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Effects of reduced salinity on the photosynthetic characteristics and intracellular DMSP concentrations of the red coralline alga, *Lithothamnion glaciale*

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Abstract Mid- to high-latitude fjordic coastal environments experience naturally variable salinity regimes. Climate projections suggest that freshwater input into the coastal ocean will increase in the future, exposing coastal organisms to further periods of reduced salinity. This study investigated the effect of low salinity on *Lithothamnion glaciale*, a red coralline alga found in mid- to high-latitude fjordic regions, during a 21-day experiment. Specific measurements included: the intracellular concentration of dimethylsulphoniopropionate (DMSP, an algal secondary metabolite and major precursor to the climatically active gas dimethylsulphide), pigment composition and photosynthetic characteristics. No significant difference in intracellular DMSP concentrations was observed between treatments, suggesting that the primary function for DMSP in *L. glaciale* is not as a compatible solute, perhaps favouring an antioxidant role. Photosynthetic parameters (including pigment composition) exhibited a mixed response, suggesting some degree of photosynthetic resilience to reduced salinity. This study provides evidence of intracellular mechanisms adopted by *L. glaciale* in response to reduced salinity. This has significant implications for the survival of *L.* *glaciale* under a projected freshening scenario and provides organism-level detail to ecosystem-level projected changes should lower-salinity conditions become more frequent and more intense in the future.

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Introduction

Atmospheric CO_2 concentrations have increased from ~280 parts per million (ppm) before the Industrial Revolution (IPCC 2013) to current levels of >395 ppm (NOAA 2014). Arguably, the higher levels of CO_2 have already led to measurable changes in atmospheric processes such as elevated temperature (IPCC 2013) and increased storm activity (Mann and Emanuel 2006). A 270 % net increase in precipitation-derived freshwater input into the North Atlantic was observed during the 1960–1990s (Josey and Marsh 2005; Bindoff et al. 2007), stimulating a debate into the future evolution of marine salinity given atmospheric CO_2 projections. By the year 2100, atmospheric temperature is projected to rise by up to 6 °C (IPCC 2013), increasing the 'moisture-holding capacity' of the atmosphere (Trenberth et al. 2007), and thus the potential for precipitation events, enhancing freshwater run-off into the coastal zone (Gillibrand et al. 2005), particularly in the mid/high latitudes (IPCC 2013). A rise in atmospheric temperatures may also lead to more pronounced seasonal ice melt (Hanna et al. 2008); run-off from the Greenland Ice Sheet into the Kangerlussaq drainage basin has increased by 113 km^3 over the last 50 years (Hanna et al. 2008). Coralline algal climate proxies from Søndre Strømfjord, western Greenland, have shown that this has resulted in a reduction in average coastal salinity by ~5 units (Kamenos et al. 2012). Fjordic landscapes are also typically characterised by a variable salinity regime: Søndre Strømfjord has a freshwater-influenced

surface layer in the upper 50–75 m of the water column; the salinity of the upper 20 m is <10 in late summer, because of ice melt (Nielsen et al. 2010). Similarly, in Loch Linnhe (western Scotland, 56.5°N), a low-salinity (~11) layer penetrates to ~10 m depth during the winter, increasing in depth through the spring as freshwater riverine discharge increases (Allen and Simpson 1998).

Non-geniculate red coralline algae (Rhodophyta: Corallinales) are widespread throughout the world's coastal oceans, from the intertidal zone to >250 m depth (Foster 2001). These algae can grow as individual, unattached thalli known as maerl or rhodoliths, or may form an encrusting layer on bed rock (Foster 2001). Red coralline algae are ecologically (Kamenos et al. 2004a, b, c) and structurally (Tierney and Johnson 2012) important components of many coastal habitats and are often used as high-resolution palaeoclimatic proxies (Kamenos et al. 2008a; Kamenos 2010; Burdett et al. 2011). *Lithothamnion glaciale*, a key ecosystem engineer for these habitats, is found in mid- to high-latitude fjordic shallow-water environments (from 55 to 80°N, <20 m depth) (Foster 2001; Teichert et al. 2012) and thus is likely to have developed intracellular mechanisms to cope with changing osmotic conditions.

