

Molecular iodine emission rates and photosynthetic performance of different thallus parts of *Laminaria digitata* (Phaeophyceae) during emersion

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Abstract The emission of molecular iodine (I_2) from the stipe, the meristematic area and the distal blade of the brown macroalga *Laminaria digitata* (Hudson) Lamouroux (Phaeophyceae) was monitored under low light and dark conditions. Photosynthetic parameters were determined to investigate both the extent of stress experienced by different thallus parts and the effects of emersion on photosynthesis. Immediately after air exposure, intense I_2 emission was detectable from all thallus parts. I_2 emission declined continuously over a period of 180 min following the initial burst, but was not affected by the light regime. The total number of mole of I_2 emitted by stipes was approximately 10 times higher than those emitted from other thallus parts. Initial I_2 emission rates (measured within 30 min of exposure to air) were highest for stipes (median values: 2,999 and 5,222 pmol g⁻¹ dw min⁻¹ in

low light and dark, respectively) and lower, by one order of magnitude, for meristematic regions and distal blades. After exposure to air for between 60 and 180 min, I_2 emission rates of all thallus parts were reduced by 70–80%. Air exposure resulted in a decrease of the maximum photosystem II (PSII) efficiency (F_v/F_m) by 3%, and in a 25–55% increase of the effective PSII quantum efficiency ($\Delta F/F'_m$); this was caused by a higher fraction of open reaction centres (q_p), whereas the efficiency of the latter in capturing energy (F'_v/F'_m) remained constant. The results indicate the presence of an iodine pool which is easily volatilised and depleted due to air exposure, even under apparently low stress conditions.

Keywords Emersion · Emission rate · IBBCEAS · *Laminaria* · Molecular iodine · Photosynthesis

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Abbreviations

IBBCEAS	Incoherent broadband cavity-enhanced absorption spectroscopy
HWHM	Half-width at half-maximum
MBL	Marine boundary layer
NPQ	Non-photochemical quenching
PFD	Photon flux density
PAR	Photosynthetic active radiation
PS	Photosystem

Introduction

Kelp species of the genus *Laminaria* are large brown algae (Phaeophyceae) representing some of the most prominent constituents of Atlantic and Pacific rocky shores in temperate regions. *Laminaria* species form ecologically

important, extensive seaweed beds which occur from the upper sublittoral fringe downwards (Lobban and Harrison 1994). The vertical distribution of kelps within the sublittoral zone is determined by light penetration into water, the morphological capacity of the thallus to withstand mechanical stress caused by wave action, and its capacity to cope with periods of emersion (Lüning 1985). *Laminaria digitata* is particularly well adapted to the environmental conditions found in the upper sublittoral zone. The sporophyte of *L. digitata* consists of a holdfast which anchors the alga to hard substrata, a smooth and flexible stipe that prevents the alga from major mechanical damage during wave exposure, and a long and large blade, single for a plantlet and divided into several laminae for an older individual. The meristematic growth region is located at the base of the blade (van den Hoek et al. 1995). During low spring tides, *L. digitata* is exposed to the atmosphere for short periods. Emersion involves changes in the abiotic environment, and *L. digitata* is subjected to potential water loss, different carbon regimes, temperature fluctuations and increased irradiances. This requires an appropriate adjustment of the metabolism to prevent inhibition of photosynthesis (Lüning 1985). A combination of such stressors results in cumulative stress, i.e. higher stress levels experienced by the alga.

Similar to many other brown macroalgae, *L. digitata* accumulates high amounts of iodine (Saenko et al. 1978) from the surrounding seawater. With iodine concentrations in tissue more than 30,000 times those in seawater (Küpper et al. 1998; Verhaeghe et al. 2008), *L. digitata* is the most effective known iodine accumulator among living organisms (Ar Gall et al. 2004). Iodine uptake by *L. digitata* is mediated by an extracellular haloperoxidase (Küpper et al. 1998); within the thallus, iodine is retained as inorganic iodide (I^-), probably associated not only in a non-covalent way with carbohydrates, polyphenols or proteins, in the apoplast but also in intracellular, non-specialised vesicles and physodes (Kyllin 1929; Pedersén and Roomans 1983; Küpper et al. 2008; Verhaeghe et al. 2008). I^- is a potent quencher of aqueous oxidants and ozone and, therefore, acts as an inorganic antioxidant in *L. digitata* (Küpper et al. 2008). Both exogenously induced and endogenous oxidative stress lead to the release of iodine from *L. digitata* into the surrounding seawater, which can be easily measured (Küpper et al. 2008), or into air in the form of molecular iodine (I_2) and various volatile iodo-carbons (Laternus et al. 2004; Palmer et al. 2005; Leblanc et al. 2006). The latter process is generally referred to as 'iodovolatilisation' (Küpper et al. 1998). Significant mixing ratios of I_2 were measured from several air-exposed brown macroalgae under reasonably low stress conditions: the algae were temperature controlled in weakly oxidative atmospheres and studied in the dark or at low light levels to avoid cumulative effects of several abiotic stressors (Bale et al. 2008; Dixneuf et al. 2009; Ball et al. 2010).

Besides the role of iodine in algal stress responses (Laternus et al. 2004; Küpper et al. 2008), atmospheric I_2 has been identified as a vital actor in the photochemistry of the MBL (Carpenter 2003). The daylight photolysis of I_2 leads to the formation of atomic iodine known to be one of the most important catalytic species of a complex chemistry which promotes the destruction of ozone and the formation of marine aerosols (O'Dowd et al. 2002; McFiggans et al. 2004; O'Dowd and Hoffmann 2005; McFiggans et al. 2010). These processes potentially affect the Earth's radiation balance and therefore may have an impact on the global climate (Kolb 2002). During field campaigns on the Irish west coast, significant concentrations of tropospheric iodine oxide (IO) and I_2 were observed at times of low tide, suggesting that I_2 emitted by the air-exposed kelp beds could potentially play a more important role than the volatile iodo-carbons in the atmospheric photochemistry of coastal regions (McFiggans et al. 2004; Saiz-Lopez and Plane 2004; Bitter et al. 2005; Huang et al. 2010; Seitz et al. 2010). Furthermore, according to Palmer et al. (2005) and Küpper et al. (2008), the iodine identified in the particles formed above air-exposed *L. digitata* originated only from I_2 ; the concomitant emission of volatile iodo-carbons did not contribute to particle production. Thus, the emission of I_2 from kelp deserved a closer examination due to its key role in atmospheric chemistry processes (O'Dowd and Hoffmann 2005). Even though quantification techniques of biogenic iodine emissions are still being debated (Ball et al. 2010), only few I_2 emission rates have been published for brown macroalgae (Palmer et al. 2005; Ball et al. 2010). These data suggest that I_2 emission rates from macroalgae need to be considered in relation to several different parameters such as the corresponding species, biotic and abiotic environmental impacts and the physiological status of the algae (Laternus et al. 2004).

