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ORIGINAL ARTICLE

Iodine contributes to osmotic acclimatisation in the kelp *Laminaria digitata* (Phaeophyceae)

Udo Nitschke · Dagmar B. Stengel

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Abstract Iodide (I^-) retained by the brown macroalga *Laminaria digitata* at millimolar levels, possesses antioxidant activities, but the wider physiological significance of its accumulation remains poorly understood. In its natural habitat in the lower intertidal, *L. digitata* experiences salinity changes and osmotic homeostasis is achieved by regulating the organic osmolyte mannitol. However, I^- may also holds an osmotic function. Here, impacts of hypo- and hypersaline conditions on I^- release from, and accumulation by, *L. digitata* were assessed. Additionally, mannitol accumulation was determined at high salinities, and physiological responses to externally elevated iodine concentrations and salinities were characterised by chl *a* fluorometry. Net I^- release rates increased with decreasing salinity. I^- was accumulated at normal (35 S_A) and high salinities (50 S_A); this coincided with enhanced $rETR_{max}$ and q_P causing pronounced photoprotection capabilities via NPQ. At 50 S_A elevated tissue iodine levels impeded the well-established response of mannitol accumulation and prevented photoinhibition. Contrarily, low tissue iodine levels limited photoprotection capabilities and resulted in photoinhibition at 50 S_A, even though mannitol was accumulated. The results indicate a, so far, undescribed osmotic function of I^- in *L. digitata* and, thus, multifunctional principles of this halogen in kelps. The osmotic function of mannitol may have been substituted by that of I^- under hypersaline conditions, suggesting a complementary role of inorganic and organic solutes under salinity stress. This study also provides first evidence that iodine accumulation in *L. digitata* positively affects photo-physiology.

Keywords Brown macroalga · Chlorophyll fluorescence · Iodine · NPQ · Mannitol · Salinity stress

Abbreviations

chl *a*	Chlorophyll *a*
DW	Dry weight
E_{50}	Irradiance at which 50 % of NPQ_{max} is attained
E_k	Light saturation coefficient of *P*/*E* curve
E_{PAR}	Irradiance in the photosynthetically active wavelength range of 400–700 nm
FW	Fresh weight
F_v/F_m	Maximum PSII efficiency measured after dark acclimation
NPQ	Non-photochemical quenching of chl *a* fluorescence
P/*E* curve	Photosynthesis vs. irradiance curve with rETR as a function of E_{PAR}
PSII	Photosystem II
q_P	Photochemical quenching of chl *a* fluorescence
rETR	Relative electron transport rate through PSII
RGR	Relative growth rate
S_A	Absolute salinity
vIPO	Vanadium-dependent iodoperoxidase

Introduction

The element iodine was discovered from brown macroalgae (Phaeophyceae) (Courtois 1813) some of which have the ability to retain it at high concentrations (Kylin 1929; Saenko et al. 1978). Specifically kelp species within the Laminariales accumulate iodine at levels higher than other

U. Nitschke (✉) · D. B. Stengel
Botany and Plant Science, School of Natural Sciences, and Ryan Institute for Environmental, Marine and Energy Research, National University of Ireland Galway, Galway, Ireland
e-mail: udo.nitschke@nuigalway.ie

brown macroalgae (Kundel et al. 2012). On average, levels of 0.5–1.0 % iodine per dry weight (DW) have been determined in *Laminaria* and *Saccharina* (formerly *Laminaria*) species (Kylin 1929; Saenko et al. 1978; Küpper et al. 1998; Ar Gall et al. 2004), but iodine contents can vary with season (Haug and Jensen 1954; Ar Gall et al. 2004), between populations (Kylin 1929; Haug and Jensen 1954; Ar Gall et al. 2004) and were further dependent on size of specimens (Küpper et al. 1998; Ar Gall et al. 2004).

In brown macroalgae, iodine appeared to be present in both inorganic and organic forms (Kylin 1929); in Laminariales a large fraction (>80 %) occurs as labile inorganic iodide (I^-) ion (Gamallo-Lorenzo et al. 2005; Shah et al. 2005; Küpper et al. 2008, 2013; Hou et al. 2009) which is non-covalently associated with polyols, phenols, amides, protein-like molecules and sulphated polysaccharides (Shah et al. 2005; Verhaeghe et al. 2008; Küpper et al. 2008, 2013). However, the exact speciation and complexation of iodine in algae are still debated (Verhaeghe et al. 2008; Küpper et al. 2011).

Highest iodine concentrations were detected in the outer cortex of *Laminaria* species (Kylin 1929), reaching up to 57 mg g^{-1} DW in stipes of *Laminaria digitata* (Küpper et al. 2013); the inner cortex contained 20 % less iodine (Küpper et al. 2013). Pedersén and Roomans (1983) showed that iodine can be retained intracellularly in physode-like vesicles and, in addition, Verhaeghe et al. (2008) reported that a large fraction can also occur in the apoplasm of *L. digitata*. However, to ascertain the localisation of iodine on sub-cellular levels, current techniques available require further improvement (Verhaeghe et al. 2008).

Iodine is a fundamental element in various biologically driven processes (Leblanc et al. 2006; Küpper et al. 2011) and plays a key role in the oxidative metabolism of Laminariales (Küpper et al. 1998, 2008, 2013). Intact cell walls, small quantities of hydrogen peroxide (H_2O_2) and vanadium haloperoxidase activity, particularly that of iodoperoxidases (vIPO) (Colin et al. 2003, 2005), are required for efficient I^- uptake (Küpper et al. 1998). *L. digitata* also released I^- after a treatment with 2 mM H_2O_2 (Küpper et al. 2008); levels of iodate (IO_3^-) and organic iodine in the surrounding seawater remained nearly unchanged (Küpper et al. 2008). During emersion, *L. digitata* emits molecular iodine (I_2) into air (e.g. Dixneuf et al. 2009; Nitschke et al. 2011; Ashu-Ayem et al. 2012) and ozone (O_3) can trigger elevated I_2 emission rates (Palmer et al. 2005). Thus, iodine functions as inorganic antioxidant in *L. digitata* (Küpper et al. 2008, 2013). Iodinated compounds may also be involved in chemical defence mechanisms (Küpper et al. 2001, 2002) and oxidised halogens might control biofilms via antimicrobial activities (Borchardt et al. 2001). However, the wider physiological advantage and a potential multifunctional role of accumulating iodine at millimolar levels remains largely unexplored (Küpper et al. 1998, 2011).

L. digitata inhabits the physically challenging lower intertidal of temperate rocky shores. Depending on tidal state, this species is either submersed or, during spring low tides, emersed implying considerable variations in several abiotic factors (Davison and Pearson 1996) including the external salinity regime (Karsten 2012). On a small scale, the microlayer of seawater surrounding algal thalli during air-exposure can evaporate which then leads to increased ion concentrations (i.e. increased salinities) on algal surfaces (Kirst 1990). By contrast, precipitation can decrease the external salinity regime resulting in extreme low values (Kirst 1990; Karsten 2012). The combination of precipitation and wave action, causing a rapid re-submersion with full-strength seawater, can further result in large salinity fluctuations.

