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Implications of mycosporine-like amino acid and antioxidant defenses in UV-B radiation tolerance for the algae species *Ptercladiella capillacea* and *Gelidium amansii*

Tse-Min Lee and Chia-Tai Shiu

Institute of Marine Biology, National Sun Yat-sen University, Kaohsiung, Taiwan 80424, Republic of China
The Kuroshio Research Group of the Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung, Taiwan 80424, Republic of China

Corresponding author: Tse-Min Lee; tel: 886-7-5252000 ext. 5110; fax: 886-7-5251509; e-mail: tmlee@mail.nsysu.edu.tw

Running title: MAA and UV-B-induced oxidative responses

1 Abstract

2

3 Ultraviolet-B (UV-B) radiation (0.5, 1.0, 1.5, 3.0 W m⁻²) induced higher H₂O₂ production and
4 lipid peroxidation in alga *Gelidium amansii* inhabiting in lower subtidal regions than upper subtidal
5 alga *Ptercladiella capillacea*. Compared to *G. amansii*, mycosporine-like amino acid (MAA)
6 concentration in *P. capillacea* was higher and can be increased by 0.5-1.0 W m⁻² UV-B, while
7 carotenoid concentration was lower but also increased by 1.5-3.0 W m⁻² UV-B. UV-B increased
8 ascorbate concentration, but to a higher degree in *P. capillacea*. UV-B decreased glutathione
9 concentration, but to a higher degree in *G. amansii*. UV-B increased ascorbate peroxidase (APX)
10 and glutathione reductase (GR) activities in *P. capillacea* but decreased them in *G. amansii*. UV-B
11 increased superoxide dismutase and catalase activities, but to a higher degree in *G. amansii*. So, *G.*
12 *amansii* suffered greater oxidative stress from UV-B radiation. *Ptercladiella capillacea* can
13 effectively reduce UV-B sensitivity by increasing sunscreen ability and antioxidant defense
14 capacity.

15

16 *Keywords:* Antioxidant; carotenoid; *Gelidium amansii*; *Ptercladiella capillacea*; UV-B radiation

17

18 1. Introduction

19

20 The stratospheric ozone layer is vital to life on Earth because it is the principal agent that
21 absorbs ultraviolet radiation in the Earth's atmosphere. The depletion of the stratospheric ozone
22 layer due to anthropogenic and natural causes since 1979 has led to increasing levels of solar
23 ultraviolet-B (UV-B) radiation (280-320 nm) reaching the Earth's surface, especially in the Arctic,
24 Antarctic and temperate regions (Seckmeyer and McKenzie, 1992; Kerr and McElroy, 1993;
25 Russell et al., 1996), where UV-B and UV-A (320-400 nm) radiation are capable of penetrating the
26 water column to an ecologically-significant depth (Calkins and Thordardottir, 1982; Whitehead et

1 al., 2000).

2 Since UV-B radiation damages DNA, algae evolved counteract mechanisms for the repairing
3 of UV-induced damage of DNA by photoreactivation and excision repair (Britt, 1995; Kim and
4 Sancar, 1995). Most algae synthesize mycosporine-like amino acids (MAAs) as an effective UV
5 sunscreen compound that absorbs harmful UV radiation and emits heat or a fluorescence emission
6 to prevent UV-induced photodamage (Riegger and Robinson, 1997; Dunlap and Shick, 1998;
7 Karsten et al., 1998). MAAs are water-soluble substances characterized by a cyclohexenone or
8 cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or its
9 imino alcohol, having absorption maxima ranging from 310 to 360 nm (Nakamura et al., 1982). The
10 MAA content shows a negative correlation with water depth, but the ability for MAA synthesis in
11 response to environmental changes is variable among algae (Hoyer et al., 2001). MAAs also act as
12 antioxidants, for example, mycosporine-glycine shows a moderate antioxidant potential (Dunlap
13 and Yamamoto, 1995).

14 UV-B radiation has been shown to reduce the photosynthetic electron transport and dark
15 reaction activity (Brandle, 1977; Foyer and Harbinson, 1994), and thus leads to excess ROS
16 formation (Langebartels et al., 2000). As a result, the cellular homeostasis of ROS production is
17 disrupted and causes oxidative stress, that is, oxidative damage to macromolecules (Foyer et al.,
18 1994; Rijstenbil, 2002). Induction of ROS over-production and oxidative stress by UV-B radiation
19 has been observed in microalgae and macroalgae (Tschiersch and Ohmann, 1993; Foyer et al., 1994;
20 Lesser, 1996; Malanga et al., 1997; He et al., 2002; Rijstenbil, 2002; Shiu and Lee, 2005).