Thus, the survival of coralline algal habitats in variable coastal environments depends, in part, on an organisms' response to periodic exposure to reduced salinity conditions. One possible mechanism may be through the production and regulation of compounds which can act as osmoprotectants (compatible solutes), such as dimethylsulphoniopropionate (DMSP) (Kirst 1996). DMSP can be produced by a range of marine algae and has been proposed to serve numerous cellular functions within algal cells, such as a compatible solute (Kirst 1996), an antioxidant (Sunda et al. 2002), a cryoprotectant (Karsten et al. 1996) and a grazing deterrent (Van Alstyne et al. 2001). The majority of studies investigating DMSP's proposed compatible solute function have focussed on hyper-salinity (resulting in increased intracellular DMSP concentrations, e.g. Karsten et al. 1992). However, in mid- to high-latitude coastal systems, a hypo-salinity scenario is more ecologically relevant because of ice melt and land run-off. In general, it is thought that DMSP is regulated in response to longer-term, chronic changes in salinity (Edwards et al. 1988; Kirst 1989), rather than as a short-term 'stress' response, perhaps due to the energy outlay required to instigate changes in intracellular DMSP concentrations (Yoch 2002). In support of this, short-term hypo-salinity conditions do not appear to result in significant declines in intracellular DMSP in the macroalga *Ulva lactuca* (Van Alstyne et al. 2003; Ross and Alstyne 2007). Red coralline algae typically maintain high intracellular DMSP concentrations (Kamenos et al. 2008b). The effect of reduced salinity on intracellular DMSP concentrations in these organisms has not yet been investigated, but previous studies have shown a response to periodic changes in other environmental variables such as light (Rix et al. 2012; Burdett et al. 2014) or pCO_2 (Burdett et al. 2012a, 2013). Regulation of intracellular DMSP concentrations may also depend on photosynthetic activity, as the precursor to DMSP, methionine, is an indirect product of photosynthesis (Wirtz and Droux 2005) and is an essential component of photosynthetic proteins such as RuBisCO and the D1-protein (Wirtz and Droux 2005).

Reductions in salinity can also have major implications for photosynthesis, particularly in the red algae (Larsen and Sand-Jensen 2006). The photosynthetic efficiency (F_v/F_m) of *L. glaciale* was reduced after 5-week exposure to a salinity of 3 (Wilson et al. 2004); however, prolonged periods at such low salinities are unlikely to regularly occur in situ. In brown algae, a reduction in photosynthetic efficiency has been observed in the microscopic life stages (zoospores) of the Arctic kelp *Alaria esculenta*, but not during adult life stages when exposed to a salinity of 20 (Fredersdorf et al. 2009). However, following prolonged exposure to low-salinity conditions, higher tolerance thresholds may develop. Optimal salinities for maximum electron transport rate and relative growth rate were higher in *Fucus vesiculosus* from Ireland (ambient salinity of 35, optimal salinity = 20–35), when compared to the same species in the Baltic Sea (ambient salinity of 5, optimal salinity = 10–20) (Nygård and Dring 2008). The physiology of the red alga *Gelidium coulteri* appeared to at least partially recover after a 5-week exposure to a low-salinity environment, despite initial decreases in photosynthetic parameters and initial increases in respiration (Macler 1988).

This study investigated the impact of reduced salinity on the intracellular DMSP concentration, pigment composition and photosynthetic characteristics of *L. glaciale*. It was hypothesised that, following prolonged exposure to reduced salinity conditions, DMSP concentrations would decrease (supporting the proposed compatible solute function) and photosynthetic characteristics would not ultimately be affected (indicating a degree of tolerance).

Materials and methods

Specimen collection

Free-living *Lithothamnion glaciale* thalli were collected from Loch Sween on the west coast of Scotland, UK (56°01.99′N, 05°36.13′W), in the summer of 2011 using SCUBA from a depth of 5 m. The west coast of Scotland is characterised by a typical post-glacial landscape, with steep valleys, thin soils and narrow fjords. Thalli were transported to the University of Glasgow in seawater at ambient temperature (12 °C), salinity (32) and light (40 µmol

photons m^{-2} s^{-1}). Thalli were transferred to 120-litre (0.80 × 0.35 × 0.40 m) re-circulating seawater tanks also maintained at ambient conditions of temperature (12 °C), light (40 µmol photons m^{-2} s^{-1}, 16:8 h light: dark cycle) and salinity (32). Thalli were acclimated to laboratory conditions for 10 days before the experiment began. Light and water temperature followed natural field conditions as they would otherwise be additional confounding factors within the experiment.