Seaweeds have evolved pronounced mechanisms to maintain their osmotic homeostasis by controlling concentrations of both inorganic ions ("fast" component of osmotic acclimatisation: hours) and small organic molecules ("slow" component of osmotic acclimatisation: days). In response to cellular water fluxes caused by external osmotic changes, most marine macroalgae adjust the concentration of osmotically active compounds (i.e. osmolytes) to achieve new steady-state conditions (Kirst 1990). As reported to date, the main osmolytes engaged in the "fast" salinity stress response of most seaweeds are monovalent ions such as K^+, Na^+, Cl^- and NO_3^- (Davison and Reed 1985a, b; Kirst 1990); the "slow" response of most Phaeophyceae involves the accumulation the polyol mannitol upon hypersaline conditions (Karsten 2012). Mannitol is generally considered the main metabolically regulated osmolyte in *L. digitata* (Reed et al. 1985).

To date, it is unknown whether I^- is engaged in processes of osmotic acclimatisation in *L. digitata*; however, the large I^- pool may significantly contribute to the cellular osmotic potential. The hypothesis that algal iodine may be associated with, or regulated by, the external salinity regime could be supported by early field studies (Kylin 1929) in which iodine contents in *Laminaria* sp. probably depended on the local salinity regime.

In the present study we hypothesised that accumulated iodine functions as an inorganic osmolyte in *L. digitata*. The physiological significance of iodine in processes of osmotic acclimatisation was investigated by subjecting *L. digitata* to modified external salinity regimes while monitoring I^- release ("fast" component). Iodine and mannitol contents were additionally determined at normal and hypersaline conditions ("slow" component); mannitol concentrations were then related to tissue iodine contents. The physiological performance of *L. digitata* was characterised

by measuring photo-physiological responses (chlorophyll *a* (chl *a*) fluorometry) and growth.

Materials and methods

Algal material and experimental design

Intact sporophytes of *Laminaria digitata* (Hudson) Lamouroux (Phaeophyceae, Heterokontophyta) including holdfasts, stipes and blades were collected on the west coast of Ireland at Finavarra, Co. Clare (53°09′25″N, 09°06′58″W), during spring low tide. All algae sampled were ~1.7 m in length and free of visible epiphytes, grazers and grazing marks.

"Fast" osmotic acclimatisation: I^- release as a function of external salinity

To determine short-term (24 h) impacts of the external salinity on the I^- release of *L. digitata*, punched discs, sampled from meristems (3.0 cm in diameter, 200–300 mg DW), were subjected to salinities of (1) 20 S_A (lowered by dilution with deionised water), (2) 35 S_A (control) or (3) 50 S_A (achieved by adding natural sea salt, Sel de Guérande, Refflets de France, Guérande, France). Prior to use in experiments, algal samples were kept in normal, sterilised seawater (35 S_A) for 20 min to remove potentially leaked I^- ions or oxidising agents. The net release of I^- into seawater (I^- uptake and release were not examined separately) was determined at an irradiance of 50 μmol photons m^{-2} s^{-1} (E_{PAR}: 400–700 nm; provided by cool white fluorescent tubes, General Electric Company, Fairfield, Connecticut, USA) and 10 °C. The light:dark cycle was 12 h:12 h. The culture media used (70 ml) were filtered (0.2 μm; Whatman GmbH, Dassel, Germany) and tyndallised and replaced after 6 h. The experimental exposure was then continued for additional 18 h, i.e. total exposure time was 24 h. I^- concentrations in the surrounding seawater had increased after 6 h and continued to increase over the following 18 h; this indicates that the release of I^- was not caused by wounding as discussed in detail by Nitschke et al. (2013). After 24 h, the maximum photosystem II (PSII) efficiency (F_v/F_m) and the effective PSII quantum efficiency ($\Delta F/F_m'$) were determined on submersed samples to assess physiological including salinity stress (Maxwell and Johnson 2000; Karsten 2007). Samples of media (collected after 6 and 24 h) were stored in polypropylene tubes (Sarstedt AG & Co, Nümbrecht, Germany) in darkness at 4 °C until I^- concentrations were determined; under these storage conditions, I^- concentrations remain stable in seawater for several months (Campos 1997). Net I^- release was corrected against initial I^- concentration in seawater (<0.12 μM), normalised to DW and exposure time, and release rates (calculated from the total amount of I^- released with 24 h) are presented in nmol g^{-1} DW h^{-1}.

"Slow" osmotic acclimatisation: iodine and mannitol accumulation and physiological performance

The potential contribution of iodine to the osmotic adjustment at hypersaline conditions through the accumulation of I^- by *L. digitata* was further investigated over a period of 9 days. Meristematic areas of 10.00 ± 0.04 g fresh weight (FW) were placed into 800 ml of filtered (0.8 μm; Whatman GmbH) and tyndallised seawater of a normal salinity (35 S_A, control) or an increased salinity (50 S_A; by adding the above natural sea salt), either with or without the additional supply of I^- (KI, 99.5 %, Fisher Scientific, Dublin, Ireland) at both salinity regimes. External I^- concentrations were <0.01 μmol g^{-1} FW (where no I^- was added) or 4 μmol g^{-1} FW (where I^- was added); the latter concentration is in accordance with I^- uptake kinetics reported from *L. digitata* by Küpper et al. (1998). The experimental temperature was 16 °C, E_{PAR} was 40 μmol photons m^{-2} s^{-1} (same fluorescent tubes as above) and the light:dark cycle was 12 h:12 h. After 9 days, iodine contents, mannitol concentrations and photosynthetic performance of algal samples were determined. Growth was measured as outlined below. Media were continuously aerated and changed every 24 h to avoid a potential depletion of nutrients and/or I^-.

Iodine concentrations in seawater and algae

Iodine concentrations in natural seawater samples (i.e. I^-) and freeze-dried *L. digitata* (total iodine content in μmol g^{-1} DW) were analysed at the Institut des Sciences Analytiques, Département Service Central d'Analyse, Solaize, France, by ion chromatography (accuracy: 3 %; detection limit: 15 μg l^{-1}). Seawater samples were analysed without pretreatment, algal samples were analysed after applying Schöninger combustion. Currently available techniques do not allow a quantification of iodine in different cell compartments, i.e. the determination of intra- and extracellular iodine remains, to date, difficult (Verhaeghe et al. 2008).