21 The increases in carotenoids, detoxifying enzymes and antioxidants provide UV-B protection
22 by scavenging ROS (Mittler and Tel-Or, 1991; Middleton and Teramura, 1993). Ascorbate (AsA),
23 glutathione (GSH), α -tocopherol, and carotenoids are considered effective antioxidants (Noctor and
24 Foyer, 1998; Smirnoff and Wheeler, 2000). Carotenoids play an essential photoprotective role by
25 quenching triplet state chlorophyll molecules and scavenging singlet oxygen and other toxic oxygen
26 species formed within the chloroplast (Young, 1991). To scavenge ROS enzymatically, superoxide

1 dismutase (SOD; EC 1.15.1.1) is the first line of detoxifying enzymes that converts $O_2^{\cdot -}$ to a less
2 toxic ROS, H_2O_2 . Then, ascorbate peroxidase (APX; EC 1.11.1.11) degrades H_2O_2 via AsA
3 oxidation and oxidized AsA, such as dehydroascorbate (DHA), is reduced to regenerate AsA by
4 dehydroascorbate reductase (DHAR; EC 1.8.5.1) using GSH as the electron donor (Asada, 1999).
5 Oxidized GSH, GSSG, is reduced to GSH by glutathione reductase (GR; EC 1.6.4.2) utilizing
6 reducing equivalents from NAD(P)H. Catalase (CAT; EC 1.11.1.6) is also responsible for H_2O_2
7 removal (Willenken et al., 1997).

8 At low latitudes, ambient UV-B radiation that is high due to the high solar angle and the
9 relatively small amount of stratospheric ozone and can reach more deeply into oligotrophic tropical
10 water column because of the relatively low quantities of dissolved and particulate organic matter
11 (Baker et al., 1980). It is expected that seaweeds from tropical regions encounter higher UV
12 radiation than those occurring in temperate and Polar regions even under the normal ozone layer
13 (Häder, 1993). In subtropical waters, the coastline along northeastern Taiwan is dominated by the
14 rhodophyte *Pterocladia capillacea* (S.G. Gmelin) Santelices & Hommersand in the upper subtidal
15 region (Fig. 1A) while another rhodophyte *Gelidium amansii* (J.V. Lamouroux) J.V. Lamouroux is
16 abundant in the lower subtidal region (Fig. 1C). These two algae belong to the Family Gelidiaceae
17 but different genus (Gelidiales, Rhodophyta). *Pterocladia capillacea* is ascribed to the genus
18 *Pterocladia* and *G. amansii* is ascribed to the genus *Gelidium*. Our surveys during 2004-2005 in
19 four sites along northeastern Taiwan coast (see 2.1. Algal materials and treatments) showed that *P.*
20 *capillacea* was significantly abundant in the subtidal region of 1-m water depth while *G. amansii*
21 was abundant in the regions of 3-m and 5-m water depth ($p < 0.0001$). Besides, they appeared
22 significant temporal variation ($p < 0.0001$); *P. capillacea* was abundant in the summer (September,
23 2004) while *G. amansii* was absent in the summer. It is therefore interesting for us to understand the
24 factors influencing the temporal and spatial differences between the two Gelidiaceae algae. We
25 hypothesize that one of factors driving this differential distribution is the sensitivity to high
26 UV/solar radiation, especially in the summer. The MAA contents of *P. capillacea* (Fig. 1B) were

Figure 1

1 significantly higher than those of *G. amansii* (Fig. 1D), especially in the summer. It is known that
2 physiological responses of macroalgae to UV-B are related to their zonation pattern. A survey of the
3 UV tolerance of marine rhodophyte from tropical habitats showed that UV exposure caused less
4 pronounced effects in intertidally-occurring species than in subtidal species (van de Poll et al.,
5 2003). The Arctic marine macroalgal species from eulittoral and upper sublittoral regions had
6 higher antioxidant enzyme activities upon exposure to UV radiation than those species from lower
7 sublittoral regions (Aguilera et al., 2002). We therefore propose that differences in UV-B tolerance
8 in term of UV screening capacity and antioxidant defense ability are one of the mechanisms that
9 forces different habitation patterns for *P. capillacea* and *G. amansii* in northeastern Taiwan. In the
10 attempts to elucidate the presence of MAA in the regulation of carotenoid synthesis and antioxidant
11 defense responses in algae differing in UV-B tolerance and their roles in mitigating UV-B/solar
12 radiation toxicity, algal thalli were sampled in the field and immediately transferred to the
13 laboratory for UV-B tolerance experiment and the contents of MAA, carotenoid and antioxidant,
14 and the activities of antioxidant enzymes were examined.

15

16 **2. Materials and methods**

17

18 *2.1. Algal materials and treatments*

19

20 In the attempt to determine temporal and spatial changes in the abundance and MAA contents
21 of *P. capillacea* and *G. amansii* in northeastern Taiwan, four sampling sites (Lung-tung, Her-mei,
22 Mo-auo, and Shea-cherng) and four sampling times (June 16-22, 2004, September 21-30, 2004,
23 March 17-23, 2005, and June 3-11, 2005) were set. For each sampling site, three 50-m sampling
24 lines parallel to coastal line were set on 1-m, 3-m and 5-m water depth (MHWS), respectively (a
25 sampling line for each water depth) and 4 random sampling stations were set on each line for the
26 determination of algal abundance (biomass). To estimate algal biomass in each sampling station,

1 algae in a 50×50 cm² quadrat were scraped and *P. capillacea* and *G. amansii* were weighed and
2 calculated as g wet weight (w. wt.) m⁻². After weighting, algae were fixed in liquid nitrogen and
3 stored in a -70°C freezer until analysis of MAAs.