Experimental set-up

Three salinity treatments were used to assess the effect of chronic reductions in salinity: control (salinity = 32.1 ± 1.1, mean ± SD), low (21.5 ± 0.6, representative of precipitation run-off into a fjord; Allen and Simpson 1998) and very low (11.7 ± 0.9, representative of late summer ice melt into a fjord; Nielsen et al. 2010). A nested experimental design was adopted: three tanks (120 l volume) were used per treatment, each containing 50 thalli. Individuals were sampled at only one timepoint; thus, each timepoint is composed of samples independent from other timepoints. As with the acclimation, light (40 µmol photons m^{-2} s^{-1}, 16:8 h light: dark cycle) and water temperature (12 °C) followed natural field conditions.

Water changes (25 %) were performed every 2 days throughout the experimental period to maintain water quality, and a constant water flow was maintained (circulation rate: 450 l h^{-1}). Seawater was made from artificial sea salt (TropicMarin Pro Reef sea salt) according to manufacturer's instructions. In the low-salinity treatments, the amount of salt in the water stock for water changes was reduced accordingly. Salinity in the treatment groups was gradually reduced during the first 7 days and maintained at the treatment level for another 14 days (total experimental period: 21 days). The salinity of the treatment tanks and water stock for water changes was monitored using a YSI Pro2030 conductivity probe (temperature compensated). This method of salinity reduction may also have reduced the level of nutrients in the treatment aquaria.

Intracellular DMSP

Algal branches were sampled for intracellular DMSP at 0, 3, 7, 14 and 21 days. Ten branches from 10 individuals were sampled from each treatment tank at each timepoint, providing 30 branches per treatment, per timepoint. Each branch was gently cleaned with a soft brush before storage in 2 ml of 10 M sodium hydroxide in 14-ml glass vials. Vials were immediately crimped shut with gas-tight Pharma-Fix septa (Grace Alltech) to hydrolyse cellular DMSP to the gas dimethylsulphide. This method may yield DMS from other tertiary sulphonium compounds (e.g.

Gage et al. 1997), but it is widely assumed that DMSP is the primary source of DMS (Van Alstyne and Puglisi 2007). Samples were incubated in the dark for 48 h before analysis. Intracellular DMSP (as dimethylsulphide) was quantified using gas chromatography (Shimadzu GC-2014 gas chromatograph) equipped with a flame photometric detector (200 °C, hydrogen gas pressure: 5.1 psi, air gas pressure 15.2 psi) and capillary column (5 % diphenyl–95 % dimethyl polysiloxane; length 25 m; inner diameter 0.25 mm; film thickness 0.25 µm, 45 °C). Samples were analysed by direct injection of the vial headspace (100 µl) into the GC (injector temperature: 45 °C; nitrogen carrier gas; total flow: 38.6 ml min^{-1}). Concentrations were calibrated against DMSP standards (DMSP obtained from Research Plus Inc., Barnegat, USA). The standard and sample detection limit was 30 nmol of sulphur per injection; sample and standard precision was within 1 %. Results are presented as µM DMSP g^{-1} biomass to aid in comparison with fleshy macroalgae; the biomass of *L. glaciale* is ~3.50 % of the total fresh mass of the thallus (Burdett et al. 2012a).

