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Mycosporine-like amino acids vs carrageenan yield in *Mazzaella laminarioides* (Gigartinales; Rhodophyta) under high and low UV solar irradiance

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Abstract: The effects of increased solar photosynthetically active radiation (PAR) and ultraviolet radiation (UVR) on biomass, mycosporine-like amino acid (MAA) content, and carrageenan yield were studied for 14 days in tank-cultivated tetrasporophytes and gametophytes of *Mazzaella laminarioides* from the Magellan Strait. The initial values of carrageenan yield were similar between gametophytes and tetrasporophytes, whereas the MAA content was higher in gametophytes than that in tetrasporophytes. After 14 days of exposure to different combinations of solar radiation (PAR, PAR + UV-A, and PAR + UV-A + UV-B) and two irradiance levels (high and low), differences between gametophytes and tetrasporophytes of *M. laminarioides* in growth rate (GR), MAA, and carrageenan yield were observed. Higher GRs were observed in gametophytes than in tetrasporophytes under all radiation and irradiance treatments. A significant decrease in total MAA content was observed in both reproductive phases, with these decreases being more evident in gametophytes under all radiation and irradiance treatments (20 to 30% decrease compared with the initial value). Changes in the proportion of each MAA were also observed. The asterina-330 and palythine content decreased in gametophytes in all radiation and irradiance treatments, whereas tetrasporophytes exhibited an increase of these MAAs, mainly under PAR + UV-A at low irradiance. The carrageenan yield decreased in tetrasporophytes in all radiation treatments, whereas gametophytes exhibited an increase in this parameter under UV radiation at high irradiance. These results suggest that both reproductive phases have different strategies to cope with light stress, mainly under high UVR. Furthermore, both reproductive phases are adapted to high solar radiation, and the differences between gametophytes and tetrasporophytes could result from the vertical distribution on shore and from intrinsic differences related to ploidy level.

Key Words: Carragenan, Irradiance, MAA content, *Mazzaella laminarioides,* Reproductive phases, Rhodophyta, Sub-Antarctic region, UV solar radiation

INTRODUCTION

Algal biomass from natural populations and those produced under culture conditions can be used to extract substances of commercial interest. Red algae (Rhodophyta) are known as the source of C-(polysaccharides) and N-compounds [bili-proteins and mycosporine-like amino acids (MAAs)]. Most of the polysaccharides are unique sulfated galactans, such as agar, agarose, and carrageenans. These polysaccharides have wide practical applications based on their ability to form gels in aqueous solutions (Selby & Whistler 1993; Therkelsen 1993). Carrageenans have potential application as photo-protective agents in addition to being used as excipients (Thevanayagam *et al.* 2014). In the case of N-compounds, MAAs have attracted attention because of both their ultraviolet (UV) screening and antioxidant properties (Dunlap & Yamamoto 1995; Bandaranayake 1998; Shick & Dunlap 2002; Jahan *et al.* 2017), being used as a source of commercial sunscreens. Furthermore, it was reported that MAAs may act as UV-absorbing compounds, modulating the expression of genes associated with oxidative stress, inflammation, and skin aging caused by UV radiation (UVR) (Suh *et al.* 2014).