4 For laboratory experiments, *P. capillacea* and *G. amansii* of 10-cm plant height were collected
5 in September, 2004 and August, 2005 from the intertidal regions at Her-mei and the subtidal regions
6 at Mo-auo in northeastern Taiwan, respectively. Plants were transported to the laboratory, washed
7 with natural seawater to remove attached sands and incubated at 22-26°C for 14 days in 35‰ (parts
8 per thousand) nutrient-enriched artificial seawater (ASW) containing 403.5 mM NaCl, 10 mM KCl,
9 10 mM CaCl₂, 30 mM MgSO₄, 10 mM Tris-HCl (pH 8.0), N- (NH₄⁺ and NO₃⁻) and P- (PO₄⁻³) free
10 Provasoli nutrient solutions (Provasoli, 1968), 3 mM NaHCO₃, 400 μM NaNO₃, and 20 μM
11 Na₂HPO₄. The light time was 14 h (photosynthetically active radiation (PAR, 400-700 nm) of
12 100-120 μmol photons m⁻² s⁻¹ in the absence of algae, achieved by 20 W cool-fluorescent lamps
13 (FL20D, China Electric Apparatus Ltd., Taiwan, ROC)). Before UV-B radiation, thallus segments
14 were cultured in a Petri dish (internal diameter: 30 cm) containing 2 L of 35‰ nutrient-enriched
15 ASW for one day (14 h light/10 h dark) incubation (150 μmol photons m⁻² s⁻¹ PAR provided by 40
16 W cool-fluorescent lamps (FL40D, China Electric Apparatus Ltd., Taiwan)). Thallus segments of
17 0.15 g wet weight (w. wt.) were transferred to a Petri dish (2.5 cm in diameter) containing 5 mL of
18 35‰ nutrient-enriched ASW for UV-B radiation (280-320 nm) (0, 0.5, 1.0, 1.5, and 3.0 W m⁻²),
19 achieved by exposure to UV-B lamps (TLD 15W/08, UVP, Japan) covered with cellulose acetate
20 film (to cut off UVC) of 0.75 mm thickness under 150 μmol photons m⁻² s⁻¹ PAR provided by 40 W
21 cool-fluorescent lamps (FL40D, China Electric Apparatus Ltd., Taiwan). After 14 h of ‘UV-B +
22 PAR’ and ‘PAR’ treatments, thalli were sampled for further analysis. The biologically weighted
23 UV-B radiation over 14 h according to generalized plant action spectrum (normalized to 300 nm;
24 Caldwell, 1971) for low-, medium- and high-UV-B radiation were 0.5-1.0 W m⁻² (25.67-50.17
25 kJ/m²/14 h), 1.5 W m⁻² (75.83 kJ/m²/14 h) and 3.0 W m⁻² (149.33 kJ/m²/14 h), respectively. Thallus
26 segments were fixed in liquid nitrogen and stored in a -70 °C freezer until analysis. The samples for

1 MAA analysis and antioxidant enzyme activity were subjected to lyophilization and stored in -70 °C
2 freezer. Each Petri dish was a replicate; there were 3 replicates for each treatment.

3

4 *2.2. Determination of lipid peroxidation and H₂O₂ production*

5

6 Thalli of 0.05 g w. wt. were ground in liquid nitrogen and 1 mL of 5% (w/v) trichloroacetic
7 acid was added. After centrifugation at 12,000 × g for 10 min at 4°C, the supernatant was collected.
8 The thiobarbituric acid reacting substance (TBARS) resulting from reaction with thiobarbituric acid
9 (TBA) was determined as lipid peroxidation according to Health and Packer (1968). TBARS
10 content was calculated based on the value of A₅₃₂-A₆₀₀ with an extinction coefficient of 155 mM⁻¹
11 cm⁻¹.

12 H₂O₂ content was determined by peroxidase-mediated decomposition of H₂O₂ (Okuda et al.,
13 1991). The pH of 0.2 mL of supernatant was adjusted to 7.5 by adding 11.51 µL of 4 M KOH and
14 then centrifuged at 12,000 × g for 1 min at 4°C. The supernatant was collected and applied to a 1
15 mL column of Amberlite IRA-410, and residual H₂O₂ was washed out with 800 µL of distilled
16 water. H₂O₂ content in the eluent was determined within 10 min after elution. Four hundred µL of
17 12.5 mM 3-dimethylaminobenzoic acid (DMAB), 0.4 mL of 10 mM 3-methyl-2-benzothiazoline
18 hydrazone (MBTH) and finally 0.02 mL of 0.25 U mL⁻¹ horseradish peroxidase (Sigma, USA) were
19 added and measured at 590 nm for 3 min. A H₂O₂ standard curve was determined following the
20 same procedure.