Mannitol content in algae

Mannitol was extracted from freeze-dried (Labconco, Kansas City, Missouri, USA) algal material according to Karsten et al. (1991) with minor modifications. Briefly, ground algal samples (DW) were extracted in 70 % aqueous ethanol (*v*/*v*) (HPLC grade, Fisher Scientific) for 4 h at 70 °C. After centrifugation at 10,000*g* for 10 min, supernatants were evaporated to dryness using a vacuum

evaporator. Dried extracts were reconstituted in deionised water before conducting mannitol analyses.

Mannitol was determined using an isocratic HPLC system (Agilent 1200 Series) coupled with an electrochemical detector (amperometric analytical cell, pulse mode detection, ESA Coulochem III, Dionex Corporation). A gold target electrode was used with palladium as reference electrode. Mannitol was separated on a CarboPac PA100 column (250 × 4.0 mm I.D., 8.5 µm particle size, Dionex Corporation) protected with a guard column (CarboPac PA100, 50 × 4.0 mm I.D., Dionex Corporation) and eluted using 250 mM aqueous NaOH (*v*/*v*) (HPLC grade, Fisher Scientific) at a flow rate of 0.6 ml min^{-1} and a temperature of 22 °C. Mannitol was identified by comparison of retention time with a commercial standard (99.5 %, Sigma-Aldrich) and quantified by peak area. Mannitol concentrations were normalised to DW and are expressed as µmol g^{-1} DW.

Measuring variable chlorophyll *a* fluorescence

In vivo chl *a* fluorescence readings were conducted using a PAM-2000 fluorometer (Heinz Walz GmbH, Effeltrich, Germany) based on the methodology by Schreiber et al. (1986), and described in detail for steady-state light curves in Nitschke et al. (2012). The present study follows the nomenclature recommended by Kromkamp and Forster (2003). Relative electron transport rate through PSII (rETR) was calculated according to Genty et al. (1989), *P*/*E* curves with rETR as a function of E_{PAR} were fitted to the model of Walsby (1997); $rETR_{max}$ and the light saturation coefficient of *P*/*E* curves E_k were determined as outlined by Nitschke et al. (2012).

Photochemical (q_P) and non-photochemical quenching of chl *a* fluorescence (NPQ) were obtained during the measurement of *P*/*E* curves. NPQ as a function of E_{PAR} was fitted to the model of Serôdio and Lavaud (2011) and NPQ_{max} and E_{50} (i.e. the E_{PAR} at which 50 % of NPQ_{max} was attained) were determined after Serôdio and Lavaud (2011).

Relative growth rates of algae

Relative growth rates (RGR) were determined in a non-destructive manner, as change in FW over the course of the experiment (9 days), and were calculated as percentage increase in FW per day (% d^{-1}) (DeBoer et al. 1978).

Statistical analysis

"Fast" processes of osmotic acclimatisation (i.e. 24 h I^- release studies) were carried out in triplicate; all data presented are means and one standard deviation of three replicated samples ($n = 3$). Effect of salinity on the net I^- release rate, F_v/F_m, and $\Delta F/F_m'$ was analysed by applying 1-way ANOVAs.

Experiments investigating "slow" salinity stress responses and the impact of additional I^- supply on *L. digitata* (exposed to normal or increased salinities over 9 days) were carried out using five replicates for each treatment. All data are presented as means and one standard deviation of five replicated samples ($n = 5$). Effect of the salinity-I^--treatment on the total iodine content, mannitol concentration, F_v/F_m, $rETR_{max}$, E_k, NPQ_{max}, E_{50}, and RGR was analysed by applying 1-way ANOVAs.

Regarding all ANOVAs performed, data were normally distributed (Kolmogorov–Smirnov test) and variances were homogenous (Levene test). Tukey tests were used to identify a posteriori homogenous sub-groups, which mean values differ significantly at $P < 0.05$.

Results

"Fast" osmotic acclimatisation: I^- release as a function of external salinity

Within 24 h, the net amount of I^- released into seawater by *L. digitata* was dependent on the external salinity regime ($P = 0.002$). The lower the salinity, the higher the I^- release rate (Fig. 1a). At 20 S_A the I^- release rate was 46.1 nmol g^{-1} DW h^{-1}; this was 2.3 and 3.7 times higher than at 35 S_A (18.0 nmol g^{-1} DW h^{-1}) and 50 S_A (11.4 nmol g^{-1} DW h^{-1}), respectively.

Both F_v/F_m ($P = 0.003$) and $\Delta F/F_m'$ ($P = 0.001$) also depended on the external salinity (Fig. 1b). After 24 h at 35 S_A (control), F_v/F_m was 0.70 and similar to values detected at 50 S_A, but at 20 S_A a significant 22 % decrease in F_v/F_m was observed.

"Slow" osmotic acclimatisation: iodine and mannitol accumulation

Additionally supplied I^- was accumulated by *L. digitata*, resulting in elevated iodine contents (Fig. 2a). When grown at low external I^- concentration for 9 days, tissue iodine contents were 30.9 µmol g^{-1} DW (35 S_A) and 31.2 µmol g^{-1} DW (50 S_A). When additional I^- was provided under either salinity regime, iodine contents had increased by ~112 µmol g^{-1} DW after 9 days.

At 35 S_A and low or high external I^-, mannitol concentrations in *L. digitata* were ~534 µmol g^{-1} DW (Fig. 2b). After exposure to 50 S_A and low external I^-, mannitol contents increased significantly by ~145 µmol g^{-1} DW. Such mannitol accumulation was not observed (at 50 S_A) when additionally supplied I^- was accumulated by *L. digitata* (Fig. 2a, b).

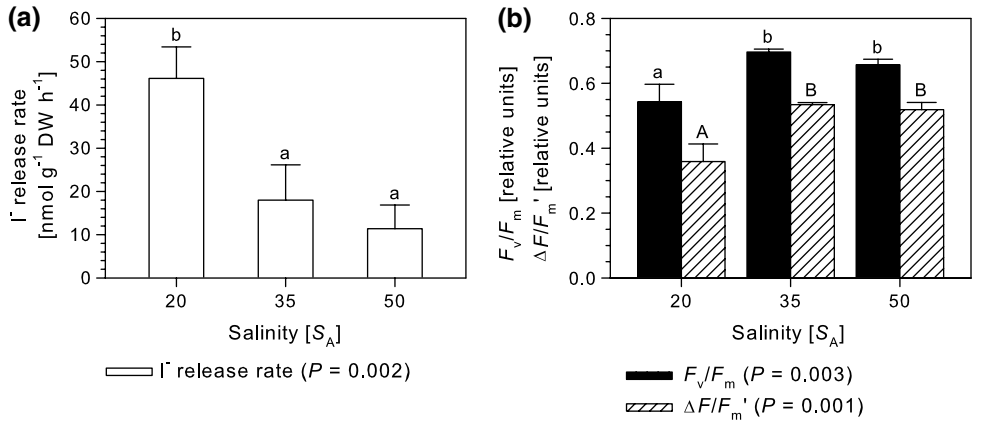

Fig. 1 **a** Net I^- release rates and **b** photosynthetic performance of *L. digitata* subjected to reduced (20 S_A), normal (35 S_A, control), and increased (50 S_A) salinities for 24 h at 50 µmol photons m^{-2} s^{-1} and 10 °C. Net I^- release rates were corrected for the initial I^- concentration (<0.12 µM); cellular uptake and release mechanisms were not investigated separately. The maximum PSII efficiency F_v/F_m and the efficient PSII quantum efficiency $\Delta F/F_m'$ were determined after 24 h; the photoperiod was 12 h. Data are means and one standard deviation (n = 3). Effect of salinity on the net I^- release rate and each photosynthetic parameter was analysed by applying 1-way ANOVAs; P values are given. Different letters indicate significant differences as revealed by Tukey's post hoc tests