Pigment composition

The reflectance spectra of *L. glaciale* branches ($n = 10$ per treatment, randomly chosen from each of the three replicate treatment tanks) were measured at the beginning (day 0) and again end of the experiment (day 21, same individuals, but different branches to minimise repeat sampling errors) using a USB 2000+ Ocean Optics spectrometer following the protocol outlined in Burdett et al. (2014). Light (Arcadia T5 Marine White, 24 W) was directed at the algal branch via a 5-mm fibre optic probe (Walz GmbH, Effeltrich, Germany). Reflected light was transmitted to the spectrometer via a 400-µm single-fibre optic probe (Ocean Optics). Due to the small diameter and nonlinearity of *L. glaciale* branches, it was logistically difficult to maintain a fixed angle between the two fibre optic probes. Thus, for each sample, the probes were positioned to achieve maximum reflectance output based on the real-time spectrometer trace (Burdett et al. 2014). Percentage reflectance was calculated by comparison with a white standard (0 % absorbance across the whole spectra). The wavelengths of pigment absorbance were obtained from Hedley and Mumby (2002).

Photosynthetic characteristics

Chlorophyll-*a* fluorescence measurements were conducted using a Diving-PAM fluorometer (Walz GmbH) and used to calculate photosynthetic characteristics of the algal thalli. Measurements were taken following the methodology outlined, and notation described, in Burdett et al. (2012b). A 5-mm-diameter fibre optic probe was used for all measurements, positioned 10 mm from branch tips. This approach

maximises the signal-to-noise ratio and provides fluorescence data integrated over the whole branch length; along-branch heterogeneity has previously been observed in *L. glaciale* (Burdett et al. 2012b).

Rapid light curves (RLCs)

Rapid light curves (RLCs), where organisms are exposed to pulses of saturating actinic light interspersed with 10–20 s of increasing levels of irradiance, have become well established within PAM fluorometry (Ralph and Gademann 2005). RLCs provide information on energy dissipation from light-limiting through to light-saturating conditions. However, due to the short exposure time at each irradiance step, steady-state conditions are not achieved during RLCs (Ralph and Gademann 2005). Thus, in contrast to traditional light curves, results from RLCs reflect actual, rather than optimal, photosynthetic state (Ralph and Gademann 2005).

RLCs ($n = 15$ per timepoint, per treatment) were conducted at 0, 3, 7, 14 and 21 days using eight irradiance steps ranging from 2 to 997 µmol photons m^{-2} s^{-1}. Thalli were dark acclimated for 5 min in the experimental tanks prior to running the RLCs, which is sufficient time to induce full dark acclimation in *L. glaciale* (Burdett et al. 2012b). Each RLC produced a series of effective quantum efficiency measurements $\left(F_q'/F_m'\right)$ that were fitted to a non-linear least squares regression model to describe the light response of quantum efficiency (Hennige et al. 2008; Burdett et al. 2012b):

$$F_q'/F_m' = \left[\left(F_q'/F_m' \ \max \times E_k \right) (1 - \exp \ (-E/E_k)) \right] / E \tag{1}$$

where E_k is the minimum saturation intensity (µmol photons m^{-2} s^{-1})[59]—the irradiance level where light shifts from being photosynthetically limiting to photosynthetically saturating. E is equivalent to photosynthetically active radiation (PAR, µmol photons m^{-2} s^{-1}). For the first step of the RLC (where the algae were dark acclimated), F_v/F_m was used instead of F_q'/F_m'. Equation 1 was also used to calculate the theoretical maximum effective quantum efficiency, $F_q'/F_m' \ \max$. As $F_q'/F_m' \ \max$ was derived from the RLC illumination, differences observed represent differences in light acclimation rather than environmental light availability (Suggett et al. 2007).

Electron transport rate (ETR, µmol electrons m^{-2} s^{-1}) was also calculated from F_q'/F_m' measurements at each actinic light intensity (E) of the RLC:

$$\text{ETR} = F_q'/F_m' \times \text{PAR} \times 0.15 \times A \tag{2}$$

where PAR is the RLC irradiance (µmol photons m^{-2} s^{-1}), 0.15 is a multiplication factor to take into account that 15 % of chlorophyll-*a* in red algae is associated with PSII

(Goldstein et al. 1992; Figueroa et al. 2003; Burdett et al. 2012b) and *A* is the corrected total algal absorbance. For the first step of the RLC (where the algae were dark acclimated), F_v/F_m was used instead of F_q'/F_m'. The maximum obtained ETR (ETR$_{MO}$) values from the RLCs are presented.