21

22 *2.3. Extraction and determination of MAA*

23

24 MAA was extracted and analyzed according to Carreto et al. (2005) with one modification. A
25 sample of 0.005 g dry weight (d. wt.) was homogenized in liquid nitrogen and extracted with 1 mL
26 of 25% (v/v) methanol for 2 h in a 45 °C water bath. The extract was centrifuged at 5,000 × g for 5

1 min and the supernatant was collected. The pellet was re-extracted twice following the same
2 procedure. Then, the pooled extracts were evaporated to dryness under vacuum (Speed Vac
3 Concentrator SVA 100H, Savant Instruments Inc., Holbrook, NY, USA) and re-dissolved in 500 μL
4 of 25% methanol (v/v). After being centrifuged at $15,000 \times g$ for 5 min, the sample was used for
5 HPLC analysis on a Merck RP-8 column (5 μm , 250 mm \times 4 mm I.D.). The mobile phase consisted
6 of 1% methanol (v/v) run at a flow rate of 0.8 mL min^{-1} . MAAs were detected at 330 nm.
7 Identification was confirmed by spectra, retention time (RT) and by co-chromatography with
8 standards, extracted from the algae *Laurencia papillosa* (C. Agardh) Greville and *Porphyra*
9 *umbilicalis* (L.) Kützing obtained from Taiwan (supplementary data). Molar extinction coefficients
10 were obtained from Karsten et al. (1998). MAA concentration was expressed as $\mu\text{mol g}^{-1} \text{ d. wt.}$

11

12 2.4. Determination of ascorbate and glutathione concentrations

13

14 Thalli of 0.02 g w. wt. were ground in liquid nitrogen and 1 mL of 5% (w/v) trichloroacetic
15 acid (TCA) was added. After centrifugation at $12,000 \times g$ for 10 min at 4°C , the supernatant was
16 collected for determination of its antioxidant content. The measurement of reduced (AsA) and
17 oxidized (DHA) ascorbate concentration were performed by a modification of the method of
18 Hodges et al. (1996). Total ascorbate was determined in 1 mL mixture containing 200 μL TCA
19 extract, 50 mM potassium phosphate buffer (pH 7.4), 3 mM EDTA and 1 mM dithiothreitol (DTT).
20 The mixture was incubated at 25°C for 10 min, 100 μL of N-ethylmaleimide, 400 μL of 0.61 M
21 TCA, 400 μL of 0.8 M orthophosphoric acid and 400 μL of α,α' -bipyridyl were then added. Finally,
22 200 μL of FeCl_3 was added and the reaction mixture was incubated in a 55°C water bath for 10 min
23 and the absorbance was detected at A_{525} . AsA concentrations were determined by adding distilled
24 water instead of DTT and N-ethylmaleimide and then following the above method. Total AsA and
25 AsA concentrations were estimated from a standard curve of 0-40 nmole L-AsA. DHA
26 concentration was calculated by subtraction of AsA concentration from total AsA concentration.

1 Total glutathione was determined by the method of Griffiths (1980). The concentration of
2 glutathione (reduced form) was estimated from a standard curve of 0-20 nmole GSH. After the
3 removal of GSH by 2-vinylpyridine derivatization, GSSG concentration was determined and GSH
4 concentration was calculated by the subtraction of GSSG concentration from total glutathione
5 concentration.

6 7 *2.5. Determination of enzyme activity*

8
9 Enzyme activity was determined according to Shiu and Lee (2005). Lyophilized thalli of 0.006
10 g d. wt. were homogenized in liquid nitrogen and 0.6 mL of 0.1 M sodium phosphate buffer (pH 6.8)
11 containing 1% (w/v) polyvinylpolypyrrolidone (PVPP) and then 0.25% (v/v) Triton X-100 was
12 added. After centrifugation at $12,000 \times g$ for 10 min at 4 °C, the supernatant was used in activity
13 assays for SOD, CAT and GR. For the APX assay, thalli of 0.005 g d. wt. were homogenized in
14 liquid nitrogen and 0.6 mL of extraction buffer (0.1 M sodium phosphate buffer (pH 6.8) containing
15 1% (w/v) PVPP, 0.25% (v/v) Triton X-100 and 0.5 mM L-ascorbate) was added. The supernatant
16 after centrifugation at $12,000 \times g$ for 10 min at 4 °C was used for the APX activity assay. Protein
17 content was determined according to Bradford (1976) with bovine serum albumin as the standard.

18 CAT activity was measured at A_{420} for H_2O_2 decomposition rate using $40 \text{ mM}^{-1} \text{ cm}^{-1}$ as the
19 extinction coefficient. SOD activity was determined by the photochemical inhibition of nitro blue
20 tetrazolium. APX activity was determined by the decrease in ascorbate concentration, which was
21 detected at A_{290} (extinction coefficient: $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$). GR activity was monitored by A_{340} for
22 β -NADPH oxidization as a measure of GSSG reduction.