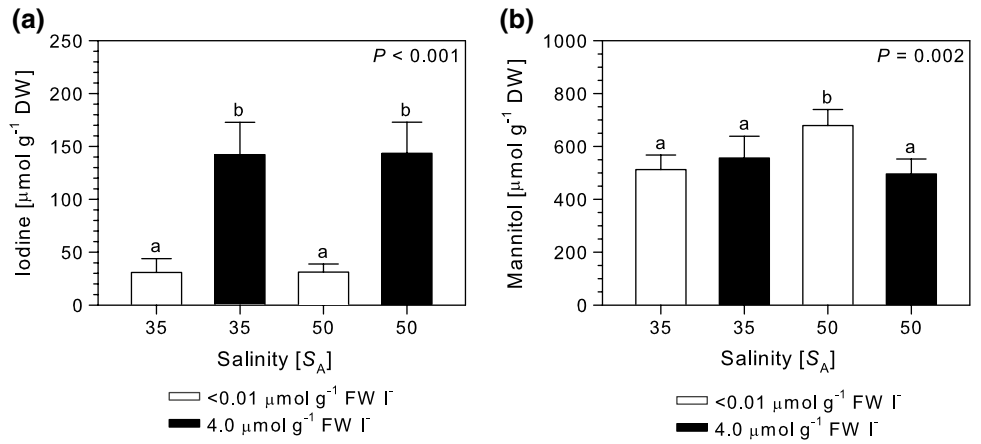

Fig. 2 **a** Total iodine and **b** mannitol content of *L. digitata* subjected to normal (35 S_A) and increased salinities (50 S_A) for 9 days and the effect of additionally provided I^-. Meristematic areas were grown in natural seawater with defined salinities and either without additional I^- supply (i.e. <0.01 µmol g^{-1} FW) (*unfilled bars*), or with additional supply of I^- (4 µmol g^{-1} FW) (*filled bars*) at 16 °C and 40 µmol photons m^{-2} s^{-1}; the photoperiod was 12 h. Data represent means and one standard deviation (n = 5). Effect of the treatment on iodine and mannitol content was analysed by applying 1-way ANOVAs; P values are given. Different letters indicate significant differences as revealed by Tukey's post hoc tests

"Slow" osmotic acclimatisation: physiological performance

After 9 days, the photosynthetic performance of *L. digitata* was impacted by both external I^- and salinity (Table 1). At 35 S_A and low or high external I^-, F_v/F_m was ~0.72; it was significantly reduced by 6 % at 50 S_A, but only when tissue iodine concentrations were low (Fig. 2a). Such a reduction in F_v/F_m was not observed when I^- was additionally present and incorporated at 50 S_A (Table 1).

When grown for 9 days at low external I^- $rETR_{max}$ was ~27, but enhanced by ~24 % at high external I^- levels, regardless of salinity (P = 0.012; Table 1). By contrast, E_k was independent of the treatment (P = 0.142) and remained at ~95 µmol photons m^{-2} s^{-1} under all growth conditions (Table 1).

The addition (and accumulation) of I^- also noticeably elevated q_P (Fig. 3a) and NPQ (Fig. 3b) during the measurement of *P*/*E* curves between 200 and 800 µmol photons m^{-2} s^{-1}, irrespective of the external salinity.

NPQ_{max} was significantly enhanced at high external I^- (Table 2). At both 35 S_A and 50 S_A and low external I^- concentrations, NPQ_{max} was ~4.0 and ~4.4, respectively, but

Table 1 Photosynthetic performance of *L. digitata* grown under normal (35 S_A) and increased salinities (50 S_A) and the effect of additionally provided I^-

Salinity [S_A]	Provided I^- [μmol g^{-1} FW]	F_v/F_m [relative units]	$rETR_{max}$ [relative units]	E_k [μmol photons m^{-2} s^{-1}]
35	<0.01	0.716 ± 0.017^b	28.7 ± 4.7^{ab}	86.0 ± 6.7
	4.0	0.720 ± 0.008^b	35.0 ± 3.4^b	100.6 ± 11.4
50	<0.01	0.677 ± 0.016^a	26.7 ± 1.4^a	92.1 ± 13.2
	4.0	0.713 ± 0.025^b	33.8 ± 4.2^b	100.7 ± 11.6
		$\boldsymbol{P = 0.005}$	$\boldsymbol{P = 0.012}$	$P = 0.142$

Shown are the maximum PSII efficiency (F_v/F_m), the maximum relative electron transport rate through PSII ($rETR_{max}$) and the light saturation coefficient of photosynthesis (E_k); these parameters were determined after a 9-day exposure at 16 °C and 40 μmol photons m^{-2} s^{-1} (E_{PAR}). Data represent means and one standard deviation ($n = 5$). Effect of treatment on each parameter was analysed by applying 1-way ANOVAs; *P* values are given (bold if significant). Different letters indicate significant differences amongst treatments as revealed by Tukey's post hoc tests