In the present study, I_2 emission from air-exposed *L. digitata* was quantified under controlled environmental conditions by applying incoherent broadband cavity-enhanced absorption spectroscopy (IBBCEAS) (Fiedler et al. 2003; Dixneuf et al. 2009). Relevant parts of the thallus, i.e. the stipe plus holdfast, the meristematic area and the distal blade, were considered individually so that their respective contribution to the total I_2 emission from the alga could be estimated for the first time. Dividing the alga into different parts in the context of I_2 emission provides useful information about the presumably multifunctional role of iodine in seaweeds.

Furthermore, effects of air exposure on photosynthetic performance of different thallus parts of *L. digitata* were investigated. In vivo chlorophyll *a* fluorescence parameters are widely used as an indicator for "health" of photosynthetic active organisms (Maxwell and Johnson 2000) and

changes can allow the quantification of potential stress experienced by the alga. Photosynthesis can be affected by air exposure due to desiccation and/or qualitative and quantitative changes in inorganic carbon supply (Dring and Brown 1982). Such effects potentially influence the efficiency of photosystem II (PSII), i.e. primary photosynthesis.

Materials and methods

Algal material and sampling strategy

Sporophytes of *L. digitata* (Hudson) Lamouroux were harvested on the west coast of Ireland at Finavarra (53°09'25"N, 09°06'58"W) during the spring low tide in early November 2009; the weather was overcast and the mean seawater temperature was ~8°C. All algae sampled were of similar age and 1.5 m long on average, and free from visible epiphytes, grazers or grazing marks. Only submersed specimens were collected and care was taken to keep the samples underwater at all times between harvesting and start of I₂ emission measurements. The latter were conducted in the Finavarra field research station ca. 500 m from the collecting site. Thus, a potential loss of iodine caused by stressful conditions during the transport to the laboratory was avoided. Prior to experiments, seaweeds were kept for up to 8 days in a 200 L tank filled with seawater from their natural habitat and stored at low irradiances at ~8°C; the water was continuously aerated by diffusers and replaced every second day with fresh, local seawater.

Experimental design

Each *L. digitata* thallus was divided into three parts: (a) the ‘stipe’ (undivided and including the holdfast), (b) the ‘meristematic area’, from the top of the stipe to where the base of the blade divides into several laminae, and (c) the ‘distal blade’, i.e. one part of the remaining laminae (cf. inset in Fig. 2). The I₂ emitted into the air was monitored by applying IBBCEAS (Fiedler et al. 2003). To investigate the effect of low irradiance on I₂ emission, the air-exposed samples were studied for 180 min under two ambient light regimes: (a) low light, i.e. a PFD of 35 μmol photons m⁻² s⁻¹ PAR (400–700 nm) provided by a fluorescent tube (Lumilux cool daylight, Osram GmbH, Munich, Germany) or (b) dark conditions. Due to the absence of radiation below 400 nm when the light was switched on, the loss of I₂ through UV photolysis inside the system was negligible. The experimental air temperature was controlled in the chamber at T_{in} ~ 10.5°C.

Prior to measurements, the relevant thallus part was cut from the rest of the alga under water, as, given the size of the experimental setup (Fig. 1), it was not feasible to study an entire specimen. Algal samples were acclimated in natural seawater to the experimental light and temperature conditions for 30–60 min prior to air exposure and I₂ emission measurements. Measurements generally started within 5 s after emersion of the sample. After 180 min, both dry weight (dw) and surface area (sa) of the sample were determined. Surface area of the meristematic area and distal blade was obtained by weighing a sample-shaped piece of paper whose surface

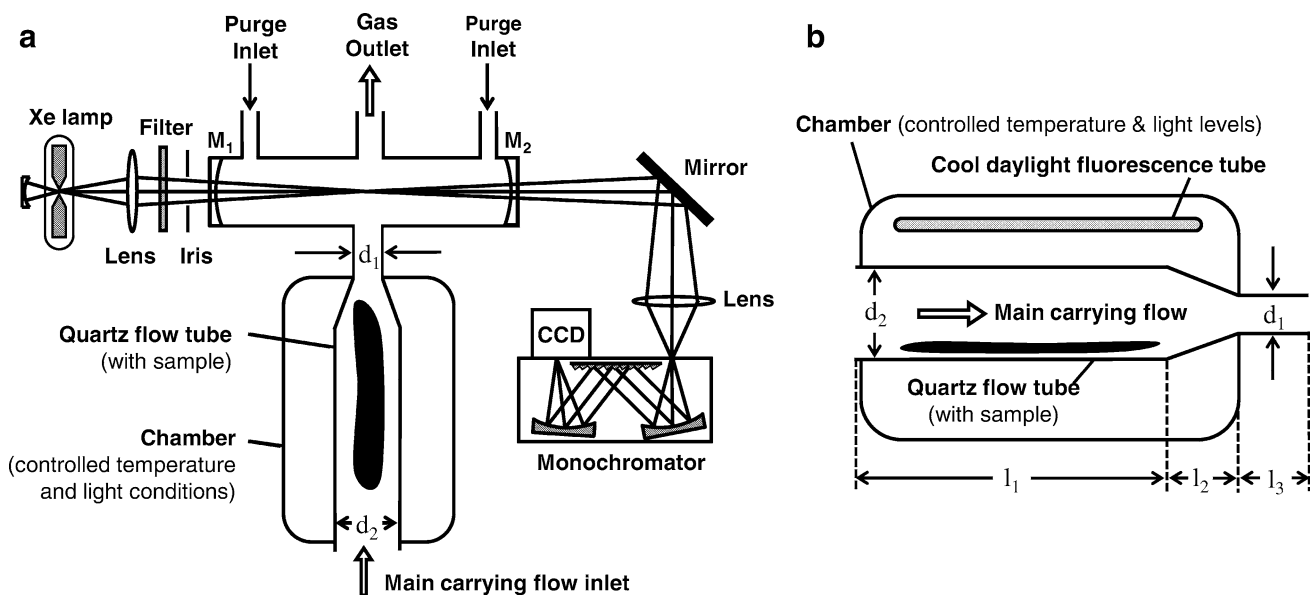


Fig. 1 Schematic representation of the IBBCEAS setup for molecular iodine flux measurements. **a** M₁, M₂: high reflectivity plano-concave mirrors (separation 76 cm). The main carrying flow (3.9 L min⁻¹) and the mirror purge flow (~0.2 L min⁻¹ in total)