23 24 *2.6. Chemicals and statistical analysis*

25
26 Chemicals were purchased from Merck (Germany) or Sigma (USA). Statistics were analyzed

1 by SAS (SAS v 8.1, NC, USA). The results presented were the mean of 3 duplicates with a Petri
2 dish as a duplicate (mean \pm SD, n=3) and the significance of treatments were tested by 1-way
3 analysis of variance (ANOVA). The biomass and total MAA concentrations for the field surveys
4 were analyzed by 3-way ANOVA (time, site, and water depth). The significance of the differences
5 between means was analyzed by Duncan's new multiple range test followed by significance
6 threshold ANOVA value of $p < 0.05$.

7

8 **3. Results**

Figure 2

9

10 *3.1. TBARS and H₂O₂ concentration*

11

12 UV-B radiation did not affect the TBARS content of *P. capillacea* ($p = 0.0672$) (Fig. 2A).
13 UV-B radiation $\geq 1.5 \text{ W m}^{-2}$ increased the TBARS content of *G. amansii* ($p = 0.0254$) (Fig. 2A).
14 H₂O₂ concentration in *P. capillacea* and *G. amansii* increased as UV-B radiation increased ($p <$
15 0.05); the increase was higher in *G. amansii* than *P. capillacea* (Fig. 2B).

16

17 *3.2. MAA and carotenoid concentration*

Figure 3

18

19 The amount of MAA is shown in Fig. 3. Shinorine, porphyra-334, and palythanol can be
20 detected in both algae. Total MAA concentration was higher in *P. capillacea* than in *G. amansii*.
21 Porphyra-334 was the dominant MAA in *P. capillacea* and shinorine was the dominant MAA in *G.*
22 *amansii*. The amounts of shinorine, porphyra-334, and palythanol in *P. capillacea* were increased by
23 UV-B radiation. A linear increase was observed as UV-B radiation increased from 0.5-1.0 W m^{-2}
24 followed by a decline as UV-B radiation $\geq 1.5 \text{ W m}^{-2}$. The amount of MAA in *G. amansii* was
25 decreased by UV-B radiation.

26

The carotenoid concentration for the control thalli was 2-fold higher in *G. amansii* than in *P.*

1 *capillacea* (Fig. 4). The carotenoid concentrations of *P. capillacea* did not change in the range of
 2 0.5-1.0 W m⁻² UV-B radiation, but increased as UV-B radiation increased from 1.5-3.0 W m⁻² (Fig.
 3 4). The carotenoid concentration of *G. amansii* increased slightly from 0.5-1.0 W m⁻² UV-B
 4 radiation followed by a decrease as UV-B radiation increased from 1.5-3.0 W m⁻² (Fig. 4).

Figure 4

6 3.3. Ascorbate and glutathione concentration

Figure 5

8 UV-B radiation increased AsA concentration ($p = 0.0131$) in *P. capillacea* (Fig. 5A) but did not
 9 affect its DHA concentration ($p = 0.1141$) (Fig. 5B). UV-B radiation increased AsA ($p = 0.0234$)
 10 and DHA ($p = 0.0130$) concentrations of *G. amansii* (Fig. 5). AsA/DHA ratios in *P. capillacea*
 11 increased as UV-B radiation increased, while those of *G. amansii* increased linearly as UV-B
 12 radiation increased from 0-1.5 W m⁻² but decreased at 3.0 W m⁻² due to an increase in DHA
 13 concentration.

14 GSH concentration in *P. capillacea* was not affected by UV-B radiation ($p = 0.1582$) (Fig. 5C)
 15 while GSSG concentration was decreased by UV-B radiation ($p = 0.0249$) (Fig. 5D). The GSH
 16 concentration of *G. amansii* was decreased by UV-B radiation ($p = 0.0435$) (Fig. 5C), but GSSG
 17 concentration was increased by UV-B radiation in the range of 0-1.5 W m⁻², followed by a drop at
 18 3.0 W m⁻² ($p = 0.0009$) (Fig. 5D). GSH/GSSG ratios in *P. capillacea* and *G. amansii* were decreased
 19 by UV-B radiation. However, the extent of the decrease was greater in *G. amansii*.

21 3.4. Enzyme activities

23 SOD activities of *P. capillacea* ($p < 0.0001$) and *G. amansii* ($p = 0.0040$) increased at UV-B
 24 radiation ≥ 1.0 W m⁻² and 0.5 W m⁻², respectively, and reached a maximum at UV-B radiation \geq
 25 1.5-3.0 W m⁻² (Fig. 6A). The increase in SOD activity caused by UV-B radiation was greater in *G.*
 26 *amansii* than in *P. capillacea*.