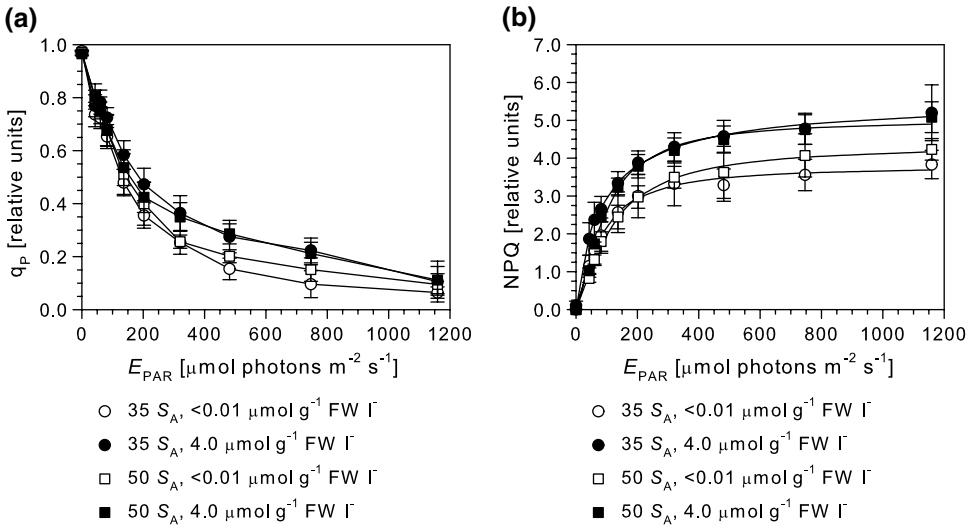

Fig. 3 **a** Photochemical (q_P) and **b** non-photochemical quenching of chl *a* fluorescence (NPQ) as a function of E_{PAR} during the measurement of *P/E* curves in *L. digitata* and the effect of additionally provided I^-. Meristematic areas were grown in natural seawater at 35 S_A (*circles*) or 50 S_A (*squares*) and either without additional I^- supply (i.e. <0.01 μmol g^{-1} FW) (*unfilled symbols*), or with additional supply of I^- (4 μmol g^{-1} FW) (*filled symbols*) for 9 days at 16 °C and 40 μmol photons m^{-2} s^{-1}; the photoperiod was 12 h. Data represent means and one standard deviation ($n = 5$). NPQ vs. E_{PAR} curves were fitted to the model of Serôdio and Lavaud (2011) (*solid lines*), respective parameters (i.e. NPQ_{max} and E_{50}) are shown in Table 2

Table 2 Effect of additionally provided I^- on parameters of non-photochemical quenching of chl *a* fluorescence (NPQ) of *L. digitata* grown under normal (35 S_A) and increased salinities (50 S_A)

Salinity [S_A]	Provided I^- [μmol g^{-1} FW]	NPQ_{max} [relative units]	E_{50} [μmol photons m^{-2} s^{-1}]
35	<0.01	3.96 ± 0.32^a	96.0 ± 40.5
	4.0	5.32 ± 0.48^b	78.3 ± 23.4
50	<0.01	4.41 ± 0.76^{ab}	119.4 ± 21.4
	4.0	5.05 ± 0.46^b	95.5 ± 14.6
		$\boldsymbol{P = 0.004}$	$P = 0.156$

Presented are the maximum NPQ (NPQ_{max}) and E_{50} of NPQ vs. E_{PAR} curves (see Fig. 3b). E_{50} is the irradiance at which 50 % of NPQ_{max} was attained. Parameters were determined after 9 days of exposure at 16 °C and 40 μmol photons m^{-2} s^{-1}. Data represent means and one standard deviation ($n = 5$). Effect of treatment on each parameter was analysed by applying 1-way ANOVAs; *P* values are given (bold if significant). Different letters indicate significant differences amongst treatments as revealed by Tukey's post hoc tests

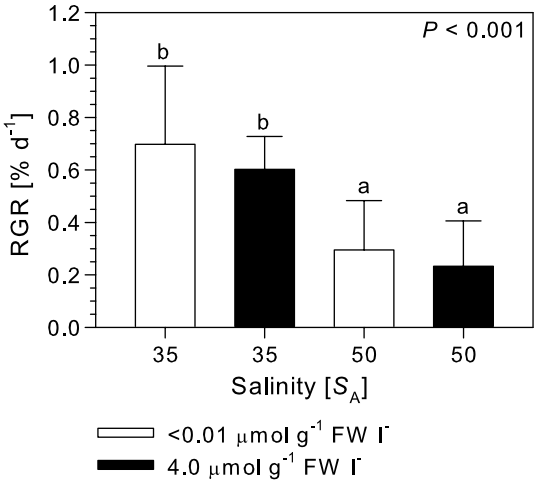

Fig. 4 Relative growth rate (RGR) of *L. digitata* subjected to normal (35 S_A) and increased salinities (50 S_A) for 9 days and the effect of additionally provided I^-. Meristematic areas were grown in natural seawater with defined salinities and either without additional I^- supply (i.e. <0.01 µmol g^{-1} FW) (*unfilled bars*), or with additional supply of I^- (4 µmol g^{-1} FW) (*filled bars*) at 16 °C and 40 µmol photons m^{-2} s^{-1}; the photoperiod was 12 h. Data represent means and one standard deviation ($n = 5$). Effect of the treatment on RGR was analysed by applying a 1-way ANOVA; the *P* values is given. Different letters indicate significant differences as revealed by Tukey's post hoc tests

higher by a factor of ~1.2 when additional I^- was present at either salinity, suggesting that it was the presence (and incorporation) of additional I^- (and not increased salinity) which caused the increase in NPQ_{max}.

The irradiance at which 50 % of NPQ_{max} was attained, i.e. E_{50}, was independent of the treatment ($P = 0.156$), and values were around 100 µmol photons m^{-2} s^{-1} (Table 2).

Growth was inhibited at increased salinities, irrespective of external (and tissue) iodine levels. RGRs of *L. digitata* were ~0.65 % d^{-1} at 35 S_A and reduced by ~60 % at 50 S_A under either I^- treatment (Fig. 4).

Discussion

The strong regulation of the net I^- release from meristems of *L. digitata* (see also Nitschke et al. 2013) by the external salinity has been documented here for the first time; results indicate that the halogen iodine possibly contributes to the adjustment the osmotic potential. The involvement of monovalent ions in the "fast" osmotic acclimatisation (i.e. hours) in Laminariales has been suggested previously (Rosell and Srivastava 1984; Bisson and Kirst 1995), but evidence of salinity affecting I^- release has been established in this study. Generally, hyposaline conditions result in rapid, intracellular-directed water fluxes (Kirst 1990), and the rapid release of osmotically active compounds (Reed and Wright 1986), including inorganic ions (Zimmermann 1978), is a common response of Phaeophyceae to achieve new steady-state conditions (Karsten 2012). The adjustment of internal K^+ and NO_3^- levels upon externally modified salinities has been documented from *L. digitata* (Davison and Reed 1985a). However, the same authors reported that the osmotic acclimatisation via NO_3^- was dependent on internal NO_3^- contents and, thus, suggested the engagement of an anion other than NO_3^- in processes of "fast" osmotic acclimatisation. Although NO_3^- was not measured here, it is probable that I^- acted as the required anion proposed by Davison and Reed (1985a).

I^- release may have occurred as an acclimative mechanism to reduce the osmotic potential at low salinities; I^- ions are retained in large quantities by *L. digitata* (Küpper et al. 2008, 2013) and were possibly present in excess at hyposaline conditions, causing a high osmotic potential with implications for water fluxes and turgor pressure (Zimmermann 1978). However, to date, there is no technique available to determine exactly the osmotic potential in living cells of brown macroalgae. Although I^- was released at lowered salinities, the observed photoinhibition indicated that hyposaline stress was experienced (Karsten 2007). On the other hand, the low I^- release rates at high salinities may suggest that I^- could have been required to maintain osmotic homeostasis at an increased salinity.