were clean compressed air. The gas outlet was open to ambient air. Temperature in the sample compartment was T_{in} ~ 10.5°C. **b** Details of the quartz flow tube: d₁ = 2.2 cm, d₂ = 9.3 cm, l₁ = 60 cm, l₂ = 25 cm, l₃ = 32 cm

area-to-weight ratio was known. Only one side of the meristematic area and distal blade was accounted for in the calculation of sa as only one face was in direct contact with air; the other was in contact with the wall of the flow tube. Approximating the stipe (and the main branches of the holdfast) by a cylinder with elliptical circumference, the surface area of these thallus parts was estimated. Dry weights were determined after freeze-drying. I_2 emission rates are expressed as both $\text{pmol g}^{-1} \text{ dw min}^{-1}$ and $\text{pmol cm}^{-2} \text{ min}^{-1}$.

In order to study the effect of air exposure on the photosynthetic performance of algal thallus parts, *in vivo* chlorophyll *a* fluorescence parameters were determined before and after I_2 emission measurements; initial fluorescence values were taken on submersed samples. Water loss of individual thallus parts was also monitored: a tissue sample (~ 1 g fresh weight) was taken before and after I_2 emission measurement and immediately frozen before freeze-drying; the water content was calculated as a reduction in weight before and after drying.

Determination of I_2 emission rates using IBBCEAS

A schematic of the experimental setup for the detection of I_2 is shown in Fig. 1. The optical cavity was formed by two plano-concave dielectric mirrors (2.5 cm diameter) with a radius of curvature of 200 cm and a separation of $d = 76$ cm. The spectrally filtered white light from a 75 W short arc Xe lamp was focused at the centre of the cavity; the light transmitted through the cavity was spectrally dispersed by a monochromator (Oriol MS125, 25 μm entrance slit, 1,200 lines mm^{-1} grating, LOT-Oriel, Darmstadt, Germany) and imaged onto a CCD detector (back illuminated DU401-BV, Andor Technology, Belfast, UK) cooled to -25°C . The spectral resolution was ~ 0.43 nm.

To achieve time-resolved flux measurements, the optical cavity was set up perpendicular to the direction of the I_2 carrying flow by means of a four-way stainless steel cross-piece with an internal diameter of $d_1 = 2.2$ cm on all sides (Fig. 1a). The seaweed sample was placed in a quartz tube (diameter $d_2 = 9.3$ cm) at right angle to the cavity axis which was connected to the central cross-piece via a conical glass-to-metal seal (Fig. 1b); the other end of the tube was sealed with a plastic cap. In Fig. 1a, the open arrows represent the inlet of the main air flow ($M_c \sim 3.9$ L min^{-1}) through a 5 mm aperture, and the only gas outlet of the system at the cross-piece. The two thin black arrows indicate the purge gas inlets (total purge flow ~ 0.2 L min^{-1}) located ~ 5 cm from the cavity mirrors. The purge flow was chosen so that the effect on the I_2 carrying flow was minimal (see below).

Both the main flow and the purge flows were supplied by ambient air compressed through an oil-free air compressor (OF302-4MD2, Jun Air Ltd., Nørresundby, Denmark) equipped with a particle filter and an adsorption

dryer. The purge air inlet line (10 m, 8 mm diameter) between the air compressor and the system also served as a scrubber for both I_2 and O_3 that may have been present in ambient air.

The quartz flow tube (i.e. the compartment where the seaweed was placed) was contained in a light-tight cylindrical copper chamber that was thermally stabilised using a closed cycle water cooler. Two thermocouples with an accuracy of $\sim 0.5^\circ\text{C}$ were used to monitor the temperature just above the seaweed (temperature $T_{\text{in}} \sim 10.5^\circ\text{C}$), and near the detection zone (temperature $T_{\text{ext}} \sim 13.5^\circ\text{C}$).

I_2 was monitored in the spectral region between 530 and 553 nm. The retrieval of the molecular iodine number density (molecules cm^{-3}), n_{I_2} , has been described in Dixneuf et al. (2009). Using cavity-enhanced absorption techniques for quantitative purposes requires the knowledge of the effective absorption path length as a function of wavelength, $L_{\text{eff}}(\lambda) = L(1 - R(\lambda))^{-1}$, where $R(\lambda)$ is the mirror reflectivity and L (cm) is the path length over which the target species in the cavity interacts with the light trapped inside the cavity. In the present study, L_{eff} was established by flowing a known concentration of I_2 into the system: a known, small flow of air was passed above iodine crystals contained in a U-shaped tube cooled to 0°C so that the iodine mixing ratio was deduced from the ratio of the vapour pressure of iodine at 0°C and atmospheric pressure. A maximum effective path length of $\sim 30 \pm 3$ m at 542 nm was found.

The detection limit for I_2 was $\approx 7 \pm 2$ nmol mol^{-1} for a 10 s average time. The systematic uncertainty on n_{I_2} (which is mostly due to the uncertainty of $L_{\text{eff}}(\lambda)$ and to the error of the I_2 reference absorption cross-sections $\sigma_{I_2}(\lambda)$; Saiz-Lopez et al. 2004) was smaller than 20% (Dixneuf et al. 2009). Time-dependent emission rates $E_{I_2}(t)$ were obtained as the product of $n_{I_2}(t)$ and the constant volumetric flow rate M_c :

$$E_{I_2}(t) = n_{I_2}(t)M_c \quad (1)$$

Each emission profile $E_{I_2}(t)$ was further characterised by averaging over two relevant time periods: (a) the interval 0–30 min where an initial I_2 emission peak was observed, and (b) the interval 60–180 min where the I_2 emission was found to gradually reach the detection limit. For each sample, both the mean initial and the mean long term emission rates, $\langle E_{I_2} \rangle$, were thus obtained.

Note that adsorption/desorption of I_2 on/from the walls of the flow cell affected the temporal emission profile. Under the present flow conditions passivating the walls with iodine was not possible. For this reason, the mean initial emission rates presented here are in principle lower limits (due to potential adsorption of I_2), while the emission rates observed during the interval 60–180 min are potential upper limits (due to the potential desorption of I_2). The I_2

wall adsorption/desorption can be accounted for when integrating the temporal emission profile $E_{I_2}(t)$ until the detection limit is reached, in the present case usually after 180 min (corresponding amounts of I_2 emitted in total, N_{I_2} , are given in Table 1). Averaging the emission profile over the periods 0–30 and 60–180 min remains a relevant way of discussing relative emission changes as a function of time.