Absorbance values (*A*) were calculated from the average absorbance of thalli branches between 400 and 700 nm (= range of PAR), determined from the spectra obtained from the pigment composition analysis. Average absorbance was corrected for non-pigment absorption by subtracting the average absorbance between 725 and 750 nm. The fraction of light absorbed by photosynthetic pigments (*A*) was calculated following Schubert et al. (2011):

$$A = 1 - 10^{-D} \tag{3}$$

where *D* is the corrected absorbance between 400 and 700 nm.

Statistics

Due to the nested experimental design, the mean of each response metric from each tank on day 21 was calculated for statistical comparisons (achieving $n = 3$ per treatment level). Neither DMSP nor photosynthetic parameter data could be transformed to meet parametric test assumptions; thus, a Kruskal–Wallis nonparametric test was used to investigate differences between treatments at the end of the experiment. Paired t-tests were used to compare reflectance spectra from each treatment at the start and end of the experiment (test assumptions met). All statistical analyses were conducted in Minitab version 15.

Results

Intracellular DMSP

The control (final salinity = 32) and low (final salinity = 22)-salinity treatments were characterised by a greater variability in intracellular DMSP concentrations than the very low-salinity treatment (final salinity = 12), particularly in the initial stages of the experiment (Fig. 1). Intracellular DMSP in the very low-salinity treatment was generally the lowest of the three treatments (~25–30 µM g^{-1} biomass from day 7, compared to >45 µM g^{-1} biomass for the low-salinity and control treatments), but was not significantly different to the control group by the end of the experiment ($H_2 = 0.62$, $p = 0.73$, Fig. 1).

Pigment composition

The absorbance spectra from all treatments at 0 and 21 days were similar in peak and trough presence/absence, although
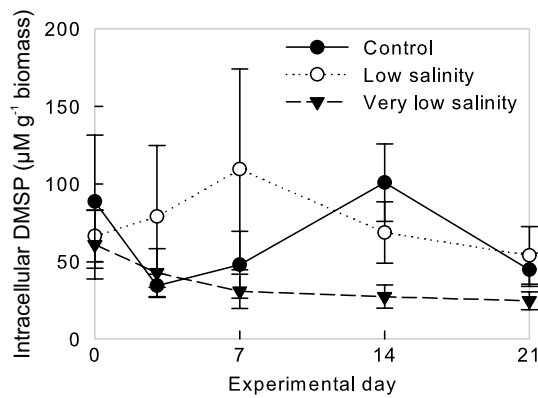
Fig. 1 Intracellular DMSP concentrations (μmol g^{-1}) of *Lithothamnion glaciale*. Thalli were maintained under control (*black circles*), low (*open circles*) and very low (*black triangles*) salinities over a 21-day experimental period. Data presented as mean ± SE

the absolute % absorbance varied (Fig. 2). Peaks in absorbance were observed at wavelengths expected for known Rhodophyta pigments: Chlorophyll-a (435 nm), phycoerythrin (488, 546, 576 nm), phycocyanin (613 nm) and allophycocyanin (652 nm). No change in the absorbance spectra was observed between the beginning and end of the experiment in the control treatment ($T = 1.54$, $p = 0.12$; Fig. 2a). In contrast, a significant increase in % absorbance across the whole spectra (PAR range: 400–700 nm) was observed in the low (final salinity = 22, $T = 95.64$, $p < 0.001$)- and very low (final salinity = 12, $T = 68.49$, $p < 0.001$)-salinity treatments by day 21 (Fig. 2b,c); this was accompanied by a modest increase in variability between thalli (Fig. 2b,c) and fouling of the epithelial surface.

