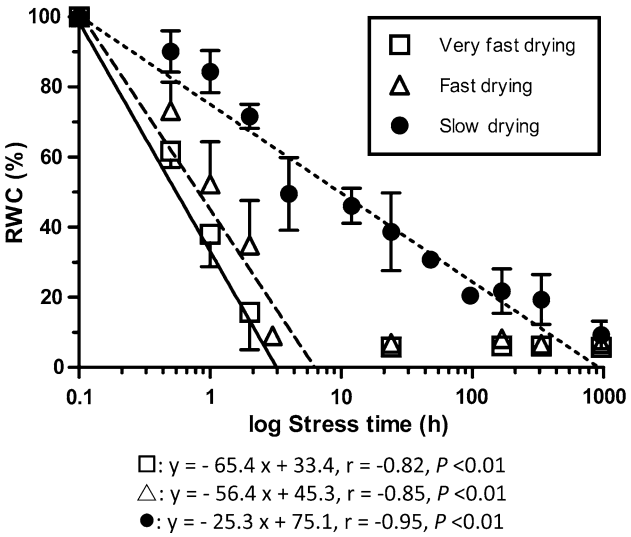

Fig. 1 Relative water content (RWC) variation as a function of stress time in *Fontinalis antipyretica*, determined at different relative humidity (RH) values (*open square* very fast drying rate, 23% RH; *open triangle* fast drying rate, 50% RH; *filled circle* slow drying rate, 95% RH). *Symbols* are means of three to six replicates in which RWC was >15%; *bars* are the standard deviation

measured after short-term recovery were analyzed with respect to RWC and stress time (Table 1). In samples submitted to slow drying rates, all indicators changed significantly, with positive correlations to RWC and negative correlations to stress time (Table 1). However, in samples submitted to faster drying rates gross photosynthesis, F_v/F_m, and NPQ correlated only with RWC (Table 1). Samples dried at intermediate rates showed a correspondingly intermediate behavior. Although photosynthetic indicators still correlated significantly with RWC and stress time, for most of them a decrease in Pearson r values with increasing stress time was observed (Table 1).

Under short-term recovery, gross photosynthesis in lab-desiccated bryophyte samples showed consistently higher correlation coefficients with RWC than with stress time, regardless of the drying rate (Table 1). Indeed, gross photosynthesis in *F. antipyretica* samples decreased linearly with declining RWC (Fig. 2a) but did not differ in response to the different drying rates (Fig. 2a).

The maximum quantum efficiency of photosystem II (F_v/F_m) correlated with variations in RWC irrespective of the drying rate. However, when a function was fitted to the data, F_v/F_m decreased linearly with RWC decline in bryophyte tips submitted to 23 and 50% RH. At 95% RH, the behavior of the bryophyte tips better fitted a logarithmic function, being F_v/F_m relatively constant and showing no significant effect of desiccation between 100 and 40% RWC (Fig. 2b). For F_v/F_m, correlations with stress time increased with decreasing drying rates (Table 1).

Table 1 Pearson correlation coefficient r between short-term recovery (30 min) of gross photosynthesis and chlorophyll a fluorescence parameters (F_v/F_m, qP, NPQ), and RWC and stress time in samples of *Fontinalis antipyretica* lab-desiccated at different rates

	23% RH ($n = 17$)		50% RH ($n = 19$)		95% RH ($n = 26$)	
	RWC	Stress time	RWC	Stress time	RWC	Stress time
Gross photosynthesis	0.85**	−0.48	0.67**	−0.53*	0.79**	−0.52**
F_v/F_m	0.79**	−0.25	0.81**	−0.63**	0.77**	−0.91**
qP	0.46	−0.30	0.40	−0.62**	0.53**	−0.89**
NPQ	0.62**	−0.38	0.81**	−0.60**	0.62**	−0.64**