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Even though the content of MAAs varies among species, the content and composition of these compounds in species of red algae have been related to environmental conditions such as quantity and quality of solar radiation [e.g. UVA and UV-B or by photosynthetically active radiation (PAR)] (Shick *et al.* 1999; Franklin *et al.* 2001; Hoyer *et al.* 2002; Korbee *et al.* 2005a), desiccation, salinity, and temperature (Karsten *et al.* 2003; Jiang *et al.* 2008). Furthermore, because they are nitrogenous compounds, a positive relationship between nitrogen availability and synthesis/accumulation of MAAs has been reported in different species of Rhodophyta (Korbee-Peinado *et al.* 2004; Korbee *et al.* 2005b; Huovinen *et al.* 2006; Figueroa *et al.* 2008; Bonomi-Barufi *et al.* 2011; Navarro *et al.* 2014). However, a negative effect on polysaccharide yield could be expected under conditions of increased nitrogen availability because the nitrogen supply does not favour carbohydrate synthesis (Lapointe & Duke 1984; Gómez-Pinchetti *et al.* 1998). Irrespective of nitrogen availability, synthesis/accumulation of polysaccharides and MAA content and composition has been thought of as protection mechanisms against UV stress. Although MAAs can absorb UVR and dissipate this energy in thermal form (Conde *et al.* 2000, 2004), reducing the photodamage (Lesser 1996, Bischof *et al.* 2000), the increase in the density and thickness of cell walls can prevent or reduce the penetration of UV-B into cells by scattering, absorption, and dispersion of radiation (Schmidt *et al.* 2009, 2012; Navarro *et al.* 2010).

Nevertheless, exposure to UV-B radiation strongly inhibited the carrageenan yield and the gel strength in *Kappaphycus alvarezii* (Doty) Doty ex P.Silva (Eswaran *et al*. 2001). However, the relationship of polysaccharide and MAA content under light stress conditions has not been studied in detail, above all not in an important carrageenan- producing red alga, as is the case for *Mazzaella laminarioides* (Bory de Saint Vincent) Fredericq.

Mazzaella laminarioides has a triphasic sporic life history with isomorphic haploid male and female gametophytes and a diploid tetrasporophyte. Fourier transform–infrared analysis has shown differences in the type of polysaccharide between both haploid and diploid phases, with tetrasporophytes producing lambda carrageenan and gametophytes producing iota–kappa carrageenan-type polysaccharides (Matsuhiro & Rivas 1993; Navarro *et al*. 2014).

The aim of this study was to evaluate the production of N-(MAAs) and C-compounds (polysaccharides) in gametophytes and tetrasporophytes of *Mazzaella laminarioides* grown in tanks under different combinations of solar radiation (PAR, PAR + UV-A, and PAR + UV-A + UV-B) and two irradiance levels (high and low).

The hypothesis that guided our investigation was that MAA concentration would increase under full solar radiation, mainly under PAR + UV, and that the content of polysaccharide (carrageenan) would increase when algae are cultured under low irradiance. However, we also hypothesized that under full solar UV-B radiation, the polysaccharide would also increase as a protection mechanism. To test our hypothesis, *Mazzaella laminarioides* was grown in tanks outside for 14 days during spring when a decrease of ozone level and a consequent increase in UV-B radiation occurs. *Mazzaella laminarioides* is an endemic intertidal Chilean species distributed on rocky shores from 28°S to 56°S. This species is considered an important carrageenan-producing red alga, and for this reason it has been harvested in southern Chile together with other carrageenophytes including *Gigartina skottsbergii* Setchell & N.L.Gardner and *Sarcothalia crispata* (Bory) Leister (Buschmann *et al*. 2001; Marín *et al*. 2002).

MATERIAL AND METHODS

Tetrasporophytic and gametophytic fronds of *Mazzaella laminarioides* were collected from the intertidal zone of Bahía Mansa (53°36′S; 70°55′W) in the Strait of Magellan during low tide and transported to the Universidad de Magallanes Marine Biology Laboratory. In the laboratory, apical portions (3-cm length) without reproductive structures were cut out and placed in tanks filled with seawater (50 litres, salinity 32) without enriched medium. The tanks were exposed to solar radiation and aerated every 30 min for 3d. Forty apical portions [10 g fresh weight (FW) in total] of *Mazzaella laminarioides* of each reproductive phase were placed into tanks filled with 50 litres of filtered seawater. Each tank received an alternating 30-min aeration period and the seawater was renewed every 2 h. Eighteen tanks were prepared for each life-history generation (gametophytes and tetrasporophytes). To assess the effects of different radiation treatments, the tanks were covered with a 395-nm cutoff filter (Ultraphan 395; Digefra GmbH. Munich, Germany), a 320-nm cutoff filter (Folex 320; Cologne, Germany), or with a 295-nm cutoff filter (Ultraphan 295; Digefra GmbH, Munich, Germany) as reported previously (Figueroa *et al*. 1997). The use of these filters results in algae exposed to different radiation treatments: PAR (P), PAR + UV-A (PA), or PAR + UV-A + UV-B (PAB), respectively. To assess the effect of light intensity, nine tanks were covered with a black mesh that filters up to 65% of the total radiation (low light treatment: LL), whereas the remaining tanks were exposed to full solar radiation (high light treatment: HL). Thus, each radiation (P, PA, and PAB) has two levels (HL and LL), and each one of those treatments has three replicates.

