



Oxidative balance in macroalgae from Antarctic waters. Possible role of Fe

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ABSTRACT

The hypothesis of this work was that exposure to diverse abiotic factors in two sites with different sediment and iron input (Peñón de Pesca: low impact; Island D: high impact, both areas in Potter Cove, King George Island, Antarctica) affects the physiological and oxidative profile of *Gigartina skottsbergii* and *Himantothallus grandifolius*. Daily metabolic carbon balance was significantly lower in both macroalgae from Island D compared to Peñón de Pesca. Lipid radical (LR•) content was significantly higher in *G. skottsbergii* collected from Island D compared to Peñón de Pesca. In contrast, *H. grandifolius* showed significantly lower values in Island D compared to Peñón de Pesca. The β-carotene (β-C) content was significantly lower in *G. skottsbergii* from Island D compared to Peñón de Pesca, and the ratio LR•/β-C showed a 6-fold increase in Island D samples compared to Peñón de Pesca. On the other hand, β-C content in *H. grandifolius* showed no significant differences between both areas. The LR•/β-C content ratio in this alga was significantly lower (26%) in Island D as compared to Peñón de Pesca. Total iron content was significantly higher in both macroalgae from Island D compared to samples from Peñón de Pesca. Results with *G. skottsbergii* suggested changes in the oxidative cellular balance, probably related to the higher environmental iron in Island D as compared to Peñón de Pesca. The species *H. grandifolius* seems to be better adapted to the environmental conditions especially through a higher antioxidant capacity to cope with oxidative stress.

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1. Introduction

Extreme seasonal variations in light regime, low temperatures, and extended periods of ice cover and snow characterize the Polar Regions (Wiencke et al., 2007). Particularly over the West Antarctic Peninsula, glacial retreat has increased over the last decades due to global warming (Rückamp et al., 2011) impacting on benthic ecosystems (Gutt et al.,

2015). Meltwater increments from glaciers due to global warming have substantial effects on light penetration, salinity, temperature, nutrient conditions and metal inputs due to higher sediment run-off in coastal waters (Dierssen et al., 2002; Quartino et al., 2013; Sahade et al., 2015). In the King George Island region, Antarctica, high sedimentation reduces underwater photosynthetically active radiation (PAR, 400–700 nm) leading to a lower carbon balance by some macroalgal species (Deregibus et al., 2016). Furthermore, the sediment and rocks in this island are rich in iron (Fe), containing 5 to 7% Fe (Tatur et al., 1999), and thus sediment ablation enriches the seawater in Fe (Ahn et al., 1996; Dierssen et al., 2002). In biological systems, Fe is an essential micronutrient for cellular functioning and growth (Templeton and Liu, 2003). It is also involved in several biochemical reactions that lead to deleterious effects through its catalytic activity. Fe²⁺ catalyzes the reduction of H₂O₂ generating the extremely reactive hydroxyl radical (•OH) (Fenton and Haber-Weiss reactions). In this way, Fe catalyzes the conversion of normal cell respiration by-products into highly cell damaging radical species.

Abbreviations: AH[•], ascorbate; β-C, β-carotene; CAT, catalase; Chl-*a*, chlorophyll-*a*; EPR, electron paramagnetic resonance; GST, glutathione-S-transferase; LR•, lipid radicals; P_{max}, net photosynthesis; PAR, photosynthetically active radiation; POBN, α-(4-pyridyl 1-oxide)-*N*-*t*-butyl nitroxide; ROS, reactive oxygen species; SOD, superoxide dismutase; α-T, α-tocopherol; TEMPOL, 4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy.

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The effects of heavy metal excess on algae include cell lysis, growth inhibition, reduced photosynthesis, disrupted calcification, disturbances in sexual reproduction, induction of the production of reactive oxygen species (ROS) and imbalance of the cellular oxidative status (Collén et al., 2003). Reactions involving ROS are responsible for alterations in the fluidity, integrity, permeability, and function of membranes that include the disturbance of ion-gradients and impairment of lipid-protein interactions naturally occurring within the cells (Halliwell and Gutteridge, 1989). Moreover, lipids are critical targets for the action of ROS, generating lipid radicals (LR•) among other cellular sub-products. In spite of the activity of the antioxidant systems (enzymatic and non-enzymatic) that regulate oxidative stress and damage, living cells could be overwhelmed by endogenous and/or exogenous oxidative challenges from the environment (Chow, 1988). As a response to the exposure to potentially damaging factors, algae could increase the activity of enzymatic antioxidants (e.g. catalase, CAT; superoxide dismutase, SOD and glutathione-S-transferase, GST) and the content of both water and lipid soluble antioxidants (e.g. ascorbate, AH^- ; α -tocopherol, α -T and β -carotene, β -C). Among them, α -T and β -C have the ability to protect polyunsaturated fatty acids from peroxidation and to scavenge free radicals (Evstigneeva et al., 1998). González et al. (2013) proposed the use of indexes such as $\text{LR}\cdot/\alpha\text{-T}$ and/or $\text{LR}\cdot/\beta\text{-C}$, as sensitive indicators of oxidative stress in the lipophilic media in marine organisms, since oxidative stress-dependent effects reflect the imbalance between oxidative damaging species and protection in the internal cellular environment. Moreover, red algae from both polar regions have been shown to produce mycosporine-like amino acids as photoprotective substances (Aguilera et al., 2002), and some Antarctic brown algae (Fairhead et al., 2005) contain phlorotannins that are a class of polyphenolic compounds that shows remarkable bioactivities such as antioxidant properties (Li et al., 2009).