Photosynthetic characteristics

ETR$_{MO}$ in the control treatment (final salinity = 32) was significantly higher (0.15 ± 0.002 μmol electrons m^{-2} s^{-1}, mean ± SE) than the low (final salinity = 22)- and very low (final salinity = 12)-salinity treatments (0.13 ± 0.003 and 0.14 ± 0.02 μmol electrons m^{-2} s^{-1}, respectively, mean ± SE) by the end of the experiment ($H_2 = 12.62$, $p = 0.002$, Fig. 3a), although the control group was characterised by a general increase in ETR$_{MO}$ over the course of the experiment (day 0: 0.12 ± 0.002, day 21: 0.15 ± 0.002 μmol electrons m^{-2} s^{-1}, mean ± SE, Fig. 3a). In contrast, no significant difference in F'_q/F'_m max was observed between treatments at the end of the experiment, which remained between 0.55 and 0.62 throughout the experimental period ($H_2 = 5.48$, $p = 0.064$, Fig. 3b). A significant difference in E_k was also observed between treatments at the end of the experiment ($H_2 = 7.81$, $p = 0.020$, Fig. 3c): in the control and low-salinity treatments, E_k rose
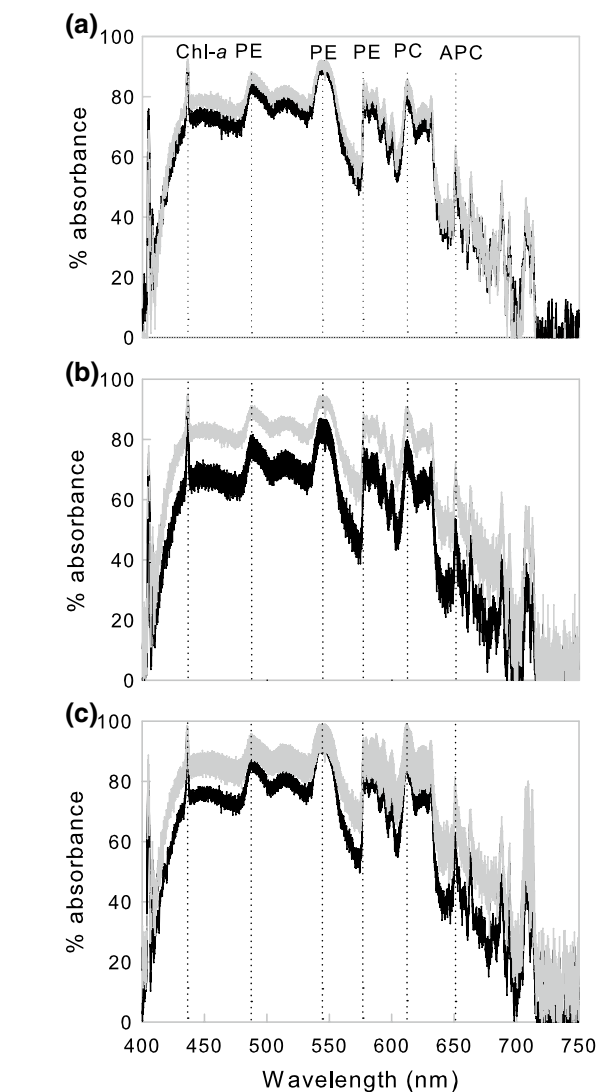

Fig. 2 Absorbance spectra of *Lithothamnion glaciale*. Measurements taken at 0 (*black line*) and 21 days (*grey line*) from samples at **a** control, **b** low and **c** very low salinities. *Width of the line* represents mean ± SD. *Vertical dotted lines* indicate the absorption wavelength of Rhodophyta pigments: Chlorophyll-a (Chl-a), phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). % absorbance is relative to a white standard, which yields 0 % absorbance across the whole spectra

to a maximum of ~45 μmol photons m^{-2} s^{-1} on day 14, whilst E_k in the very low-salinity treatment (final salinity = 12) remained relatively constant (~25 photons μmol m^{-2} s^{-1}) throughout the experiment (Fig. 3c).