1 CAT activity in *P. capillacea* increased as UV-B radiation increased from 0.5-1.0 W m⁻² and
2 then slightly decreased ($p = 0.0025$), while CAT activity in *G. amansii* was increased by UV-B
3 radiation ≥ 1.5 W m⁻² ($p = 0.0051$) (Fig. 6B). The APX activity of *P. capillacea* was increased by
4 UV-B radiation and reached a maximum at 1.0 W m⁻² UV-B radiation, followed by a slight decrease
5 to a level that was still higher than the control ($p = 0.0052$) (Fig. 6C). In contrast, the APX activity
6 in *G. amansii* decreased linearly as UV-B radiation increased ($p = 0.0408$) (Fig. 6C). GR activity in
7 *P. capillacea* increased linearly as UV-B radiation increased ($p = 0.0062$) while that of *G. amansii*
8 increased to a peak at 1.0 W m⁻², followed a sharp decline to a level below the control when
9 exposed to 1.5-3.0 W m⁻² ($p = 0.0408$) (Fig. 6D).

Figure 6

11 4. Discussion

12
13 This study shows that UV-B radiation causes oxidative stress in *P. capillacea* and *G. amansii*.
14 The over-production of ROS and the induction of oxidative stress by UV-B radiation have been
15 found in several algae and cyanobacteria (Tschiersch and Ohmann, 1993; Foyer et al., 1994; Lesser,
16 1996; Malanga et al., 1997; He et al., 2002; Rijstenbil, 2002; Shiu and Lee, 2005). The higher levels
17 of lipid peroxidation and H₂O₂ production in *G. amansii* in response to UV-B radiation evidence
18 that *G. amansii* which inhabits the lower subtidal regions was relatively more susceptible to UV-B
19 radiation than *P. capillacea* inhabiting the upper subtidal regions. A survey of the UV tolerance of
20 rhodophytes from tropical habitats showed that UV exposure caused less pronounced effects in
21 intertidally occurring species than in subtidal species (van de Poll et al., 2003). It suggests that algae
22 in the upper subtidal regions have developed mechanisms to cope with high levels of UV/solar
23 radiation. Arctic marine macroalgal species from the eulittoral and upper sublittoral regions exhibit
24 higher antioxidant enzyme activity upon exposure to UV radiation than species from lower
25 sublittoral regions (Aguilera et al., 2002). A study of UV radiation effects on photosynthetic
26 performance, growth and the capability to synthesize MAAs of the aeroterrestrial green algae

1 *Stichococcus* sp. and *Chlorella luteoviridis* that form biofilms on building facades, and compared
2 with the responses of green algae from soil (*Myrmecia incisa*) and from brackish water
3 (*Desmodesmus subspicatus*) shows that the biosynthesis and accumulation of MAAs under UV
4 radiation resulted in the reduced sensitivity of growth and photosynthesis to UV radiation, which
5 consequently may enhance survival in an environmentally harsh habitat (Karsten et al., 2007). It is
6 believed that *P. capillacea*, from the upper subtidal regions, has higher amounts of UV
7 absorbing/screening compounds as well as other mechanisms related to UV-B radiation resistance
8 than *G. amansii* from the subtidal regions.

9 Algae are known to synthesize MAAs as an effective UV sunscreen to prevent UV-induced
10 photodamage (Riegger and Robinson, 1997; Dunlap and Shick, 1998; Karsten et al., 1998). But, the
11 ability to synthesize MAAs in response to environmental changes is different for each algae species.
12 In the present study, the data of Fig. 3 have shown that *P. capillacea* contained 2-fold higher MAA
13 amounts than *G. amansii*, and MAA accumulation was induced by UV-B radiation only in *P.*
14 *capillacea* but not in *G. amansii*. A similar result was reported by Hoyer et al. (2001) in a study of
15 the distribution pattern of MAA and responses to environmental radiations in Antarctic macroalgae.
16 MAA content was found to be negatively correlated with water depth, while MAA content was not
17 changed after transplantation from deep waters to shallow waters. They have identified 3
18 physiologically different groups of algae in terms of MAA values: 1. species with no capability for
19 MAA biosynthesis; 2. species with a basic MAA content which is adjusted relative to changes in
20 environmental radiation; 3. species with constant high MAA content irrespective of environmental
21 conditions. Evidently, *P. capillacea* belongs to the group 2 and *G. amansii* is a species similar to
22 group 1. Since MAA is a primary sunscreen compound against UV-B radiation, it can be suggested
23 that *G. amansii* with less MAA is more susceptible to UV-B radiation when compared to *P.*
24 *capillacea*.