The accumulation of additionally supplied I^- modified the to-date established "slow" physiological response of *L. digitata* exposed to high salinities; this further supports that iodine may possess an osmotic function. At hypersaline conditions, most algae minimize inhibitory effects of "aggressive" ions (e.g. Na^+ and Cl^-) on cellular structures and metabolic processes (Gimmler et al. 1984) by synthesising and/or accumulation of organic osmolytes acting as compatible solutes (Kirst 1990); in *L. digitata* mannitol appeared to be the main metabolically regulated osmolyte (Reed et al. 1985). The well-documented mannitol accumulation of *L. digitata* exposed to increased salinities (Davison and Reed 1985a, b) was also observed here, but only at low tissue iodine levels. This mannitol accumulation was, however, insufficient to prevent photoinhibition, indicating (high) salinity stress (Sudhir and Murthy 2004; Karsten 2007).

The osmotic homeostasis in *L. digitata* at high salinities may have been retained by the substitution of I^- with mannitol. For example, at I^- replete conditions, tissue iodine contents were elevated by ~112 µmol g^{-1} DW; under increased salinities, this elevation coincided with the absence of mannitol accumulation. On the other hand, it is clear that processes of osmotic adjustments and the potential substitution of inorganic and organic solutes in *L. digitata* are complex since I^- accumulation also occurred at normal salinities whilst mannitol levels remained high

(i.e. as an opposed reduction). Also, considering the influence of ions on the physical behaviour of macromolecules (Hofmeister 1888), chaotropic ions, such as I^-, can destabilise proteins (Baldwin 1996). However, recent research has highlighted that effects of Hofmeister ions strongly depend on characteristics of the interacting macromolecule (e.g. hydrophobic portions) and/or on, e.g. the pH of the surrounding medium (Zhang and Cremer 2010).

High salinity had physiological implications for *L. digitata* since growth rates were reduced, regardless of tissue iodine contents. Growth integrates (abiotic) stress at multiple cellular levels over a prolonged period. For example, the light-independent reactions of photosynthesis could be impaired at increased salinities (Sudhir and Murthy 2004), although here both inorganic (I^-) and organic (mannitol) solutes appeared to be involved in osmotic adjustment. Growth rates obtained in this study were also ~10 times lower than those reported from young sporophytes (Roleda et al. 2006a, b); this can probably attributed to age differences and the strong seasonality in growth patterns of *Laminaria* species (Bartsch et al. 2008).

I^- accumulation could be an energetically efficient mechanism at high salinity stress; Erdmann and Hagemann (2001) reported that 57 ATP equivalents are required to generate 1 mol mannitol and, thus, its accumulation is energetically expensive. Contrarily, uptake of I^- is independent of direct ATP expenditure (Küpper et al. 1998).

At a cellular level, I^- could act as a competitive ion for Cl^- and as a counter ion for monovalent cations, such as K^+ or Na^+, in *L. digitata*. Previously, Verhaeghe et al. (2008) showed an inverse relationship between I^- and Cl^- concentrations in blades and stipes of *L. digitata*, i.e. high I^- contents in the outer cortex coincided with low Cl^- concentrations, and vice versa.

The concept that algal I^- is regulated by the external salinity regime in *L. digitata* is further in agreement with previous field observations. Kylin (1929) reported that *Laminaria* sp. collected from low salinity waters on the Swedish west coast (~27 S_A) contained iodine at lower levels than those from the North Sea and the Atlantic (35 S_A). Also, Davison and Reed (1985a) reported anion deficits from *L. digitata* to maintain osmotic homeostasis in autumn, and Ar Gall et al. (2004) observed higher iodine contents in *L. digitata* in autumn than during other seasons.

To our knowledge, this study is the first record of an enhanced photo-physiological performance of *L. digitata* due to I^- replete conditions since, regardless of salinity, $rETR_{max}$ and q_P were elevated. Higher $rETR_{max}$ resulted, furthermore, in greater photoprotective capabilities (as NPQ), which were sufficient to maintain a larger fraction of "open" PSII reaction centres (q_P). This physiological advantage was of particular importance under high salinity stress as it prevented photoinhibition. Wieners et al. (2012) also reported positive effects of externally elevated I^- concentrations since exposure of green algal photobionts, isolated from terrestrial lichens and cultured in seawater, to I^--enriched seawater prior to desiccation prevented photoinhibition after a high light treatment. The external supply of iodine (as IO_3^-) to vascular plants (*Lactuca sativa* cv. Philipus) grown under 100 mM NaCl activated antioxidant enzymes, which then kept levels of reactive oxygen species minimal (Leyva et al. 2011), stimulated the synthesis of phenolic compounds and improved plant growth (Blasco et al. 2013). A direct comparison of marine brown macroalgae and vascular plants remains, however, difficult since members of the two evolutionary distinct entities can exhibit large physiological differences due to adaptation to their natural habitat (Cock et al. 2010). In addition, I_2 emission studies on *L. digitata* exposed to air under presumed low-stress conditions did not reveal a direct link between iodine metabolism and chl *a* fluorescence responses (Nitschke et al. 2011), but abiotic stress conditions applied were possibly not severe enough to be reflected in chl *a* fluorescence parameters (Eggert et al. 2007; Nitschke et al. 2010, 2013).

In conclusion, the presented results suggest that iodine can contribute to processes of osmotic acclimatisation in *L. digitata*. I^- may act as an osmolyte in this species; such physiological function has not been described previously. Retaining I^- at millimolar levels is unique to *L. digitata* and provides this species with an enhanced photo-physiological performance which is of importance during (high) salinity stress. Under hypersaline conditions high iodine tissue contents seemed to be sufficient to maintain osmotic homeostasis since the energetically expensive mannitol accumulation was not initiated. The beneficial effects of iodine with regard to salinity stress response, tolerance and PSII functioning should be investigated in greater detail not only in iodine-accumulating algae, but also in vascular plants.