Discussion

Climate projections suggest that ice melt and high-intensity storm activity will increase in the future (Hanna et al. 2008; Knutson et al. 2010), increasing the input of freshwater
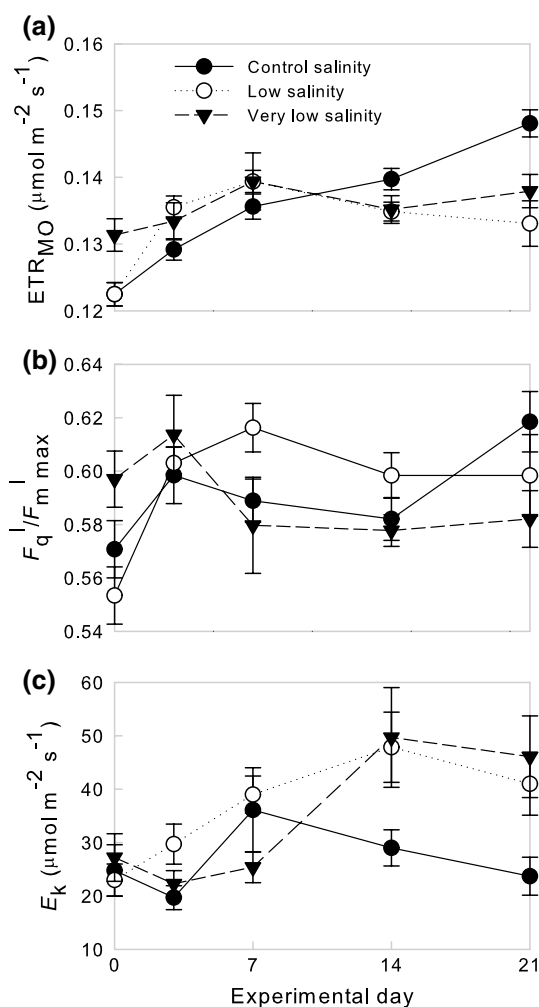
Fig. 3 Photosynthetic characteristics of *Lithothamnion glaciale*. **a** Maximum obtained ETR (μmol electrons m^{-2} s^{-1}), **b** $F'_q/F'_{m\,max}$ and **c** E_k (μmol photons m^{-2} s^{-1}) under control (*black circles*), low (*open circles*)- and very low (*black triangles*)-salinity conditions over a 21-day experimental period. Data presented as mean ± SE

into the coastal zone and exposing coastal organisms to prolonged, more frequent, periods of reduced salinity. This study has shown that the bed-forming red coralline alga *Lithothamnion glaciale*, an integral species in many temperate and polar coastal habitats, harbours intracellular mechanisms that enable it to tolerate a periodic reduction in salinity.

Intracellular DMSP concentrations of *L. glaciale* (normalised to biomass) were comparable to other high DMSP macroalgae such as the Ulvales (Van Alstyne and Puglisi 2007). DMSP is known to act as a compatible solute in marine algae, helping to protect against external changes in salinity. DMSP did not significantly decline under hyposalinity conditions, supporting previous studies of the green macroalga *Ulva lactuca* (Van Alstyne et al. 2003; Ross and Alstyne 2007), and suggesting that other carbohydrate

molecules are regulated in response to hypo-salinity (e.g. glycine betaine). These results may also have been confounded by the reduction in nutrients: DMSP can replace the role of N-containing osmolytes (e.g. proline) (Stefels 2000) and thus may increase during nutrient limitation (Stefels 2000). However, this has not been universally observed; some macroalgae exhibit no response to varying nitrogen conditions (Van Alstyne et al. 2007). This suggests that osmotic control may not be the priority function for DMSP in *L. glaciale*, even when exposed to a reduced salinity environment. However, a range of evidence is available which suggests the proposed antioxidant function for DMSP (Sunda et al. 2002) is active in red coralline algae—*L. glaciale* is often light-saturated even under ambient conditions (Burdett et al. 2012b), and intracellular DMSP concentrations are regulated in response to varying light levels (Rix et al. 2012; Burdett et al. 2014). The large variability in intracellular DMSP concentrations may also be influenced by the antioxidant function: 'self-shading' by outer branches results in photosynthetic heterogeneity (Burdett et al. 2012b), and likely heterogeneity in antioxidant requirements, which may be expressed as intra-thallus heterogeneity in intracellular DMSP concentrations.