Only samples with RWC >15% were considered

* $P < 0.05$; ** $P < 0.01$

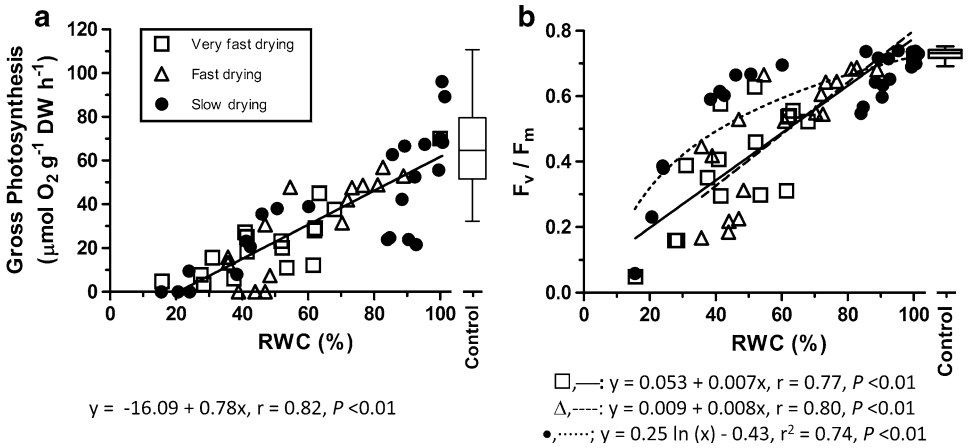

Fig. 2 Short-term recovery of gross photosynthesis (**a**) and maximum quantum efficiency of photosystem II (F_v/F_m; **b**) with changes in relative water content (RWC) at very fast (*open square* 23% RH; $n = 17$), fast (*open triangle* 50% RH; $n = 19$), and slow (*filled circle* 95% RH; $n = 26$) drying rates in lab-desiccated samples of *Fontinalis antipyretica*. Only samples with RWC >15% were considered. In the box-and-whiskers plots, the *horizontal line* represents the median, *boxes* the 25 and 75% quartiles, and *whiskers* the 5% and 95% quantiles

To evaluate the effect of desiccation on the energy captured by PSII and used for photochemical and non-photochemical processes, qP and NPQ were measured (Table 2). In lab-desiccated samples, qP (with a pre-desiccated value of ~0.85) correlated significantly with stress time for slow and fast drying rates whereas for RWC only a correlation with slowly dried samples was found (Table 1). NPQ (with a pre-desiccated value of ~1.3) correlated significantly with RWC for all three treatments and with stress time in slow- and fast-dried bryophyte samples (Table 1).

Field and lab-desiccated samples were compared based on their RWC, since it was not known for how long and at what rate the field-desiccated samples had become dehydrated. Field-desiccated bryophyte samples had a low RWC, about 10–15%. When rehydrated in the laboratory, their median values of gross photosynthesis were within the range determined for control samples (Fig. 3a) but their F_v/F_m values were lower (Fig. 3b). Field-desiccated bryophyte tips also had qP values slightly lower than those of controls and NPQ values about half those of pre-desiccated samples (Table 3).

Table 2 Photochemical quenching coefficient (qP) and non-photochemical quenching (NPQ) in lab-desiccated samples of *Fontinalis antipyretica* following short-term recovery (30 min)

	Stress time	qP	NPQ
Lab-desiccated ($n = 174$)	Non-stressed control	0.85 ± 0.11	1.29 ± 0.34
23% RH ($n = 3$–6)	0.5 h	0.85 ± 0.12	0.37 ± 0.10[a]
	1 h	0.78 ± 0.15	0.44 ± 0.08[a]
	2 h	0.88 ± 0.13	0.43 ± 0.07[a]
50% RH ($n = 3$–6)	0.5 h	0.97 ± 0.03	0.76 ± 0.15[a]
	1 h	0.92 ± 0.10	0.66 ± 0.37[a]
	2 h	0.80 ± 0.11	0.46 ± 0.08[a]
95% RH ($n = 3$–6)	0.5 h	0.83 ± 0.08	0.74 ± 0.17[a]
	1 h	0.83 ± 0.05	0.61 ± 0.07[a]
	2 h	0.89 ± 0.03	0.76 ± 0.18
	24 h	0.83 ± 0.06	0.71 ± 0.14[a]
	336 h	0.72 ± 0.11	0.23 ± 0.08[a]

ANOVA analysis (Tukey post-test) was performed to identify those values statistically different from the control. Only samples with RWC >15% were considered