Photosynthetic performance

A pulse-amplitude modulated fluorometer (PAM-2000, Heinz Walz GmbH, Effeltrich, Germany) was used for measurements of in vivo chlorophyll *a* fluorescence parameters which were determined directly before (in natural seawater) and after 180 min (in air) I_2 emission studies. The measuring principle was based on the technique by Schreiber et al. (1986). The maximum PSII efficiency, F_v/F_m , the effective PSII quantum efficiency under low light exposure ($35 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR), $\Delta F/F'_m$, the photochemical quenching, which is the fraction of open PSII reaction centres, q_p , and the efficiency of the latter in capturing energy, F'_v/F'_m , were determined for each thallus part. The nomenclature used in this study is according to Kromkamp and Forster (2003). F_0 was determined with a 1.6 kHz pulsed red measuring light ($\sim 0.2 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, 650 nm) and F_m and F'_m were measured with a 600 ms completely saturating white light pulse (20 kHz, $\sim 9,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The thallus parts were kept in the dark for 30 min and, in the case of the low light experiment, additionally for 30 min at $35 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR, prior to measuring the aforementioned photosynthesis parameters. F_v/F_m was calculated as follows:

$$\frac{F_v}{F_m} = \frac{F_m - F_0}{F_m} \tag{2}$$

where F_v is the variable chlorophyll *a* fluorescence, F_m is the maximal chlorophyll *a* fluorescence after dark acclimation, and F_0 is the minimal level chlorophyll *a* fluorescence.

$\Delta F/F'_m$ was calculated according to the following equation:

$$\frac{\Delta F}{F'_m} = \frac{F'_m - F}{F'_m} \tag{3}$$

where F'_m is the maximal chlorophyll *a* fluorescence after acclimation to light and F is the steady-state fluorescence of chlorophyll *a* in light (Genty et al. 1989; Kromkamp and Forster 2003).

The parameter q_p was computed as follows:

$$q_p = \frac{F'_m - F}{F'_m - F_0} \tag{4}$$

where F'_0 and F'_m are, respectively, the minimal and maximal chlorophyll *a* fluorescence after acclimation to light (Kromkamp and Forster 2003).

Finally, F'_v/F'_m was calculated as:

$$\frac{F'_v}{F'_m} = \frac{F'_m - F'_0}{F'_m} \tag{5}$$

Statistical analysis

I_2 emission experiments were carried out using three different thallus parts under two ambient light regimes (low light and dark conditions) and each treatment was replicated five times ($n = 5$). Due to a high variability in the obtained emission data, the I_2 emission rates $\langle E_{I_2} \rangle$ are presented as median and as minimum and maximum showing the data range. Results of the photosynthetic performance (F_v/F_m , $\Delta F/F'_m$, q_p and F'_v/F'_m) of the different thallus parts are presented as mean and one standard deviation.

The effects of the fixed factors “thallus part” (i.e. stipe, meristematic area and distal blade), “time” (i.e. 0–30 and 60–180 min) and “light regime” (i.e. low light and dark conditions) on the variables “ $\langle E_{I_2} \rangle$ ” and “ F_v/F_m ” were analysed by applying three-way analyses of variance (ANOVAs). Effects of the fixed factors “thallus part” and “time” on the variables “ $\Delta F/F'_m$ ”, “ q_p ” and “ F'_v/F'_m ” were analysed using two-way ANOVAs. The *main effects* were computed. The Tukey test was used to find a posteriori homogeneous sub-groups that differed significantly (level of significance: $P < 0.05$). Regarding I_2 emission rates $\langle E_{I_2} \rangle$, the data were logarithmically transformed prior to statistical analyses (Sokal and Rohlf 1995). All data (or, regarding $\langle E_{I_2} \rangle$, the transformed data) were normally distributed (Kolmogorov–Smirnov test) and the variances were homogeneous (Levene test).

Table 1 Amount of I_2 emitted in total, N_{I_2} , by different thallus parts of *Laminaria digitata*, relative to their dry weight (dw) or surface area, obtained by integrating the observed temporal I_2 emission profile over the entire interval 0–180 min during which the sample was exposed to air at $\sim 10.5^\circ\text{C}$ under low light or dark conditions

	Total amount of I_2 emitted (N_{I_2})	
	nmol g ⁻¹ dw	nmol cm ⁻²
Low light conditions		
Stipe	259 (148–782)	13.3 (7.8–43.3)
Meristematic area	51 (17–130)	0.89 (0.25–2.14)
Distal blade	14 (5–39)	0.22 (0.08–0.61)
Dark conditions		
Stipe	358 (171–638)	29.1 (21.2–55.9)
Meristematic area	30 (16–83)	0.52 (0.30–1.14)
Distal blade	19 (4–692)	0.37 (0.05–15.7)

Data are presented as median and the minimum and maximum ($n = 5$)

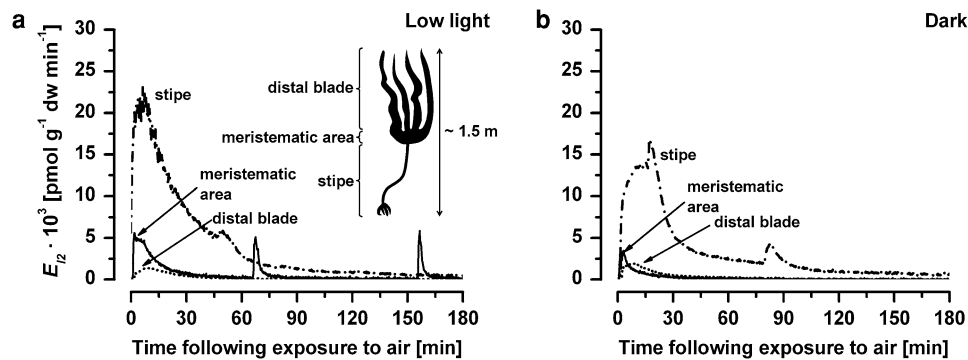


Fig. 2 The strongest I_2 emitting specimens of air-exposed *Laminaria digitata* as examples of an I_2 emission rate, $E_{I_2}(t)$ ($\text{pmol g}^{-1} \text{ dw min}^{-1}$) as a function of time, with respect to different parts of the thallus: ‘stipe’, ‘meristematic area’, and ‘distal blade’. Emission into

air at $\sim 10.5^\circ\text{C}$ was determined over 180 min. **a** Low light condition ($35 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ PAR); inset shows a schematic of a *L. digitata* thallus and relevant parts. **b** In darkness; the ‘distal blade’ measurement was terminated after 92 min