Growth rates (GRs), carrageenan yield, and MAA content and composition were assessed in apical portions exposed to solar radiation over 14 d. The algal FW was recorded with an analytical balance. Values were obtained at the beginning and after 14 d of the experimental period. The GRs were estimated according to Lignel & Pedersén (1989):

$$\mathrm{GR} = \left[(\ln \mathrm{FW_f} - \ln \mathrm{FW_i})t^{-1}\right]100$$

where $\mathrm{FW_i}$ is the initial and $\mathrm{FW_f}$ is the final weight of the tetrasporophytes of *Mazzaella laminarioides* after t days of culture under different treatments. Units of GR were percentage of fresh weight per day (% $\mathrm{FW \cdot d^{-1}}$).

MAAs were determined using 10 to 20 mg [dry weight (DW)] of alga weighed in four replicates with MAAs extracted in 1 ml of 20% aqueous methanol (v/v) for 2 h at 45°C. After extraction, 600 µl of the supernatant was evaporated in a rotaevaporator under vacuum to dryness. Dried extracts were redissolved in 600 µl of 100% methanol followed by filtration through a 0.2-µm membrane filter. MAAs were determined by injecting 30 µl of each sample into a Spheroclone C8 column (Aschaffenburg, Germany) with a precolumn (5-mm packing; 250 × 4 mm inner diameter) coupled to a Waters (Barcelona, Spain) high-performance liquid chromatography system according to Karsten *et al*. (1998) and modified by Korbee-Peinado *et al*. (2004). MAAs were detected with a Waters photodiode array detector (Waters 996; Barcelona, Spain) at a wavelength of 330 nm. Absorption spectra were recorded between 290 and 400 nm. The quantification followed the method described by Korbee-Peinado *et al*. (2004).

Polysaccharide extraction for carrageenan was carried out as described in Navarro *et al*. (2014). Briefly, polysaccharide was extracted from 2 g of dry *Mazzaella laminarioides* in distilled water at 95°C for 3 h. The digestion product was centrifuged at 10,733 × *g* (Hermle Labortechnik GmbH, Wehingen, Germany) for 10 min, and the supernatant was collected and concentrated in a vacuum rotary evaporator. Subsequently, the polysaccharides were precipitated in 100% ethanol. The precipitate was dissolved in distilled water, frozen, and freeze dried. Finally, the carrageenan yield was calculated as a percentage of algal dry mass:

$$\mathrm{Yield} = (\mathrm{DW\ carrageenan/DW\ algae}) \times 100.$$

To assess the effect of radiation treatment, light intensity, reproductive phase, and the interaction among these factors on all data (GR, carrageenan yield, and MAA content and

Table 1. Results from multifactorial analyses of variance for each dependent variable, showing simple effects result and interaction between independent variables. Data obtained from gametophytes and tetrasporophytes of *Mazzaella laminarioides* cultivated under different solar radiation treatments: PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB), and under two irradiances (HL: high light, and LL: low light) during 14 d. Dependent variables: GR, growth rate; MAAs, mycosporine-like amino acids; myc–glyc, mycosporine–glycine; shinorine; palythine; and asterine-330. Bold values mean significant effectiveness at $P < 0.05$.