[a] Statistically different from control

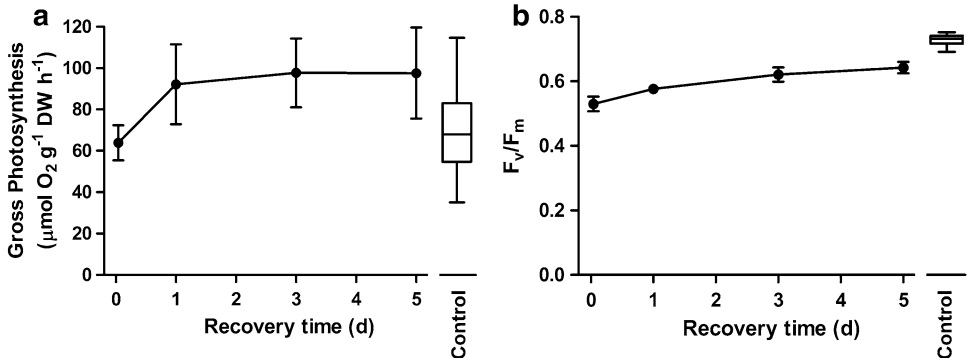

Fig. 3 Short-term (10–20 min) and long-term (5 days) recovery of gross photosynthesis (**a**) and the maximum quantum efficiency of photosystem II (F_v/F_m; **b**) in field-desiccated ($n = 5$) versus control samples of *F. antipyretica* (box-and-whiskers plots). In the box-and-whiskers plots, the *horizontal line* represents the median, *boxes* the 25 and 75% quartiles, and *whiskers* the 5 and 95% quantiles

Table 3 Photochemical quenching coefficient (qP) and non-photochemical quenching (NPQ) in field-desiccated samples of *Fontinalis antipyretica* following short-term (30 min) and long-term recovery (day 5)

	qP	NPQ
Lab-desiccated ($n = 174$)		
Non-stressed control	0.85 ± 0.11	1.29 ± 0.34
Field-desiccated ($n = 5$)		
Short-term recovery (30 min)	0.56 ± 0.04[a]	0.59 ± 0.17[a]
Long-term recovery (5th day)	0.86 ± 0.02	0.52 ± 0.13[a]

ANOVA analysis (Tukey post-test) was performed to identify those values statistically different from the control

[a] Statistically different from control

Long-term recovery

In moss samples left to recover for up to 5 days (Table 4; Fig. 4), RWC values were similar in slow- and fast-dried samples, but the response patterns of several physiological parameters differed (Table 4). Gross photosynthesis and F_v/F_m correlations with RWC and stress time were similar in short-term and long-term recovery samples but the changes showed opposing signs (Tables 1, 4). Different response patterns were observed for qP and NPQ with respect to RWC and stress time in samples dried at different rates and then allowed either a short or a long-term recovery (Tables 1, 4). After 5 days of recovery, there was no significant correlation between either qP or NPQ and RWC in samples subjected to fast or slow drying (Table 4). However, in slowly dried samples, qP did not correlate with stress time whereas a significant correlation was found for NPQ (Table 4). Gross photosynthesis levels were lower in moss samples that recovered from fast drying than from slow drying (Fig. 4a, b). The gross photosynthesis values of slowly desiccated samples subjected to long-term recovery were closer to pre-desiccation ones and always higher than those of fast dried samples for a similar RWC, with the exception of bryophyte tips dried at 95% RH for 7 days (Fig. 4b). Although in fast-dried samples there was an initial recovery (1 day) of gross photosynthesis, the levels subsequently remained unchanged even after 5 days (Fig. 4a).

Immediately after rehydration, F_v/F_m values were always higher in slow- than in fast-dried samples (Fig. 4c. d). In slow-dried samples, F_v/F_m recovery was slower than in fast-dried samples but in both cases, most of the samples had reached pre-desiccation values after 5 days (Fig. 4c, d). Despite this general pattern, it is interesting to note that slow-dried samples did not completely recover control values of F_v/F_m when the stress time was more than 1 day (Fig. 4d).

After 5 days of recovery, NPQ was almost fully restored in slow-dried samples (with the exception of 7-day dried samples) while in bryophytes subjected to fast drying lower values were recorded (Table 5). By contrast, qP recovered fully and recovery was not influenced by the drying rate or the stress time (Table 5).

In field-desiccated bryophyte samples, gross photosynthesis fully recovered after 1 day of rehydration but F_v/F_m values were lower than pre-desiccation ones (Fig. 3a), with a pattern similar to that of the slow lab-desiccated samples. Despite this difference, F_v/F_m progressively recovered over the 5-day period (Fig. 3b). The pattern observed in field-desiccated bryophytes for both gross photosynthesis and F_v/F_m more closely resembled that of slow lab-desiccated samples (Fig. 4b, d). Finally, rehydration of field-desiccated samples resulted in the recovery of qP but not of NPQ to pre-desiccation values (Table 3).

