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Seasonal effects of sun exposure and emersion on intertidal seaweed physiology: Fluctuations in antioxidant contents, photosynthetic pigments and photosynthetic efficiency in the red alga *Porphyra umbilicalis* Kützing (Rhodophyta, Bangiales)

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ABSTRACT

There is a great deal of speculation regarding the physiological and biochemical mechanisms that give certain seaweed species the ability to colonize the intertidal zone. Frequent exposure to ambient temperatures and high irradiance levels in addition to dehydration during tidal emersion generates acute physiological stress. The ability of seaweeds like *Porphya* to overcome these challenges and survive in such a harsh environment has been linked to elevated reactive oxygen metabolism. The current study focused on measuring seasonal changes in antioxidant enzymes plus alterations in pigment contents and photosynthetic efficiency of *P. umbilicalis* plants found growing in the uppermost intertidal zone.

Our results suggest that *P. umbilicalis* exhibits increased antioxidant metabolism, which could contribute to its success in colonizing such a stressful habitat. Elevated levels of glutathione reductase GTR, catalase and carotenoid contents during emersion suggested heightened protection against reactive oxygen species ROS damage is a necessary attribute for species in the upper intertidal regions. This hypothesis was further strengthened by the finding that the greatest antioxidant increases were observed during summer months when irradiance levels and temperatures were at their peak. Winter emersion did not elicit the same physiological response, as antioxidant levels were similar in submersed and emersed plants.

For the most part, photosynthetic pigments were largely affected by sun exposure and less by emersion stress. Shaded blades maintained higher concentrations of photosynthetic pigments compared to sun exposed thalli concurring with established research. Photosynthetic efficiency measurements indicated emersion and not sun exposure was the greater facilitator of photoinhibitory damage and ROS generation at PSII. The findings of this field study strengthen previous assertions that protection via elevated antioxidant metabolism and increased PSII repair are involved in providing relief from the acute environmental stresses in the intertidal zone.

1. Introduction

Tidal exposure imposes considerable environmental stress on intertidal seaweeds such as elevated irradiance levels, temperature changes and desiccation. Typically, seaweeds sensitive or intolerant to ambient stresses inhabit the lowermost intertidal zone (where emersion at low tide is brief and/or absent), while those found at higher elevations usually possess heightened tolerance to environmental fluctuations. It is likely that such spatial separation within the intertidal zone is the result of morphological (i.e. thallus shape, size and thickness; Chapman, 1986), physiological and biochemical variations among seaweed species (Dring and Brown, 1982; Lüning, 1990; Figueroa et al., 1997; Lundheim, 1997; Malm and Kautsky, 2003; Murru and Sandgren, 2004).

A decrease in thallus water content is associated with emersion, with the rate of water loss dependent on atmospheric conditions as well as the evaporating surface-to-volume ratio of the thallus (Dromgoole, 1980; Lobban et al., 1985). A decrease in water content influences emersed photosynthesis and recovery upon re-immersion (Bell, 1993; Williams and Dethier, 2005). Immediately following emersion, seaweed photosynthetic rates decline because the inorganic carbon supply is greatly restricted (Lobban et al., 1985). Apparently, once the surface water film has evaporated, CO_2 from the air can readily penetrate the cells enhancing photosynthesis (Zou and Gao, 2002), but as water loss progresses, the photosynthetic machinery becomes more stressed and is eventually damaged. As desiccation advances, the rate of photosynthesis further declines and the electron system operating between photosystem II (PSII) and photosystem I (PSI) is interrupted (Bewley, 1979).

Prolonged exposure of seaweeds to high light intensities can damage the photosynthetic system and contribute to decreases in

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Table 1
Results (p-values) of ANOVA's on antioxidants, pigments and photosynthetic efficiency (Fv/Fm)

Response	Main Effects			Interactive Effects			
	Date	Sun Exp	Emersion	Date×Emersion	SunExp×Emersion	Date×SunExp	Date×SunExp×Emersion
GTR	<0.001	0.032	<0.001	<**0.001**	0.064	**0.020**	0.109
Catalase	**0.006**	0.183	<0.001	0.328	**0.014**	0.339	0.175
APX	<0.001	0.040	<0.001	<0.001	<0.001	<0.001	**0.048**
Carotenoids	<0.001	0.907	<0.001	<**0.001**	0.382	**0.050**	0.108
R-PE	<0.001	<0.001	0.008	**0.015**	0.675	**0.001**	0.522
R-PC	<0.001	<0.001	0.061	0.116	0.529	**0.008**	0.879
Total Chl	<0.001	<**0.001**	<0.001	<**0.001**	0.735	0.155	0.550
Fv/Fm*	<0.001	0.002	<0.001	<**0.001**	0.105	<**0.001**	0.145

Experimental factors were month of collection (Date), sun exposure (SunExp; sun exposed or shaded) and emersion time (Emersion; 0 or 6 hours). Bolded values represent statistical significance. Values <0.05 that are not bolded indicate that a significant interaction with another experimental factor is present.
*Emersion Time=0 or 3 hours.

both quantum efficiency and maximum photosynthetic rates. Damage is further escalated when coupled with desiccation and/or temperature stress (Davison and Pearson, 1996). As a result, many intertidal seaweeds have developed mechanisms to prevent/avoid lethal physiological damages incurred and maintain physiological integrity during and throughout emersion.