Results

Temporal profile of I_2 emission

Two examples of the I_2 emission profiles, $E_{I_2}(t)$, obtained from each of the three parts of the strongest I_2 emitting specimens of *L. digitata* are presented in Fig. 2 with a time resolution of 10 s. Immediately after $t = 0$ when the thallus was first exposed to air, an intense initial burst of I_2 was detectable from all thallus parts, lasting about 30 min (note that initially the thallus was still surrounded by a thin water film). The median HWHM of the initial I_2 emission burst was 21 min for the stipe, 15 min for the meristematic area and 11 min for the distal blade. The strongest initial burst was observed from the stipe with a maximum E_{I_2} about 5 to 10 times higher than bursts from the meristematic area and the distal blade, respectively. I_2 emission from each part of the alga decreased continuously over time to about 30% of the initial emission rate; the decay did not follow a single exponential decay. During exposure between 60 and 180 min, emission rates continued to decrease gradually and dropped to the detection limit after 180 min. For the two specimens exemplified in Fig. 2, additional short and intense I_2 emission bursts were later observed from the stipe and the meristematic area. Similar subsequent bursts were frequently detectable from these two thallus parts, at both low light and dark conditions, but were only once observed from the distal blade. These intense, additional bursts were neither periodic nor did they follow any specific pattern; they occurred at random times t after exposure to air. The median HWHM of the additional I_2 emission bursts (on the local I_2 emission level at the time of the burst) was approximately 10 min for the stipes and only ca. 1 min for the meristematic areas and the (one) distal blade. The peak height of the short bursts ranged from 0.2 to 6 times the height of the initial peak (data not shown). Furthermore, the light regime (i.e. low

light or dark conditions) did not seem to have any impact on the general emission pattern.

I_2 emission rates from different thallus parts

The total amounts of I_2 released by different thallus parts of the algae, N_{I_2} , are presented in Table 1 relative to the dry weight and surface area. Under either light regime, the largest amount of I_2 was emitted by the stipe, followed by the meristematic area and the distal blade, i.e. $N_{I_2}(\text{stipe}) > N_{I_2}(\text{meristematic area}) > N_{I_2}(\text{distal blade})$. The difference in the amounts of I_2 emitted by the stipe and the other two thallus parts was roughly an order of magnitude. For further characterisation, the temporal emission profiles were divided into two distinct phases. Table 2 shows the I_2 emission rates $\langle E_{I_2} \rangle$ of different thallus parts, averaged over the first 30 min of the experiment (initial I_2 emission) and over the period 60–180 min.

At low light conditions, the median of the initial I_2 emission rate of the stipe was $2,999 \text{ pmol g}^{-1} \text{ dw min}^{-1}$, and therefore 3.6 and 17 times higher than that of the meristematic area and the distal blade, respectively (Table 2). Between 60 and 180 min after the initial air exposure, the I_2 emission rates of the stipes were reduced by 75% and the corresponding median was found to be $758 \text{ pmol g}^{-1} \text{ dw min}^{-1}$. Emission rates of the meristematic area and the distal blade also decreased by 70%; they were 5 and 14 times lower than those of stipes, respectively ($P < 0.001$, supplemental Table S1).

Similar results were observed under dark conditions (Table 2). The median of the initial I_2 emission rate of the stipe amounted to $5,222 \text{ pmol g}^{-1} \text{ dw min}^{-1}$ and was thus about 13 times higher than that of the meristematic area and the distal blade. Between 60 and 180 min of exposure to air, median values of I_2 emission rates of all thallus parts had decreased by 85%. Overall, I_2 emission rates were highly variable (Tables 1, 2).

Table 2 Molecular iodine emission rates (E_{I_2}) of different thallus parts of *Laminaria digitata* relative to their dry weight (dw) or surface area

Air exposure time period	I_2 emission rate (E_{I_2})			
	pmol g ⁻¹ dw min ⁻¹		pmol cm ⁻² min ⁻¹	
	0–30 min	60–180 min	0–30 min	60–180 min
Low light conditions				
Stipe	2,999 (739–14,976) ^A	758 (573–1,013) ^D	155 (39–827)	49 (30–56)
Meristematic area	830 (126–2,316) ^B	145 (77–399) ^E	12 (2–38)	2 (1–7)
Distal blade	175 (136–856) ^C	53 (35–65) ^F	6 (3–13)	<1 (<1–1)
Dark conditions				
Stipe	5,222 (720–11,035) ^A	1,045 (631–2,207) ^D	253 (70–970)	101 (66–118)
Meristematic area	436 (213–952) ^B	57 (54–215) ^E	7 (4–15)	1 (1–3)
Distal blade	370 (79–13,664) ^C	49 (3–746) ^F	7 (1–310)	1 (<1–17)

Thalli were exposed to air for 180 min at ~10.5°C and I_2 emission rates determined applying IBBCEAS. The observed temporal I_2 emission profile was split into two time periods (0–30 min: initial I_2 emission peak and 60–180 min: low I_2 emission) for which mean emissions were determined. Data are presented as median and the minimum and maximum emission rates obtained from $n = 5$ replicates. Levels of significance of the fixed factors “thallus part”, “time” and “light regime” were determined by three-way ANOVA (“thallus part” $P < 0.001$, “time” $P < 0.001$, “light regime” $P = 0.362$). Significant differences within factors as revealed by Tukey’s post hoc test (supplemental Table S1) are indicated by different superscripts

I_2 emission rates differed significantly between the three thallus parts ($E_{I_2}(\text{stipe}) > E_{I_2}(\text{meristematic area}) > E_{I_2}(\text{distal blade})$, $P < 0.001$, supplemental Table S1), but were independent of the experimental light regime ($P = 0.362$).

When considering the emission rates relative to the surface area, a high variability was also observed. As shown in Table 2, emission rates of the stipe were still the highest. A median emission rate of 253 pmol cm⁻² min⁻¹ occurred within the first 30 min of exposure to air, in darkness; this value was 36 times higher than the median values for the meristematic area and for the distal blade. Initial I_2 emission rates of the meristematic area and the distal blade were in a similar range, between 1 and 15 pmol cm⁻² min⁻¹, but a high rate was observed for one distal blade (310 pmol cm⁻² min⁻¹). After exposure to air for more than 60 min, the stipe exhibited emission rates of up to 118 pmol cm⁻² min⁻¹, i.e. 100 times higher than the other *L. digitata* thallus parts. Similar results were observed under low light conditions (Table 2).