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References

Ar Gall E, Küpper FC, Kloareg B (2004) A survey of iodine content in *Laminaria digitata*. Bot Mar 47:30–37

Ashu-Ayem ER, Nitschke U, Monahan C, Chen J, Darby SB, Smith PD, O'Dowd CD, Stengel DB, Venables SD (2012) Coastal

iodine emissions. 1. Release of I_2 by *Laminaria digitata* in chamber experiments. Environ Sci Technol 46:10413–10421

Baldwin RL (1996) How Hofmeister ion interactions affect protein stability. Biophys J 71:2056–2063

Bartsch I, Wiencke C, Bischof K, Buchholz C, Buck BH, Eggert A, Feuerpfeil P, Hanelt D, Jacobsen S, Karez R, Karsten U, Molis M, Roleda MY, Schumann R, Schubert H, Valentin KU, Weinberger F, Wiese J (2008) The genus *Laminaria* sensu lato: recent insights and developments. Eur J Phycol 43:1–86

Bisson MA, Kirst GO (1995) Osmotic acclimation and turgor pressure regulation in algae. Naturwissenschaften 82:461–471

Blasco B, Leyva R, Romero L, Ruiz JM (2013) Iodine effects on phenolic metabolism in lettuce plants under salt stress. J Agric Food Chem 61:2591–2596

Borchardt SA, Allain EJ, Michels JJ, Stearns GW, Kelly RF, McCoy WF (2001) Reaction of acylated homoserine lactone bacterial signaling molecules with oxidized halogen antimicrobials. Appl Environ Microbiol 67:3174–3179

Campos MLAM (1997) New approach to evaluating dissolved iodine speciation in natural waters using cathodic stripping voltammetry and a storage study for preserving iodine species. Mar Chem 57:107–117

Cock JM, Sterck L, Rouzé P, Scornet D, Allen AE, Amoutzias G, Anthouard V, Artiguenave F, Aury JM, Badger JH, Beszteri B, Billiau K, Bonnet E, Bothwell JH, Bowler C, Boyen C, Brownlee C, Carrano CJ, Charrier B, Cho GY, Coelho SM, Collén J, Corre E, Da Silva C, Delage L, Delaroque N, Dittami SM, Doulbeau S, Elias M, Farnham G, Gachon CMM, Gschloessl B, Heesch S, Jabbari K, Jubin C, Kawai H, Kimura K, Kloareg B, Küpper FC, Lang D, Le Bail A, Leblanc C, Lerouge P, Lohr M, Lopez PJ, Martens C, Maumus F, Michel G, Miranda-Saavedra D, Morales J, Moreau H, Motomura T, Nagasato C, Napoli CA, Nelson DR, Nyvall-Collen P, Peters AF, Pommier C, Potin P, Poulain J, Quesneville H, Read B, Rensing SA, Ritter A, Rousvoal S, Samanta M, Samson G, Schroeder DC, Ségurens B, Strittmatter M, Tonon T, Tregear JW, Valentin K, von Dassow P, Yamagishi T, Van de Peer Y, Wincker P (2010) The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. Nature 465:617–621

Colin C, Leblanc C, Wagner E, Delage L, Leize-Wagner E, van Dorsselaer A, Kloareg B, Potin P (2003) The brown algal kelp *Laminaria digitata* features distinct bromoperoxidase and iodoperoxidase activities. J Biol Chem 278:23545–23552

Colin C, Leblanc C, Michel G, Wagner E, Leize-Wagner E, van Dorsselaer A, Potin P (2005) Vanadium-dependent iodoperoxidases in *Laminaria digitata*, a novel biochemical function diverging from brown algal bromoperoxidases. J Biol Inorg Chem 10:156–166

Courtois B (1813) Découverte d'une substance nouvelle dans le Vareck. Ann Chim 88:304–310

Davison IR, Pearson GA (1996) Stress tolerance in intertidal seaweeds. J Phycol 32:197–211

Davison IR, Reed RH (1985a) Osmotic adjustment in *Laminaria digitata* (Phaeophyta) with particular reference to seasonal changes in internal solute concentrations. J Phycol 21:41–50

Davison IR, Reed RH (1985b) The physiological significance of mannitol accumulation in brown algae: the role of mannitol as a compatible cytoplasmic solute. Phycologia 24:449–457

DeBoer JA, Guigli HJ, Israel TL, D'Elia CF (1978) Nutritional studies of two red algae. I. Growth rate as a function of nitrogen source and concentration. J Phycol 14:261–266

Dixneuf S, Ruth AA, Vaughan S, Varma RM, Orphal J (2009) The time dependence of molecular iodine emission from *Laminaria digitata*. Atmos Chem Phys 9:823–829

Eggert A, Nitschke U, West JA, Michalik D, Karsten U (2007) Acclimation of the intertidal red alga *Bangiopsis subsimplex* (Stylonematophyceae) to salinity changes. J Exp Mar Biol Ecol 343:176–186

Erdmann N, Hagemann M (2001) Salt acclimation of algae and cyanobacteria: a comparison. In: Rai LC, Gaur JP (eds) Algal adaptation to environmental stresses. Springer, Heidelberg, pp 323–362

Gamallo-Lorenzo D, Barciela-Alonso MC, Moreda-Piñeiro A, Bermejo-Barrer A, Bermejo-Barrera P (2005) Microwave-assisted alkaline digestion combined with microwave-assisted distillation for the determination of iodide and total iodine in edible seaweed by catalytic spectrophotometry. Anal Chim Acta 542:287–295

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87–92

Gimmler H, Kaaden R, Kirchner U, Weyand A (1984) The chloride sensitivity of *Dunaliella parva* enzymes. Z Pflanzenphysiol 114:131–150

Haug A, Jensen A (1954) Seasonal variations in the chemical composition of *Alaria esculenta*, *Laminaria saccharina*, *Laminaria hyperborea* and *Laminaria digitata* from northern Norway. Technical Report 84, Norwegian Institute of Seaweed Research

Hofmeister F (1888) Zur Lehre von der Wirkung der Salze. Arch Exp Pathol Pharmakol 24:247–260

Hou X, Hansena V, Aldahanb A, Possnert G, Lindd OC, Lujaniene G (2009) A review on speciation of iodine-129 in the environmental and biological samples. Anal Chim Acta 632:181–196

Karsten U (2007) Salinity tolerance of Arctic kelps from Spitsbergen. Phycol Res 55:257–262

Karsten U (2012) Seaweed acclimation to salinity and desiccation stress. In: Wiencke C, Bischof K (eds) Seaweed biology: novel insights into ecophysiology, ecology and utilizations. Springer, Berlin, pp 87–107

Karsten U, Thomas DN, Weykam G, Daniel C, Kirst GOA (1991) A simple and rapid method for extraction and separation of low molecular weight carbohydrates from macroalgae using high-performance liquid chromatography. Plant Physiol Biochem 29:373–378

Kirst GO (1990) Salinity tolerance of eukaryotic marine algae. Annu Rev Plant Physiol Plant Mol Biol 40:21–53

Kromkamp JC, Forster RM (2003) The use of variable fluorescence measurements in aquatic ecosystems: differences between multiple and single turnover measuring protocols and suggested terminology. Eur J Phycol 38:103–112

Kundel M, Thorenz UR, Petersen JH, Huang R-J, Bings NH, Hoffmann T (2012) Application of mass spectrometric techniques for the trace analysis of short-lived iodine-containing volatiles emitted by seaweed. Anal Bioanal Chem 402:3345–3357

Küpper FC, Schweigert N, Ar Gall E, Legendre JM, Vilter H, Kloareg B (1998) Iodine uptake in Laminariales involves extracellular, haloperoxidase-mediated oxidation of iodide. Planta 207:163–171