The generation of reactive oxygen species (ROS) like superoxide, peroxides, hydroxyl radicals and singlet state oxygen is routine during "normal" photosynthetic and respiratory metabolism. However, during periods of elevated physiological stress such as emersion, ROS formation can escalate rapidly. ROS scavenging enzymes and substrates (i.e. antioxidants) in addition to tocopherols and polyamines are present within algal cells as protective agents against the damages commonly associated with ROS. Since ROS are highly reactive with most cellular components including proteins, lipids and nucleic acids they must be neutralized quickly and efficiently once formed.

Antioxidant enzyme and substrate responses, that scavenge and purge ROS before they react with surrounding molecules, are triggered by elevated levels of ROS at both the gene and protein levels to coordinate the most effective response (Asada and Takahashi, 1987; Halliwell and Gutteridge, 1999; Mackerness et al., 1999; Estevez et al., 2001). In both algae and higher plants, three major defense systems have evolved to protect against superoxide radicals and the subsequent formation of H_2O_2: the superoxide dismutases (SODs), peroxidases and catalases (Asada, 1999). With the exception of singlet excited O_2, all ROS species are derived from superoxide and peroxides. Because all superoxide is defensively converted into peroxides, they are at the center of ROS cellular damages. Ascorbate peroxidase (APX), in addition to its affiliated enzymes, and catalase are therefore the primary defensive enzymes against excessive ROS damage.

APX is a substrate specific, heme-containing enzyme that scavenges and reduces H_2O_2 into H_2O+O_2 within the chloroplast and cytosol of photosynthetic cells (Allen, 1995). APX utilizes ascorbate as its specific electron donor to reduce H_2O_2 (Shigeoka et al., 2002). Recycling of ascorbate between its oxidized and reduced states is essential for the proper functioning of APX. The tripeptide molecule glutathione (GSH) is heavily involved in oxidative stress defenses by reacting directly with ROS and participating in the enzymatic detoxification of H_2O_2 (Lappartient and Touraine, 1997). Oxidized glutathione (GSSG) is reduced by the NADPH-dependent enzyme glutathione reductase (GTR), which is located in the chloroplast, mitochondria and cytosol of photosynthetic cells. Recycling of glutathione between its oxidized and reduced states simultaneously replenishes the reduced ascorbate pool. Catalase differs from peroxidases in that it dismutates H_2O_2 to H_2O. In plants, catalase scavenges H_2O_2 generated during photorespiration and the β-oxidation of fatty acids and is thought to play a significant role in the protection of aerobic organisms from the toxic effects of H_2O_2. Carotenoid molecules have recently been shown to provide significant antioxidative protection at the PSII reaction center. They are responsible for quenching energy directly from excited state chlorophyll and singlet oxygen ($^1O_2$) non-photochemically (Siefermann-Harms, 1987; Hosaka et al., 2005; Penuelas and Munne-Bosch, 2005; Telfer, 2005).

Studies of stress tolerance have suggested a correlation between elevated metabolism of antioxidants and increased tolerance to environmental stresses in higher plants (Allen, 1995; Hernandez et al., 2000; Sairam and Saxena, 2000; Peltzer and Polle, 2001), green and brown algae (Malanga and Puntarulo, 1995; Collen and Davison, 1999a,b; Choo et al., 2005; Dring, 2006), as well as some red algae (Collen and Davison, 1999c, Burritt et al., 2002). Additionally, seaweed responses to high irradiance levels suggest that repairs to PSII and pigment manipulation are vital

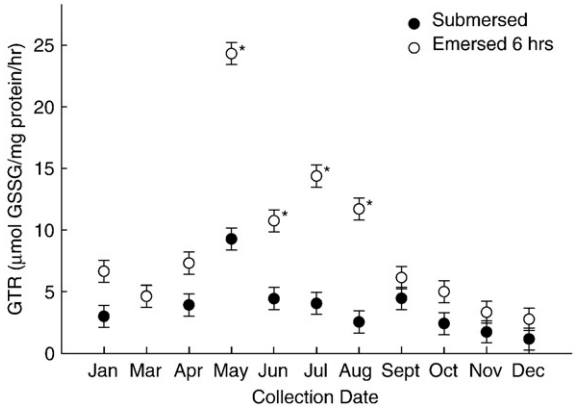

Fig. 1. Seasonal change in GTR activity of *P. umbilicalis* collected while submersed (filled circles) and following 6 hours of emersion (open circles). Data are n=8 samples per treatment. Asterisks (*) indicate significant differences between emersion times on that date (α=0.05). Error bars are ± one standard error.

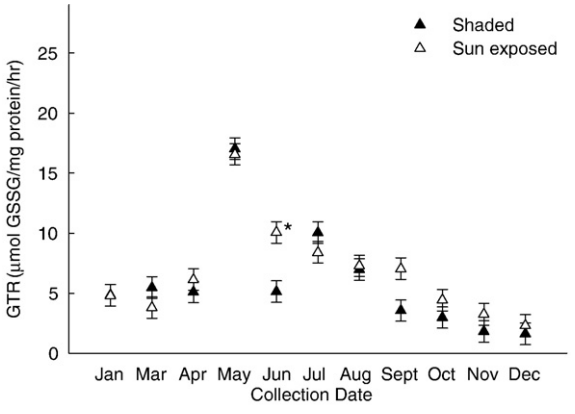

Fig. 2. Seasonal change in GTR activity collected from shaded (filled triangles) and sun exposed (open triangles) locations. Data are n=8 samples per treatment. Asterisks (*) indicate significant differences between sun exposures on that date (α=0.05). Error bars are ± one standard error.