Discussion

Fontinalis antipyretica shows partial desiccation tolerance

Although *F. antipyretica* is an aquatic bryophyte, its metabolic responses to desiccation, both under field and

Table 4 Pearson correlation coefficient r between gross photosynthesis and chlorophyll a fluorescence parameters (F_v/F_m, qP, NPQ), and RWC and stress time for samples of *Fontinalis antipyretica* lab-desiccated at fast (50% RH) and slow (95% RH) drying rates and then allowed to recover for 5 days (long-term recovery)

	Fast desiccation (50% RH) ($n = 15$)		Slow desiccation (95% RH) ($n = 30$)	
	RWC	Stress time	RWC	Stress time
Gross photosynthesis	0.84**	−0.75**	0.46**	−0.67**
F_v/F_m	0.75**	−0.79**	0.60**	−0.82**
qP	−0.22	0.12	−0.12	0.19
NPQ	0.46	−0.36	0.33	−0.54**

Only samples with RWC >15% were considered

** $P < 0.01$

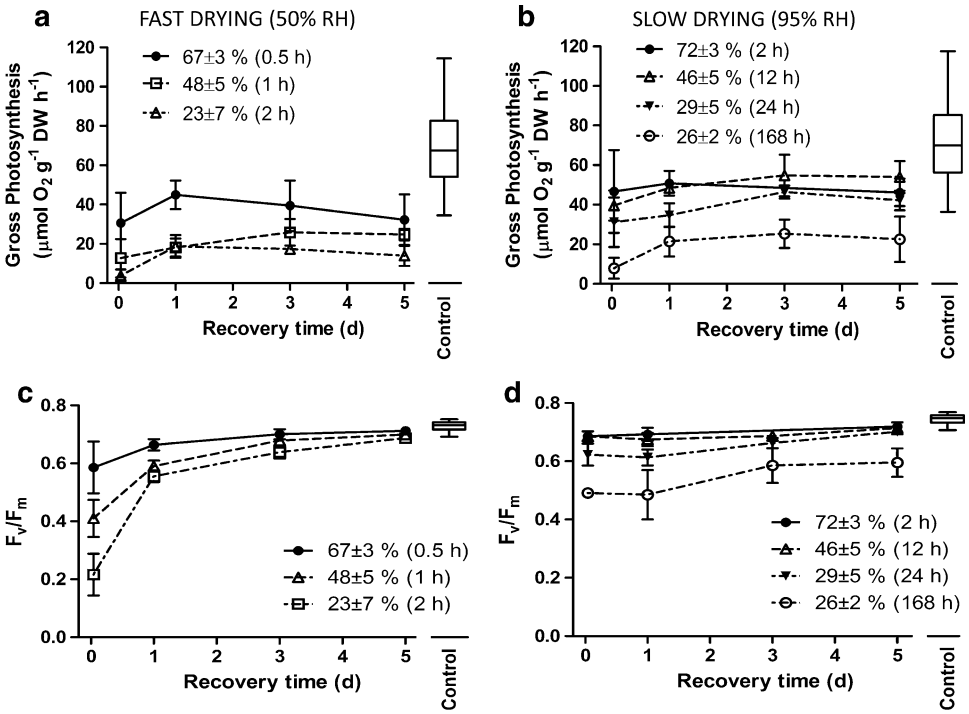

Fig. 4 Long-term recovery (5 days) of gross photosynthesis (**a**, **b**) and the maximum quantum efficiency of photosystem II (F_v/F_m; **c**, **d**) for samples of *F. antipyretica* lab-desiccated at fast (50% RH) and slow (95% RH) drying rates at different stress times. *Symbols* are the means of five replicates, and *bars* the standard deviation. In the box-and-whiskers plots, the *horizontal line* represents the median, *boxes* the 25 and 75% quartiles, and *whiskers* the 5 and 95% quantiles