	Phase (A)		Irradiance (B)		Radiation treatments (C)		A × B		A × C		B × C		A × B × C
	df:1		df: 1		df: 2		df: 1		df: 2		df: 2		df: 2
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Myc–glyc	1.07	0.306	4.97	**0.031**	2.02	0.145	2.51	0.120	0.63	0.535	2.61	0.085	4.90	**0.012**
Shinorine	55.03	**0.001**	0.00	0.967	1.09	0.344	0.31	0.579	0.89	0.415	8.71	**0.001**	10.04	**0.001**
Palythine	71.81	**0.001**	0.03	0.860	4.93	**0.010**	0.15	0.695	7.51	**0.001**	1.31	0.277	2.95	0.060
Asterina-330	145.46	**0.001**	0.50	0.482	4.36	**0.017**	0.00	0.970	6.48	**0.003**	2.66	0.078	0.64	0.531
MAAs	78.50	**0.001**	0.00	1.000	2.93	0.061	0.02	0.890	3.18	**0.049**	5.15	**0.009**	7.23	**0.002**
Carrageenan yield	271.37	**0.001**	5.55	**0.027**	3.74	**0.039**	33.69	*0.001*	1.18	0.324	1.98	0.160	4.12	**0.029**
GR	8.87	**0.005**	22.26	**0.001**	14.25	**0.001**	2.97	0.093	2.49	0.097	5.01	**0.012**	4.23	**0.022**

composition), a multifactorial analysis of variance (ANOVA) was done. The initial values of carrageenan yield and MAA content and composition of tetrasporophytes and gametophytes were compared using a Student's *t* test. Normality and homogeneity of variances were evaluated before the Student's *t* test and ANOVA procedure. In the case of the percentage of change of carrageenan and MAA content, values were arcsine transformed. An *a posteriori* Fisher (least significant difference) test was used to establish statistical differences. Dependent variables (GR, carrageenan yield, and MAA content and composition) were submitted to Pearson correlation analysis. Statistical significance was set to $P < 0.05$.

Additionally, to reduce the dimensionality of the data set and to illustrate the different responses between gametophytes and tetrasporophytes when exposed to stress factors, all data sets (GR, carrageenan yield, and MAA content in gametophytes and tetrasporophytes cultivated under cultivated under P, PA, and PAB and under HL and LL during 14 d) were incorporated in a principal component analysis (PCA). For HL and LL the values of 0 and 1 were assigned, respectively. In the same way, for radiation treatments values of 0, 1, and 2 were assigned. Afterward, all data sets were normalized to improve linearity and reduce outlier effect. All data were evaluated using the software STATISTICA 7.0 (Copyright Statsoft. Inc., Tulsa, OK, USA) except for the PCA, which was performed using PRIMER 6.1 (PRIMER-E Ltd, Plymouth, United Kingdom).

RESULTS

The multifactorial ANOVA for each of the parameters evaluated in gametophytes and tetrasporophytes of *Mazzaella laminarioides* are shown in Table 1. GR, carrageenan yield, and MAA content and composition are variables that were influenced by the interaction of reproductive phase, irradiance, and radiation treatments.

Growth rates

Relative GRs were influenced by the interactions among reproductive phase, radiation, and irradiance treatment ($F = 4.23$; $P = 0.022$). Higher GRs were observed in gametophytes than in tetrasporophytes under all radiation and irradiance treatments, with the exception under PA at HL, where the latter exhibited the highest GR during the experiment (Fig. 1).

The initial weight:area ratio was similar between gametophytes and tetrasporophytes (Fig. 2). Even though a decrease in weight:area ratio in both reproductive phases after 14 d of cultivation was observed, this decrease was not significantly different.