Photosynthetic performance and water content

As shown in Fig. 3a and b, the values for the maximum PSII efficiency (F_v/F_m) were generally high before exposure to air; mean F_v/F_m values ranged from 0.751 ± 0.010 to 0.777 ± 0.028 for all thallus parts. F_v/F_m was dependent on the thallus part ($P = 0.041$, supplemental Table S1), with the lowest values for the meristematic area and the highest for the distal blade. F_v/F_m was significantly affected by the emersion time ($P < 0.001$, supplemental Table S1) and reduced by 3% after 180 min of exposure to

air in each thallus part of *L. digitata*. No significant difference was observed between F_v/F_m obtained for the different thallus parts investigated at two light regimes ($P = 0.057$, supplemental Table S1).

The low light experiment allowed the determination of the effective PSII quantum efficiency ($\Delta F/F'_m$), the photochemical quenching (q_p), as well as the capacity to capture energy (F'_v/F'_m) at 35 μmol photons m⁻² s⁻¹ PAR (Fig. 3c–e). $\Delta F/F'_m$ was dependent on thallus part ($P < 0.001$, supplemental Table S1). Prior to air exposure, mean values for the stipe (0.345 ± 0.071) were significantly lower than values for the meristematic area (0.469 ± 0.051) and the distal blade (0.458 ± 0.058), respectively. $\Delta F/F'_m$ in the meristematic area and the distal blade was 34% higher than in the stipe and did not differ significantly from each other. After exposure to air for 180 min, $\Delta F/F'_m$ had increased by 25–55% in all thallus parts ($P < 0.001$), but remained lower in the stipe (Fig. 3c). Prior to exposure to air, the lowest q_p values were again observed from the stipe (0.560 ± 0.103) and reached 74% of q_p of the meristematic area (0.753 ± 0.052) and 81% of q_p of the distal blade (0.692 ± 0.102). As illustrated in Fig. 3d, air exposure resulted in an increase of q_p for all thallus parts of *L. digitata* ($P < 0.001$, supplemental Table S1), i.e. by 46% for the stipe, 16% for the meristematic area and 22% for the distal blade. The efficiency of open reaction centres in capturing energy (F'_v/F'_m) was significantly lower in the stipe than in the distal blade but that of other thallus parts were similar (Fig. 3e; supplemental Table S1). Furthermore, F'_v/F'_m remained nearly constant during the experiment ($P = 0.249$, supplemental Table S1; Fig. 3e).

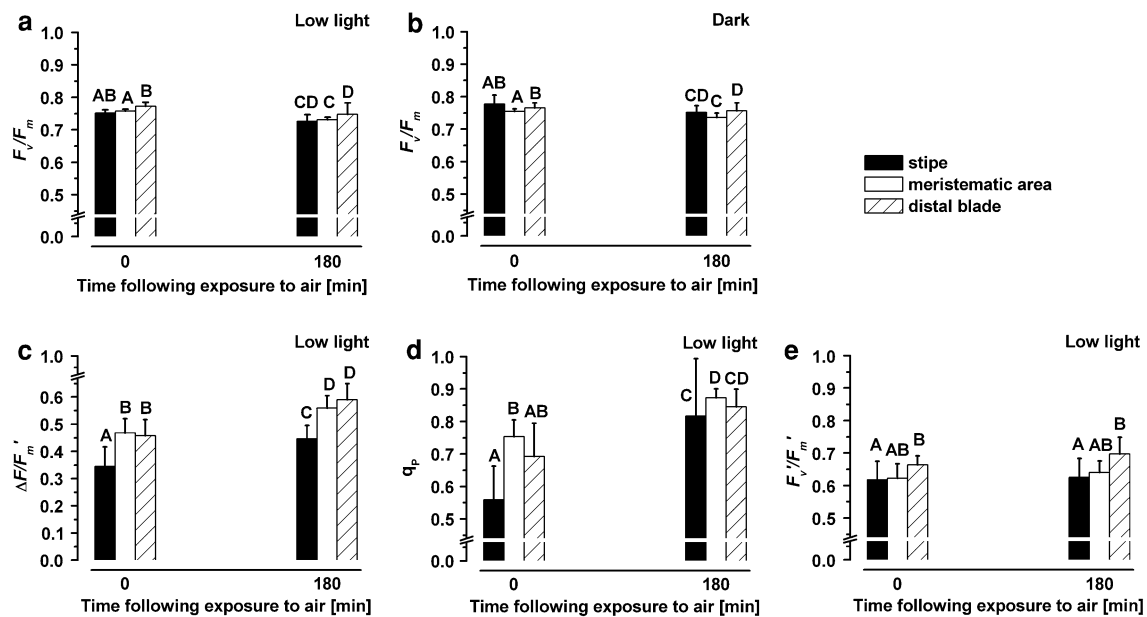


Fig. 3 Effects of air exposure on the photosynthetic performance of different thallus parts of *Laminaria digitata*. Maximum PSII efficiency (F_v/F_m), effective PSII quantum efficiency ($\Delta F/F_m'$), photochemical quenching (q_p) and efficiency of open reaction centres in capturing energy (F_v'/F_m') were determined in stipes (black bars), meristematic areas (unfilled bars) and distal blades (hatched bars) prior to air exposure, i.e. when thallus parts were submersed in natural seawater (before the time 0 of the experiment), and after 180 min in air. **a** F_v/F_m obtained in the low light experiment. **b** F_v/F_m obtained in the dark experiment. **c** $\Delta F/F_m'$, **d** q_p and **e** F_v'/F_m' obtained in the low light experiment. The experimental temperature was $\sim 10.5^\circ\text{C}$. Data are presented as mean and standard deviation ($n = 5$). Levels of

significance of the fixed factors “thallus part”, “time” and “light regime” on F_v/F_m were calculated by three-way ANOVA (“thallus part” $P = 0.041$, “time” $P < 0.001$, “light regime” $P = 0.057$; note that data shown in **a** and **b** were analysed together). Levels of significance of the fixed factors “thallus part” and “time” on $\Delta F/F_m'$, q_p and F_v'/F_m' were calculated by two-way ANOVAs ($\Delta F/F_m'$: “thallus part” $P < 0.001$, “time” $P < 0.001$; q_p : “thallus part” $P = 0.031$, “time” $P < 0.001$; F_v'/F_m' : “thallus part” $P = 0.016$, “time” $P = 0.249$). Different uppercase letters are used to indicate significant differences within variables as revealed by Tukey’s post hoc test (see supplemental Table S1)

The average water content of *L. digitata* was 83%. The water loss during exposure to air for 180 min was similar for all thallus parts, about 2%, irrespective of the light regime.