lab conditions, were consistent with a desiccation-tolerance pattern. As shown in the present work, this species was able to resume photosynthesis after both fast and slow drying, at RWC levels as low as ~20%. Previous classifications of *F. antipyretica* as desiccation-intolerant were based mostly on habitat-preference data (Kimmerer and Allen 1982; Seel et al. 1992; Franks and Bergstrom 2000) since habitat is related to desiccation tolerance. However, in ecological studies, several confounding factors can modify the expected response. Davey (1997) reported that for most bryophytes in Antarctica the rates of carbon exchange and recovery following dehydration were related to habitat water availability, but many of the responses measured were either not correlated with habitat or showed a wide spread around the general trend. Thus, physiological studies are needed to obtain a detailed understanding of the limits of a species' response to a particular environmental factor. In the few works in which the degree of desiccation tolerance was tested under controlled conditions, *F. antipyretica* was classified as desiccation sensitive (Lee and Stewart 1971; Brown and Buck 1979) since photosynthesis did not recover after dehydration (Lee and Stewart 1971) and intracellular K leakage under desiccation conditions was substantially higher than in other species (Brown and Buck 1979). However, none of these works followed the recovery of this species over the course of several days. It is known that even FDT bryophytes suffer desiccation-related membrane damage, but as metabolism is recovered quickly, membrane damage alone (as previous plasmolysis assays) is not an accurate indicator of bryophyte desiccation tolerance.

Table 5 Photochemical quenching coefficient (qP) and non-photochemical quenching (NPQ) in lab-desiccated samples of *Fontinalis antipyretica* following long-term recovery (day 5)

	Stress time	qP	NPQ
Lab-desiccated ($n = 174$)	Non-stressed control	0.85 ± 0.11	1.23 ± 0.34
50% RH ($n = 4$–5)	0.5 h	0.83 ± 0.03	0.76 ± 0.04[a]
	1 h	0.85 ± 0.03	0.75 ± 0.06[a]
	2 h	0.85 ± 0.04	0.81 ± 0.08
95% RH ($n = 4$–5)	2 h	0.74 ± 0.03	0.90 ± 0.03
	12 h	0.82 ± 0.02	0.98 ± 0.15
	24 h	0.78 ± 0.02	0.91 ± 0.13
	168 h	0.80 ± 0.02	0.59 ± 0.26[a]

ANOVA analysis (Tukey post-test) was performed to identify those values statistically different from the control

[a] Statistically different from control

If a DT bryophyte is defined as one that survives desiccation and recovers its metabolic activity upon rehydration (Bewley 1979; Crowe et al. 1998; Alpert and Oliver 2002; Proctor et al. 2007b), then *F. antipyretica* is undoubtedly a member of this group. However, "recovery" can be defined as a return (within few days) to a normal rate of carbon fixation (positive net photosynthesis) or as the full restoration of metabolic systems, and thus to a pre-desiccation state (Proctor and Pence 2002). According to these criteria, the recovery of *F. antipyretica*, measured as a return of photosynthesis to pre-desiccation values, requires a slow drying process (more than 2 h). Under conditions of fast drying (<2 h) and 5 days of recovery, the photosynthetic rate in this aquatic bryophyte was 25–50% of pre-desiccation values. Thus, *F. antipyretica* cannot be classified as exhibiting MDT (a category that includes DT vascular plants) since, unlike DT angiosperms, it is able to survive desiccation if water loss occurs in <12 h (Oliver et al. 2000).

Oliver (2008) reported that even DT species require a relatively slow drying process in order to allow cells to survive desiccation, implying that the rate of water loss also plays an important role in subsequent recovery. Since not all previous works provided a detailed accounting of the experimental conditions to which the studied bryophytes were submitted during desiccation, namely, the rate of water loss, temperature, and atmospheric RH, comparisons of our data with the results of those studies is difficult. In *Atrichum androgynum* dried over silica gel (0% RH), 25% RWC was reached in about 8 h (Mayaba et al. 2002) whereas in *F. antipyretica* the same RWC was achieved in 2 h at 50% RH. This difference implicates bryophyte morphology and cell organization as determinants of the rate of water loss in different bryophytes, as recently suggested by Pressel and Duckett (2010). *F. antipyretica* is an aquatic bryophyte with only a single layer of cells; this simple structure is the probable explanation for its very high rate of water loss. Furthermore, *F. antipyretica* cannot be compared with desert mosses such as *Tortula ruralis* and *Tortula ruraliformis*, both of which are able to survive desiccation occurring in <3 h and fully recover their photosynthetic rate (Schonbeck and Bewley 1981; Seel et al. 1992). Nevertheless, even for *T. ruralis* the rate of water loss is important since it suffers less damage if desiccation occurs in 3 h rather than 1 h (Schonbeck and Bewley 1981). *F. antipyretica* is, however, similar to *Plagiothecium succulentum* and *Mnium stellar*, as these species only recover from slow, and not fast drying (Abel 1956), by a process referred to as hardening (Wood 2007). The aeroterrestrial green alga *Klebsormidium crenulatum* retrieved from alpine regions, where water availability frequently fluctuates (between precipitation, condensation, and water vapour), also presents some similarities to *F. antipyretica* since even after fast dehydration some of the cells are able to survive and recover (Karsten et al. 2010).