25 Carotenoid is involved in UV-B radiation defense in *P. capillacea*. As MAA concentration
26 decreased when exposed to 1.5-3.0 W m⁻² UV-B radiation, carotenoid concentration in *P.*

1 *capillacea* increased (see Figs. 3 and 4). *Gelidium amansii* did not accumulate carotenoid in
2 response to UV-B radiation; it linearly decreased when exposed to 0.5-3.0 W m⁻² UV-B radiation.
3 Carotenoid performs a photoprotective role by quenching triplet state chlorophyll molecules and
4 scavenging singlet oxygen and other toxic oxygen species formed within chloroplasts (Young,
5 1991). The induction of carotenoid and detoxifying enzymes or radical quenchers and antioxidants,
6 as well as the repair of UV-induced DNA damage, are modulated to counteract the toxicity of UV
7 radiation (Singh et al., 2008). By increasing the capacity for carotenoid synthesis through the
8 transformation of tobacco plants with a heterologous carotenoid gene encoding β -carotene
9 hydroxylase from *Erwinia uredovora* under constitutive promoter control for the conversion of
10 β -carotene into zeaxanthin, Gotz et al. (2002) suggest that the enhancement of zeaxanthin formation
11 in the presence of functional xanthophyll cycle contributes to UV protection and the prevention of
12 UV damage. Thus, carotenoids are accumulated in *P. capillacea* to avoid photodamage when it fails
13 to accumulate MAA in response to 1.5-3.0 W m⁻² UV-B radiation. As a result, UV-B-induced
14 photodamage in *P. capillacea* is alleviated.

15 Higher antioxidant concentrations and antioxidant enzyme activities are associated with higher
16 stress tolerance in algae (Butow et al., 1994; Collén and Davison, 1999a, b). However, the present
17 results based on the data of Fig. 5 do not support the above view. We found that higher antioxidant
18 regeneration ability is associated with higher UV-B tolerance. As compared to *G. amansii*, *P.*
19 *capillacea* had higher AsA and GSH regeneration ability in response to UV-B radiation. The
20 magnitude of the UV-B-induced increase of AsA concentration in *P. capillacea* was also higher
21 than that of *G. amansii*.

22 The increases of SOD and CAT activities by UV-B radiation were greater in *G. amansii* than in
23 *P. capillacea*. The response of SOD, the first line of defense against ROS in plants (Alscher et al.,
24 2002), to UV-B stress depends on the algal species and the exposure time. SOD activity in the green
25 macroalga *Ulva fasciata* was increased by UV-B radiation (Shiu et al., 2005). UV-B radiation also
26 increased SOD activity in symbiotic dinoflagellate (Lesser and Shick, 1989) but longterm UV-B

1 exposure decreased SOD activity in *Chlorella vulgaris* (Malanga and Puntarulo, 1997). Arctic
2 macroalgae (*Monostroma arcticum*, *Acrosiphonia penicilliformis*, *Coccotylus truncates*, *Phycodrys*
3 *rubens*, *Palmaria palmate*, *Devaleraea ramentacea*) show less SOD induction by UV-A+UV-B, and
4 it is even depressed in some species (Aguilera et al., 2002). The induction of CAT by UV-B
5 radiation was also observed in land plants such as maize (Bold and Scandalios, 1997) and sunflower
6 (Costa et al., 2002). Because of the low affinity of CAT for H₂O₂ but the high affinity of APX for
7 H₂O₂, the higher induction of CAT activity accompanied by depression of APX activity and higher
8 H₂O₂ production in *G. amansii* seems to indicate that *G. amansii* faces greater oxidative stress upon
9 exposure to UV-B radiation than does *P. capillacea*.

10 APX and GR contribute to H₂O₂ detoxification in *P. capillacea*. GR activity was increased by
11 UV-B radiation to regenerate GSH needed for the reduction of DHA to AsA that is the substrate of
12 APX. In contrast, a decrease in GR activity along with a significant GSSG accumulation in *G.*
13 *amansii* demonstrates that the regeneration of GSH in *G. amansii* was inhibited by UV-B radiation.
14 Accordingly, the regeneration of AsA in *G. amansii* was relatively low than that of *P. capillacea*,
15 and the action of APX on H₂O₂ decomposition concomitantly decreased. So, the capacity for
16 scavenging ROS became lower in *G. amansii* than in *P. capillacea* on exposure to UV-B radiation

17 A comparative study of antioxidant enzyme activity among Arctic macroalgae exposed to UV
18 radiation showed that algal tolerance to UV-induced oxidative stress was correlated with high SOD,
19 APX, and CAT activities (Aguilera et al., 2002). The present study delineated that APX and GR
20 activities are positively correlated with UV-B radiation tolerance in these 2 red algae, but SOD and
21 CAT activities are negatively correlated with UV-B tolerance. It seems that the strategy for UV-B
22 defense is different for subtropical algae compared to Arctic macroalgae.

23 In conclusion, MAA is involved in the regulation of oxidative responses in red macroalgae in
24 subtropical waters in northeastern Taiwan. As MAA accumulates, UV-B is screened and ROS
25 production is reduced. Higher MAA concentration and the ability to accumulate MAA are primarily
26 used by the upper subtidal alga *P. capillacea* for effective screening of UV-B radiation, and when

1 MAA accumulation ability decreased, carotenoid levels were increased. The lower subtidal alga *G.*
2 *amansii*, whose MAA concentration and antioxidant defense capacity were low, suffers severe
3 oxidative stress from UV-B radiation. It seems to suggest that either *G. amansii* inhabits lower
4 subtidal regions because of less MAA concentration or it produces less MAAs because in the lower
5 level it does not need them. It needs more work to prove it.