Discussion

In this study, I_2 emission rates of different thallus parts of *L. digitata* sporophytes were monitored at low light ($35 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ PAR) and dark conditions at $\sim 10.5^\circ\text{C}$ applying a highly sensitive cavity-enhanced absorption spectroscopy technique (Fiedler et al. 2003; Dixneuf et al. 2009). To estimate the extent of stress experienced by each thallus part over the course of the experiment, photosynthetic performance was determined by measuring parameters of primary photosynthesis using chlorophyll *a* fluorescence.

For all thallus parts, I_2 emission systematically showed a strong initial emission peak lasting about 30 min, which was followed by a gradual decrease. The temporal profiles are, therefore, consistent with those recently observed

(Bale et al. 2008; Dixneuf et al. 2009; Ball et al. 2010). For a complete *L. digitata* thallus, Ball et al. (2010) observed a decrease of the maximum I_2 emission rate to half its value in 5 min. The HWHM observed in this study was different for different thallus parts and ranged from 11 to 21 min. During incubation experiments, an intense initial I_2 emission peak from entire thalli of *L. hyperborea* and *Saccharina latissima* (Laminariales) was also observed (Ball et al. 2010) immediately after first exposure to air, followed by an exponential decay over a period of 30–40 min. Thus, for the species of the order Laminariales investigated so far, exposure to air resulted in an immediate I_2 emission. It has been shown that iodine is mostly retained as I^- in external mucilage of epidermal cell layers (Küpper et al. 2008; Verhaeghe et al. 2008). The apoplastic stock of I^- is believed to be associated with charged polymers for a rapid remobilisation and (bio)availability (Verhaeghe et al. 2008). Temporal I_2 emission profiles from *L. digitata* and other Laminariales (Bale et al. 2008; Dixneuf et al. 2009; Ball et al. 2010; present study) indicate the occurrence of either a rapidly mobilised or a directly iodovolatilisable iodine pool, that is being depleted during emersion.

Besides the strong initial I_2 emissions, additional short, intense and sporadic I_2 emission bursts were detectable during the extended period of exposure to air. These additional bursts had been previously observed for the first time from an entire *L. digitata* thallus in a static chamber (Dixneuf et al. 2009). In the present study, such bursts were frequently observed from the stipe and the meristematic area, but observed only once from the distal blade. Iodine has been found in intracellular, vesicle-like structures, which are mainly located in meristodermic cells of the stipe and, much lower in density, in the blade (Pedersén and Roomans 1983; Verhaeghe et al. 2008). It has been suggested that the outer cells of the stipe have a distinctive ability to store iodine and that the content of such vesicle-like structures can probably easily be leached (Verhaeghe et al. 2008). A sporadic release of iodine by intracellular vesicles (Evans and Holligan 1972) might have been a physiological origin for the short additional I_2 bursts that were mainly detected from the stipe.

As I_2 emission rates were found to be not affected by the experimental light regime (low light and darkness), the suggested depletion of an easily mobilised iodine pool indicates a physiologically multifunctional role of iodine in *L. digitata*. The physiological significance for the higher I_2 emission rates observed from the stipe and the meristematic area remains, however, speculative.

At the beginning of the measurement, *L. digitata* thalli were surrounded by a thin seawater layer. Iodine exists in coastal waters mainly as iodate (IO_3^- , up to $0.35 \mu\text{mol L}^{-1}$), iodide (I^- , up to $0.20 \mu\text{mol L}^{-1}$), and to a smaller extent as organic-bound iodine (Hou et al. 2009), dependent on the local water chemistry. Therefore, iodine is generally considered a trace element in seawater, in particular when compared to the concentrations found in living marine organisms (Hou et al. 2009). Thus, in the present study, the remarkable amounts of I_2 detected in the gaseous phase originated mainly from labile iodine made available by the thallus parts; the contribution of I_2 elution from the thin layer of seawater to the total amount of I_2 detected was most likely negligible.

In response to environmental stress and as part of chemical defence mechanisms, the release of iodine from macroalgae is generally linked with the quenching of hydrogen peroxide and other reactive oxygen species (ROS) (Palmer et al. 2005; Küpper et al. 2001, 2008). Emission of inorganic and organic iodine species was previously observed when algae were exposed to aqueous oxidants, ozone and ultraviolet radiation (Laternus et al. 2004; Palmer et al. 2005; Küpper et al. 2008). The mechanisms and chemical reactions leading to the retransmission of iodine to the gaseous environment, mainly in its molecular form (I_2), are still subject of ongoing research (e.g. Leblanc et al. 2006). Since the ozone mixing ratio in the main carrying flow was not monitored in the present

study and no information on the composition of the aqueous surface layer surrounding the seaweed was available, the immediate strong I_2 emission cannot be unambiguously attributed to a specific oxidising process.

In *S. latissima*, iodine has been demonstrated to be translocated from the distal blade towards the meristematic area (Amat and Srivastava 1985). In *L. digitata*, previous observations of higher average iodine contents in the stipe than in the blade (Küpper et al. 1998; Verhaeghe et al. 2008) correlate to the observed I_2 emission rate differences for different thallus parts observed here (i.e. dw- and sa-normalised emission of stipe > meristematic area > distal blade). Ar Gall et al. (2004) have shown that haloperoxidase activity and iodine uptake rates were highest in distal parts of the blade and lower in the meristematic area. The gradual increase of haloperoxidase activity, which accompanies increasing iodine uptake rates, from meristem towards distal blade parts is consistent with the fact that lowest I_2 emission rates were observed for the distal blade in the present study.

Different I_2 emission rates have been reported for thalli of *L. digitata* from different European populations under presumed low stress conditions (i.e. low light levels and/or controlled temperature). Average emissions of $3.7 \text{ pmol g}^{-1} \text{ fresh weight (fw) min}^{-1}$ (Palmer et al. 2005) and $3.0 \text{ pmol g}^{-1} \text{ fw min}^{-1}$ (Ball et al. 2010) were measured for a towel-dried strip of *L. digitata* from the west coast of Scotland and for an entire alga from French Brittany, respectively. Bale et al. (2008) have reported I_2 mixing ratios comparable to the ones found in this investigation, i.e. a maximum of 300 ppbv for a whole *L. digitata* thallus (50 g fw), also from the west coast of Ireland, that corresponds to a peak emission rate of ca. $2,500 \text{ pmol g}^{-1} \text{ fw min}^{-1}$ for a 10 L min^{-1} carrying air flow. These data cannot easily be compared with the results presented here as emission rates presented in this study were normalised by the dry weight or surface area, and median values of I_2 emission rates from three separate thallus parts were calculated.