Although the extent of dehydration is clearly important, the rate at which it occurred appears to be the key factor determining the recovery response. Regardless of the RWC reached by the studied mosses during desiccation, they were able to recover to their pre-desiccation state if the drying process took more than 2 h. This finding is in accordance with the observations of other authors (Oliver and Bewley 1997; Oliver et al. 2005).

This work is one of the few studies (Glime 1971) comparing lab-desiccated samples with field-desiccated ones. We found that the physiological response of the latter was consistent with a slow drying process since the recovery response resembled that of the slow-dried laboratory sample. Indeed, in both field- and lab-desiccated *F. antipyretica*, photosynthesis and fluorescence completely recovered to control values after 24 h of rehydration. It should be noted that under field conditions fast drying most probably does not occur, as *F. antipyretica* is aquatic, with a habitat in streams that, at least in Mediterranean areas, do not dry out in <2 h.

PSII as a target for DT protection mechanisms

PSII reaction centers are obvious targets for protection by DT mechanisms (Proctor 2008). In FDT bryophytes such as *Racomitrium lanuginosum* (Proctor and Smirnoff 2000) and *Polytrichum formosum* (Proctor et al. 2007a), full recovery (10–20 min) of F_v/F_m is extremely fast (10 and 18 days post-desiccation, respectively), although the authors of that study did not specify the drying rate. Basal fluorescence, F_0, one of the parameters used to calculate F_v/F_m ($F_v = F_m - F_0$), is very sensitive to changes in the

spatial organization of supramolecular thylakoid complexes (Havaux and Strasser 1992), which, according to Proctor et al. (2007a) is related to the maintenance of grana-stroma thylakoid networks in chloroplasts. Rapid recovery of F_v/F_m also occurred in our samples of slowly dried *F. antipyretica*. Although F_v/F_m also recovered in fast-dried samples, recovery was much slower and was achieved only after 24–48 h, underlining the importance of drying rate in maintaining an intact chloroplast membrane structure. Nevertheless, after 5 days recovery these particular F_v/F_m presented lower F_0 and F_m values relatively to control, indicating fewer cells contributing to the ratio. However, in the same slowly dried samples, photosynthesis did not recover as quickly as F_v/F_m, with normal levels restored only after 3 days. This delay may have been due to the involvement of a later step in photosynthetic electron transport or photosynthetic carbon assimilation process, since both are mediated by enzymes, which may take more time to be repaired. After a few days of rehydration, F_v/F_m fully recovered to pre-desiccation values in both slow- and fast-dried bryophytes, in contrast to photosynthesis, which in the latter samples did not fully reach pre-desiccation values. The decrease in photosynthesis in absolute terms was due to the presence of damaged cells, whereas photosynthesis was functional and F_v/F_m restored to normal pre-desiccation values in the remaining active cells. The death of some of the cells especially in the fast-dried samples was confirmed by preliminary microscopy observations (data not shown) and is consistent with their simple structure, which results in rapid water loss and bryophyte cells more vulnerable to irreversible injury.

We also observed that in slow-dried samples the storage time at low RWC influences F_v/F_m recovery, perhaps reflecting damage to the D1 protein of PSII (Smirnoff 1993). Alternatively, there may be down-regulation of PSII itself (Deltoro et al. 1998; Hamerlynck et al. 2002) in order to protect the complex while the cell maintains energy levels sufficient to counteract ROS formation during recovery. This would explain why in field-desiccated bryophytes (drying time and rate unknown) low F_v/F_m values persisted after a few days of recovery. According to this scenario, PSII regulation and structural maintenance might be part of the induced, protective mechanism of desiccation tolerance that allows slowly dried *F. antipyretica* to eventually recover.