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- 26

1

2 **Figure Legends**

3

4 Fig. 1. Temporal (sampling times were 2004-J: June 16-22, 2004, 2004-Sept: September 21-30,
5 2004, 2005-Mar: March 17-23, 2005, and 2005-Jun: June 3-11, 2005) and spatial (sampling sites
6 were L: Lung-tung, H: Her-mei, M: Mo-auo, and S: Shea-cherng) changes in biomass (A, C) and
7 total MAA contents (B, D) (means, n=4) in *Ptercladiella capillacea* and *Gelidium amansii*.

8

9 Fig. 2. Changes in TBARS (A) and H₂O₂ (B) contents (means±SD, n=3) in *Ptercladiella capillacea*
10 (●) and *Gelidium amansii* (∇) in response to UV-B radiation. Different letters indicate significant
11 difference among treatments (large font for *Ptercladiella capillacea* and small font for *Gelidium*
12 *amansii*).

13

14 Fig. 3. The total MAA concentrations of (A, E), shinorine (B, F), porphyra 334 (C, G), and
15 palythanol (D, H) in *Ptercladiella capillacea* (●) and *Gelidium amansii* (∇) in response to UV-B
16 radiation. Data are presented as mean±SD (n=3) and different letters indicate significant difference
17 among treatments (large font for *Ptercladiella capillacea* and small font for *Gelidium amansii*).

18

19 Fig. 4. The carotenoid concentration in *Ptercladiella capillacea* (●) and *Gelidium amansii* (∇) in
20 response to UV-B radiation. Data are presented as mean±SD (n=3) and different letters indicate
21 significant difference among treatments.

22

23 Fig. 5. The AsA (A), DHA (B), GSH (C), and GSSG (D) concentrations in *Ptercladiella capillacea*
24 (●) and *Gelidium amansii* (∇) in response to UV-B radiation. Data are presented as mean±SD (n=3)

1 and different letters indicate significant difference among treatments (large font for *Ptercladiella*
2 *capillacea* and small font for *Gelidium amansii*).

3

4 Fig. 6. Effects of UV-B radiation on SOD (A), CAT (B), APX (C), and GR (D) activities (mean \pm SD,
5 n=3) in *Ptercladiella capillacea* (●) and *Gelidium amansii* (∇). Different letters indicate difference
6 among treatments (large font for *Ptercladiella capillacea* and small font for *Gelidium amansii*).

7

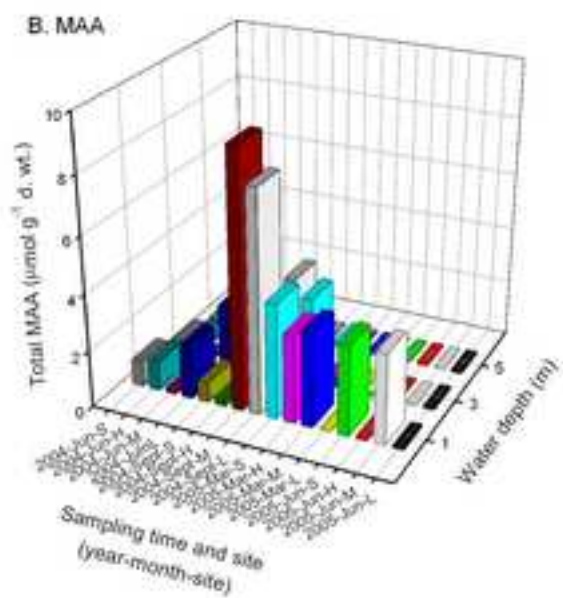
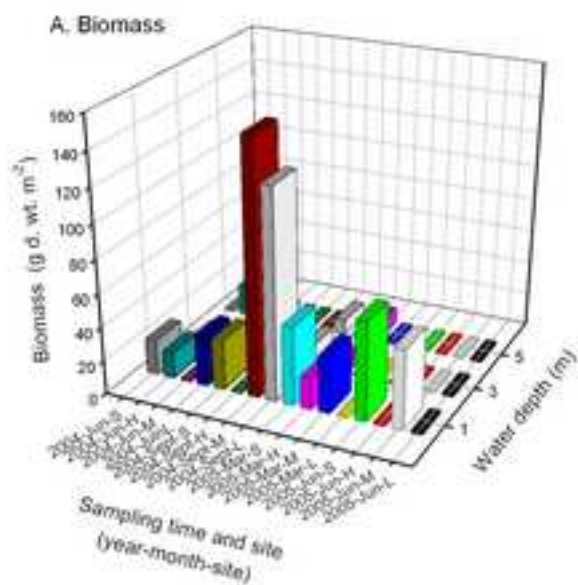
8 **Supplementary data:**

9 Fig. 1. HPLC profile of MAAs of *Ptercladiella capillacea* and *Gelidium amansii* in response to
10 UV-B radiation.

11

12

13

Pteridiella capillacea*Gelidium amansii*