Küpper FC, Kloareg B, Guern J, Potin P (2001) Oligoguluronates elicit an oxidative burst in the brown algal kelp *Laminaria digitata*. Plant Physiol 125:278–291

Küpper FC, Müller DG, Peters AF, Kloareg B, Potin P (2002) Oligoalginate recognition and oxidative burst play a key role in natural and induced resistance of sporophytes of Laminariales. J Chem Ecol 28:2057–2081

Küpper FC, Carpenter LC, McFiggans GB, Palmer CJ, Waite TJ, Boneberg E-M, Woitsch S, Weiller M, Abela R, Grolimund D, Potin P, Butler A, Luther GW III, Kroneck PMH, Meyer-Klaucke W, Feiters MC (2008) Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry. Proc Natl Acad Sci USA 105:6954–6958

Küpper FC, Feiters MC, Olofsson B, Kaiho T, Yanagida S, Zimmermann MB, Carpenter LJ, Luther GW III, Lu Z, Jonsson M, Kloo L (2011) Commemorating two centuries of iodine research: an interdisciplinary overview of current research. Angew Chem Int Ed 50:11598–11620

Küpper FC, Carpenter LC, Leblanc C, Toyama C, Uchida Y, Maskrey BH, Robinson J, Verhaeghe EF, Malin G, Luther GW III, Kroneck PMH, Kloareg B, Meyer-Klaucke W, Muramatsu Y, Megson IL, Potin P, Feiters MC (2013) In vivo speciation studies and antioxidant properties of bromine in *Laminaria digitata* reinforce the significance of iodine accumulation for kelps. J Exp Bot 64:2653–2664

Kylin H (1929) Über das Vorkommen von Jodiden, Bromiden und Jodidoxidasen bei den Meeresalgen. Hoppe-Seylor's Z Physiol Chem 186:50–84

Leblanc C, Colin C, Cosse A, Delage L, La Barre S, Morin P, Fiévet B, Voiseux C, Ambroise Y, Verhaeghe E, Amouroux D, Donard O, Tessier E, Potin P (2006) Iodine transfers in the coastal marine environment: the key role of brown algae and of their vanadium-dependent haloperoxidases. Biochimie 88:1773–1785

Leyva R, Sánchez-Rodríguez E, Ríos JJ, Rubio-Wilhelmi MM, Romero L, Ruiz JM, Blasco B (2011) Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. Plant Sci 181:195–202

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence: a practical guide. J Exp Bot 51:659–668

Nitschke U, Boedeker C, Karsten U, Hepperle D, Eggert A (2010) Does the lack of mannitol accumulation in an isolate of *Rhodella maculata* (Rhodellophyceae, Rhodophyta) from the brackish Baltic Sea indicate a stressed population at the distribution limit? Eur J Phycol 45:436–449

Nitschke U, Ruth AA, Dixneuf S, Stengel DB (2011) Molecular iodine emission rates and photosynthetic performance of different thallus parts of *Laminaria digitata* (Phaeophyceae) during emersion. Planta 233:737–748

Nitschke U, Connan S, Stengel DB (2012) Chlorophyll *a* fluorescence responses of temperate Phaeophyceae under submersion and emersion regimes: a comparison of rapid and steady-state light curves. Photosynth Res 114:29–42

Nitschke U, Dixneuf S, Ruth AA, Schmid M, Stengel DB (2013) Molecular iodine (I_2) emission from two *Laminaria* species (Phaeophyceae) and impact of irradiance and temperature on I_2 emission into air and iodide release into seawater from *Laminaria digitata*. Mar Environ Res 92:102–109

Palmer CJ, Anders TL, Carpenter LJ, Küpper FC, McFiggans GB (2005) Iodine and halocarbon response of *Laminaria digitata* to oxidative stress and links to atmospheric new particle production. Environ Chem 2:282–290

Pedersén M, Roomans GM (1983) Ultrastructural localization of bromine and iodine in the stipes of *Laminaria digitata* (Huds.) Lamour., *Laminaria saccharina* (L.) Lamour. and *Laminaria hyperborea* (Gunn.) Fosl. Bot Mar 26:113–118

Reed RH, Wright PJ (1986) Release of mannitol from *Pilayella littoralis* (Phaeophyta: Ectocarpales) in response to hypoosmotic stress. Mar Ecol Prog Ser 29:205–208

Reed RH, Davison IR, Chudek JA, Foster R (1985) The osmotic role of mannitol in the Phaeophyta: an appraisal. Phycologia 24:35–47

Roleda MY, Hanelt D, Wiencke C (2006a) Growth and DNA damage in young *Laminaria* sporophytes exposed to ultraviolet radiation: implication for depth zonation of kelps on Helgoland (North Sea). Mar Biol 148:1201–1211

Roleda MY, Wiencke C, Hanelt D (2006b) Thallus morphology and optical characteristics affect growth and DNA damage by UV radiation in juvenile Arctic *Laminaria* sporophytes. Planta 223:407–417

Rosell K-G, Srivastava LM (1984) Binding of inorganic elements to kelp residues. Hydrobiologia 116:505–509

Saenko GN, Kravtsova YY, Ivanenko VV, Sheludko SI (1978) Concentration of iodine and bromine by plants in seas of Japan and Okhotsk. Mar Biol 47:243–250

Schreiber U, Schliwa U, Bilger W (1986) Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth Res 10:51–62

Serôdio J, Lavaud J (2011) A model for describing the light response of the nonphotochemical quenching of chlorophyll fluorescence. Photosynth Res 108:61–76

Shah M, Wuilloud RG, Kannamkumaratha SS, Caruso JA (2005) Iodine speciation studies in commercially available seaweed by coupling different chromatographic techniques with UV and ICP-MS detection. J Anal At Spectrom 20:176–182

Sudhir P, Murthy SDS (2004) Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42:481–486

Verhaeghe EF, Fraysse A, Guerquin-Kern J-L, Wu T-D, Devés G, Mioskowski C, Leblanc C, Ortega R, Ambroise Y, Potin P (2008) Microchemical imaging of iodine distribution in the brown alga *Laminaria digitata* suggests a new mechanism for its accumulation. J Biol Inorg Chem 13:257–269

Walsby A (1997) Modelling the daily integral of photosynthesis by phytoplankton: its dependence on the mean depth of the population. Hydrobiologia 349:65–74

Wieners PC, Mudimu O, Bilger W (2012) Desiccation-induced nonradiative dissipation in isolated green lichen algae. Photosynth Res 113:239–247

Zhang Y, Cremer PS (2010) Chemistry of Hofmeister anions and osmolytes. Annu Rev Phys Chem 61:63–83

Zimmermann U (1978) Physics of turgor- and osmoregulation. Ann Rev Plant Physiol 29:121–148