Despite the observed epithelial fouling, no change in Rhodophyta-specific pigment composition (phycoerythrin, phycocyanin and allophycocyanin) was observed at the end of the 21-day experiment, providing confidence that epiphytic micro-organisms (e.g. cyanobacteria) did not confound the results. This is in contrast to other red macroalgae (e.g. *Gelidium coulteri*, Macler 1988) and suggests that *L. glaciale* pigmentation is more robust to reduced salinity than other red macroalgae. However, some evidence for dynamic photoinhibition in response to salinity reduction was observed, as an increase in the overall absorbance of *L. glaciale* thalli in the low- and very low-salinity treatments. This may have affected the alga's light-harvesting capacity and supports the observed reduction in E_k. Absorbance data may have also been affected by the epithelial fouling; branches were not cleaned of fouling material prior to conducting the spectral analysis. However, as Rhodophyta pigments were still easily detected, this was not considered a major artefact. Dynamic photoinhibition has also been observed in other temperate macroalgae (Edwards and Kim 2010), tropical red coralline algae (Burdett et al. 2014) and seagrass plants (Belshe et al. 2007); such mechanisms may be critical for survival in naturally variable coastal environments.

Photosynthetic characteristics of the control treatment thalli were within the range observed by other laboratory and field PAM studies on *L. glaciale* (Burdett et al. 2012b). PAM fluorometry does not provide direct measurements of photosynthetic output (e.g. oxygen production) so PAM-derived ETR may be more indicative of photosynthetic

capacity rather than actual photosynthetic rate (Enríquez and Borowitzka 2010). However, ETR values derived from PAM techniques can correspond well to photosynthesis rate, particularly at low irradiances (Figueroa et al. 2003; Nielsen and Nielsen 2008). F'_q/F'_m max in all treatments was comparable to that observed for *L. glaciale* thalli in the field (Burdett et al. 2012b). This indicates that photosynthetic mechanisms were not severely affected by reduced salinity, further highlighting the tolerance of *L. glaciale* to reduced salinity conditions compared with other red macroalgae.

The gradual, but modest, increase in ETR in the control treatment may have been caused by the static light regime of laboratory conditions and highlights the moderate impact of reduced salinity on the photosynthetic characteristics of *L. glaciale*. By day 14, the E_k of thalli in the control and low-salinity treatments was similar to the experimental conditions (40 µmol photons m^{-2} s^{-1}) and to that observed for *L. glaciale* in the field (Burdett et al. 2012b), indicating that (1) full acclimation to the static laboratory environment took 3–4 weeks and should be taken into account in future red coralline algal studies and (2) moderate decreases in salinity did not affect the optimal irradiance required for photosynthesis in *L. glaciale*. Continued acclimation to the laboratory conditions during the beginning of the experiment is also indicated by the relatively large variation in intracellular DMSP concentrations for the first 7 days. Pre-experimentation acclimation periods are typically <10 days; this study shows that a prolonged acclimation period is a necessary consideration in red coralline algal studies. In the very low-salinity treatment, E_k remained lower than the experimental irradiance level of 40 µmol photons m^{-2} s^{-1}. Thus, at this irradiance level, the algae will have been exposed to light-saturating conditions, increasing the likelihood of oxidant production and photodamage, reinforcing the proposed antioxidant function for DMSP in red coralline algae.

Despite low numbers of truly individual replicates ($n = 3$ experimental tanks with a nested design), at the organism level, this study suggests that *L. glaciale* may be able to survive periodic freshening events, although repeated exposure may compromise its survival by permitting excessive fouling on the alga's surface and reducing its antioxidant capacity. During prolonged exposure to reduced salinity conditions, the photosynthetic apparatus remained operational, and it appeared that only marginal regulation of intracellular metabolites was required in response to the hypo-salinity regime. The results of this study also have broader, ecosystem-level, implications. Juvenile invertebrates such as the queen scallop *Aequipecten opercularis* (Kamenos et al. 2004b, c) preferentially settle on live *L. glaciale* beds and tropical red coralline algae provide important settlement cues for invertebrate larvae (Huggett

et al. 2006; Steller and Cáceres-Martinez 2009). The exact cues for settlement are unknown, but may be affected by changes in intracellular DMSP (Steinberg and De Nys 2002; Kiehn and Morris 2010) or surface fouling, affecting adult invertebrate recruitment rates and the subsequent development of reefal ecosystems. Lastly, ecosystem grazing dynamics may be mediated by algal-derived DMSP (Van Alstyne and Houser 2003) and thus may also be affected when intracellular DMSP concentrations are regulated in response to environmental drivers such as salinity reduction.

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