Antarctic macroalgae colonize nearshore areas with hard substrates (e.g. rocks and boulders), and occur in distinct vertical zonation mainly between the intertidal and the subtidal zone down to 30 m depth (Wiencke and Clayton, 2002). The red alga *Gigartina skottsbergii*, Setchell and Gardner 1936, is a subtidal species that occurs in the Antarctic Peninsula, the South Shetland Islands and the South Orkney Islands (Billard et al., 2015). It is morphologically similar to the algae named *G. skottsbergii* from South America, but probably represents a separate species (Billard et al., 2015; Hommersand et al., 2009). It is a pseudoperennial macroalga whose blades may reach up to 60 cm or more (Wiencke and Clayton, 2002). The brown macroalga *Himantothallus grandifolius* (Gepp and Gepp) Zinova 1959, inhabiting Antarctic waters, is the largest Antarctic seaweed (up to 10 m thallus length). Whereas *G. skottsbergii* occurs predominantly in shallow waters, *H. grandifolius* mostly occurs in deeper waters on vertical rocks and boulders in conditions of moderate to low turbulence and on less stable substrata, such as pebbles and gravels (Wiencke et al., 2007). Both species have completely different life strategies. While *G. skottsbergii* is a typical opportunistic species known as a seasonal responder that reacts mainly to environmental conditions and grows in the Antarctic summer, *H. grandifolius* is a seasonal anticipator that grows in late winter/spring based on photoperiodic and circannual rhythms using stored carbon (Wiencke and Amsler, 2012). Moreover, they belong to two algal divisions highly divergent in terms of phylogeny, depth distribution, morpho-functional attributes and ecological functions. The different life strategies may also influence the antioxidant responses of both species.

The hypothesis of this work was that the exposure to diverse abiotic factors, as a consequence of the glacier impact, affects the physiological and oxidative profiles of both *G. skottsbergii* and *H. grandifolius*. Since higher sediment load, which implies less light and higher Fe values, is present in Island D compared to Peñón de Pesca, Potter Cove, King George Island, Antarctica, algae present in Island D could be more affected than in Peñón de Pesca. The main objective was to analyze photophysiological parameters (Chlorophyll *a*, Chl-*a*, content, photosynthetic

efficiency, saturation and compensation points, respiration and daily metabolic carbon balance) and the oxidative profile (LR• content and antioxidants) of the two macroalgae living in Antarctic waters (*G. skottsbergii* and *H. grandifolius*), in different areas. The putative development of adaptive factors was analyzed.

2. Materials and methods

2.1. Study area

The study was conducted at Potter Cove (62°14'S, 58°38'W, King George Island/Isla 25 de Mayo, South Shetland Islands, Antarctica) in two different areas (Peñón de Pesca and Island D) at the north-east side of the cove. These two sites were chosen due to their different positions and abiotic characteristics. Peñón de Pesca (S 62°14'21"; W 58°42'91") is located outside the cove far from the retreating glacier, where the clear water of Maxwell Bay penetrates clockwise; while Island D (S 62°13'25.9"; W 58°38'38.2") is a rocky island at the inner end of the cove near the glacier, heavily influenced by sediment inflow, which appeared approximately in 2003 following deglaciation (Fig. 1; supplementary maps available at Deregibus et al., 2015, <http://dx.doi.org/10.1594/PANGAEA.853859>).

2.2. Quantum irradiance, salinity and temperature measurements

Underwater photosynthetically active radiation (PAR, 400–700 nm) was recorded every 15 min over 7 (seven) consecutive days during sampling at 5 m depth in each area. Measurements were performed using an Odyssey Photosynthetic Irradiance Recording System (Data Flow Systems, Christchurch, New Zealand). Light meters were calibrated 7 (seven) days prior to deployment against a LI-COR LI 1400 datalogger equipped with a LI-COR 190 PAR sensor (Deregibus et al., 2016). After calibration, light loggers were positioned standing upright on tripods and secured by SCUBA divers. During the same period, salinity (PSU) and water temperature (°C) were measured using a CTD (Sea-Bird, SBE19plus, USA) in each area.

2.3. Algal collection and maintenance

Three adult individuals of *H. grandifolius* and *G. skottsbergii* were collected at 5 m water depth in both areas by SCUBA diving during summer 2011–2012. All individuals were covered by dark plastic bags and kept in aerated 0 °C seawater from the cove in a culture room. Subsequently, algal pieces of approximately 0.5 g were cut from the middle part of the lamina, between the distal and the basal parts, and kept overnight in the dark in filtered seawater at 0 °C to avoid wounding effects (Drew, 1983). The algal pieces were stored at –70 °C until used for measurement of oxidative parameters.

2.4. Content of Chl-*a*

Extraction of Chl-*a* was carried out using *N*, *N*-dimethyl formamide, as described by Inskeep and Bloom (1985). Optical density was measured in a spectrophotometer (Pharmacia Biotech Ultrospec 3000, Cambridge, England) at $\lambda = 664$ nm, and the Chl-*a* content was calculated according to Inskeep and Bloom (1985).

2.5. Photosynthetic measurements and quantification of the daily metabolic carbon balance

Photosynthesis and dark respiration were measured in a constantly stirred plastic chamber (30 mL) fitted with a fiber-optic O₂ mini sensor, connected to an OXY-4 (4 channel Fiber Optic Oxygen Meter, PreSens, Regensburg, Germany). The chamber was placed in a water bath kept at constant temperature (2.00 ± 0.01 °C) with a thermostat (Haake DC3, Karlsruhe, Germany). A slide projector (Leica, Pradovit CA2502,

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