Seasonal variation in the iodine content in *L. digitata* has also been documented; e.g. Ar Gall et al. (2004) found higher iodine levels in various European populations in autumn and winter and lower contents during spring and summer months. Similarly, in a Norwegian *L. digitata* population, the maximum level of iodine concentration was observed in February and the minimum in summer (Haug and Jensen 1954). Since the present study was performed in early November, the observed high I_2 emission may have been related to a high (autumn) initial iodine content. However, further investigations are required to elucidate aspects of potential seasonal variability of biogenic I_2 emission.

Exposure to air imposes a number of constraints or benefits on algal physiology in terms of inorganic carbon

supply, irradiance, temperature changes and cellular water loss (Johnson et al. 1974; Harker et al. 1999; Gómez et al. 2004; Hunt and Denny 2008). Upper distribution limits of intertidal algae are notably determined by their capacity to tolerate extended periods of emersion (Schonbeck and Norton 1978; Wiltens et al. 1978). To assess the physiological performance of algae during a tidal cycle, many studies have focused on photosynthesis using chlorophyll *a* fluorescence-based techniques due to the potential sensitivity of parameters of primary photosynthesis (e.g. F_v/F_m , $\Delta F/F'_m$) to environmental changes (Harker et al. 1999; Gevaert et al. 2002; Gómez et al. 2004). In the present study, F_v/F_m values in different thallus parts of the subtidal alga *L. digitata* ranged from 0.751 to 0.777 at either submersed conditions and even after 180 min of emersion. Such values are typical for unstressed brown macroalgae (Büchel and Wilhelm 1993). Algal physiology is significantly affected by a combination of numerous abiotic and biotic factors (Dethier and Williams 2009); it seems that the ambient experimental conditions during the emersion were not stressful enough to substantially inhibit photosynthesis.

The results of primary photosynthesis presented here were based on overall chlorophyll *a* fluorescence signals measured from several cell layers (depending on the thickness of the alga); they, therefore, indicate an integrated status of the photosynthetic performance of the thallus part investigated, without allowing for a distinction between surface and subsurface cells. However, since desiccation proceeded most likely from the thallus surface inwards, photosynthesis of the surface cells may have been more influenced by the emersion than photosynthesis of the subsurface cells. Given that the inner cells of blades of *L. digitata* were shown to have generally lower F_v/F_m values than surface cells (Garbary and Kim 2005), it appeared difficult to quantify the stress in *L. digitata* by measuring F_v/F_m only.

In contrast to F_v/F_m , $\Delta F/F'_m$ is the proportion of absorbed energy which is used in photochemistry and relates to achieved photosynthetic efficiency. As such it can give an indication of the overall photosynthesis (Maxwell and Johnson 2000). Unexpectedly, in the present study, $\Delta F/F'_m$ was increased significantly in all thallus parts of *L. digitata* after 180 min of emersion, suggesting an overall increase in photosynthesis. An enhanced photosynthetic activity was also found by Dring and Brown (1982) in *Fucus serratus* and *L. digitata* during emersion; in that instance, the increase lasted between 30 and 40 min and was followed by a continuous decline, which only occurred if thalli were allowed to dry out (Dring and Brown 1982). However, under the present experimental conditions, intracellular water was mainly retained by *L. digitata*.

Hence, a critical water status for severe inhibition of overall photosynthesis was probably not achieved, but photosynthetic activity most likely depended on the distance of the cells to the thallus surface. The response of $\Delta F/F'_m$ is defined by either, the fraction of open reaction centres (q_p), their efficiency in capturing energy (F'_v/F'_m) or a combination of both (Krause and Weis 1991; Linger and Brüggemann 1999). Here, exposure to air had pronounced positive effects on q_p in all thallus parts of *L. digitata*, whereas F'_v/F'_m remained unchanged. An increase in $\Delta F/F'_m$ is, thus, primarily caused by a higher fraction of open reaction centres suggesting an enhanced performance of the photosynthetic dark reactions (Linger and Brüggemann 1999). The efficiency of energy capture (F'_v/F'_m) depends on the thermal energy dissipation in the antennae and interrelates with F_v/F_m and the NPQ of chlorophyll *a* fluorescence. As F'_v/F'_m was not affected, the observed slight reduction in F_v/F_m had to be based on an altered efficiency in NPQ. In conclusion, air exposure of *L. digitata* at low light conditions ($35 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) and a moderate temperature ($\sim 10.5^\circ\text{C}$) resulted most likely in changes in the NPQ efficiency, causing, in turn, a minimal decline in F_v/F_m which was yet sufficient to maintain a high fraction of open reaction centres (q_p). Moreover, the efficiency of primary photosynthesis ($\Delta F/F'_m$) was more pronounced in aerial than in submersed conditions, due to an increase in open reaction centres, whereas energy capture efficiency in the antennae of open reaction centres (F'_v/F'_m) was unaffected. This pronounced performance may have been caused by an increase in the capacity of photosynthetic dark reactions caused by qualitative and/or quantitative changes in inorganic carbon supply (e.g. CO_2).

In conclusion, the sporophyte of *L. digitata* released remarkable amounts of I_2 even under presumed low-stress conditions. The stipe, which exhibited a reduced photosynthetic performance, emitted highest amounts of I_2 , whereas emission rates observed from the meristematic area and the distal blade were lower by one order of magnitude. The temporal profile of I_2 emission indicates the occurrence of an iodine pool which is easily mobilised and becomes depleted upon air exposure. Since iodine is mainly retained as apoplastic I^- in *L. digitata* (Küpper et al. 2008), a respective chemical oxidation is required to generate I_2 . According to Küpper et al. (1998), the intracellular production of hydrogen peroxide seems to be not the major source for the oxidation of apoplastic I^- . In the present study, exogenous oxidative sources, e.g. gaseous ozone or oxidative compounds in the thin water film surrounding the alga particularly during early stages of emersion, were not investigated. Besides the high I_2 emission, air exposure enhanced primary photosynthesis of

all investigated thallus parts under the experimental conditions. Since chlorophyll *a* fluorescence did not detect any potential stress experienced by the alga, it has so far not been possible to establish a direct relationship between I_2 emission and physiological stress. ROS that would cause the retransmission of iodine to the gaseous phase remained below a stressful level at which the efficiency of the PSII would have been inhibited.

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