Energy-flow regulation as a DT mechanism

As discussed above, the regulation of energy flow may contribute to the mechanism of desiccation tolerance. The decrease in qP with dehydration extent during the initial moments of recovery, especially in the slowly dried samples, indicated that the fraction of absorbed energy used in photochemistry is smaller than in unstressed samples and the plastoquinone pool more reduced (Schreiber et al. 1986), resulting in excess energy production at PSII compared to its consumption downstream. In tracheophytes, photosynthetic electron flux is controlled in the interchain between PSII and PSI, during electron transfer either between plastoquinol and cytochrome b6/f (Foyer 2002) or, as more recently proposed, between cytochrome b6/f and plastocyanin (Schöttler et al. 2004). A hypothetical time-dependent decrease of qP combined with slow drying may be a short-term adaptation response directed at avoiding excessive electron pressure at PSI and the concomitant production of superoxide by the Mehler reaction. Nevertheless, a few days after rehydration, qP had fully recovered in both fast- and slow-dried samples, implying that energy was being channeled to photosynthesis.

In the first few minutes of *F. antipyretica* recovery, NPQ values were lower than those measured in the pre-desiccation stage were. Deltoro et al. (1998) observed that, in *Frullania dilatata*, NPQ recovered to pre-desiccation levels rather quickly while in *Pellia endiviifolia* a large increase was registered. Low NPQ values have been positively correlated with desiccation tolerance (Deltoro et al. 1998; Hamerlynck et al. 2002). However, more recently Proctor and Smirnoff (2011) observed in bryophytes from unshaded more exposed to desiccation habitats, like *Andreaea rothii* or *Schistidium apocarpum*, that protection against high radiation involves high photosynthetic electron transport to oxygen and high NPQ. NPQ is an indicator of photosynthetic electron-transport-chain protection mechanisms in response to excessive light energy in the PSII antenna system (Demmig-Adams 1990). The main component of NPQ is usually qE (for review see Horton et al. 1996), the high-energy-state quenching that is dependent on the transthylakoidal pH gradient. The decrease in NPQ during the initial moments of rehydration was paralleled by a decrease in the activity of the PSII reaction center, as shown by the decrease in F_v/F_m. According to this scenario, there may have been an increase in energy pressure over the PSII antenna that could not be dissipated by NPQ mechanisms. This would suggest that *F. antipyretica* is more susceptible to light damage within the first moments of rehydration. Thus may be due to xanthophyll degradation but also to a lack of membrane organization at this early stage, when it is difficult to properly quench the excess energy. However, in nature, water acts as a film and thus as a light filter (Glime 2007) as does the riparian vegetation bordering the small streams, in both cases reducing the incident light (Cruz de Carvalho, personal observation). Therefore, under field conditions, the energy pressure over the PSII antenna may be less than expected. After a few days of recovery, NPQ was almost fully restored in slow dried samples, with the exception of the

7-day dried samples. The lack of full NPQ recovery following fast drying supports the hypothesis that xanthophyll integrity and/or its functionality can only be restored following slow drying. In field-desiccated samples, the NPQ did not show any recovery signs, reaching only about half of the value in the unstressed control bryophytes and values similar to those determined in 7-day dried samples. This might reflect the effect of stress time on non-photochemical energy dissipation protection mechanisms or on energy flow and thus on the shift to photochemical processes mediating recovery. We suggest that the decrease in qP alleviates the effects of excess energy on PSI that occurs during the initial moments of rehydration and that low NPQ allows an energy shift that enables recovery over the following days. This process becomes increasingly important as desiccation continues and it contributes to the partial desiccation tolerance displayed by *F. antipyretica*.

In summary, this work demonstrates, for the first time, that *F. antipyretica* exhibits partial desiccation tolerance, in contrast to what has been reported previously. Being aquatic, this bryophyte is able to induce DT mechanisms, mainly the protection of the PSII reaction centers, and to fully recover only if drying is slow. While the extent of dehydration affects metabolism, the rate at which it occurs determines the degree of recovery. Our findings were confirmed in field-desiccated samples, whose behavior more closely resembled slow rather than fast lab-desiccated samples. Bryophytes have been of utmost importance in the study of desiccation tolerance. This physiological study of a widely distributed aquatic bryophyte periodically subjected to desiccation contributes to improving our knowledge of the role played by desiccation rate in moss survival. Moreover, aquatic bryophytes such as *F. antipyretica* allows us to gain insight into both the existence of desiccation tolerance mechanisms in mosses presently adapted to very humid habitats and the evolutionary implications of these processes.

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