MAA content and composition

The initial MAA concentration of gametophytes (3.0 ± 0.4 mg g^{-1} DW) was higher than in tetrasporophytes (2.3 ± 0.5 mg g^{-1} DW) ($P = 0.037$). These concentrations were affected by the reproductive phase and by the interaction among reproductive phase, irradiance, and radiation treatment

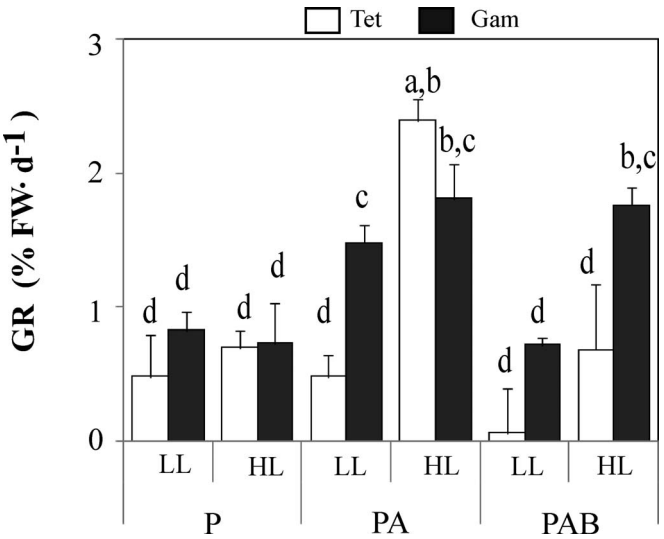


Fig. 1. Growth rate (GR: % FW d^{-1}) of tetrasporophytes (Tet) and gametophytes (Gam) of *Mazzaella laminarioides* cultivated under different solar radiation treatments: PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB), and under two irradiances (HL: high light, and LL: low light) during 14 d. Data are expressed as mean values ± standard error of the mean ($n = 3$). Different letters indicate differences ($P < 0.05$).

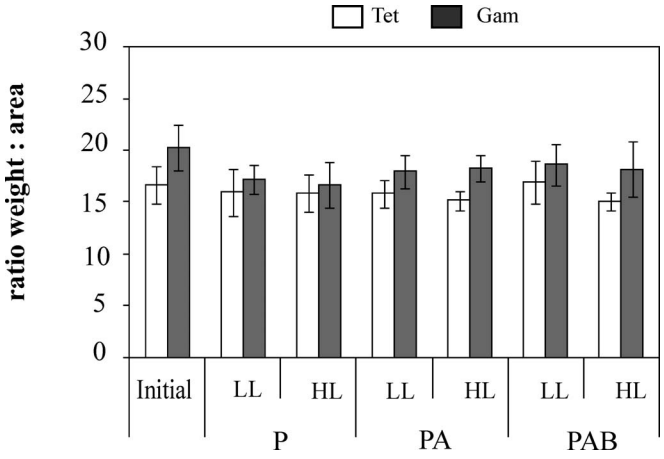


Fig. 2. Weight:area ratios of tetrasporophytes (Tet) and gametophytes (Gam) of *Mazzaella laminarioides* cultivated under different solar radiation treatments: PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB), and under two irradiances (HL: high light, and LL: low light) during 14 d. Data are expressed as mean values ± standard error of the mean ($n = 3$).

(Table 1). Higher values were observed in tetrasporophytes than in gametophytes under all radiation treatments, but mainly under PA at LL treatment (Fig. 3). A significant decrease in MAA content was observed in gametophytes under all radiation and irradiance treatments (20 to 30% of decrease related to initial value) (Fig. 4).

Four different MAAs were identified in *Mazzaella laminarioides*: shinorine, palythine, asterina-330, and mycosporine–glycine. Variations in terms of concentration (Figs 5–12) and proportion of each MAA relative to total MAA concentration (Figs 13–16) were observed during the experiment. Whereas gametophytes showed a slight decrease in palythine content under all radiation and irradiance treatments, tetrasporophytes exhibited an increase of this MAA, mainly under PA at LL treatments (Figs 7, 8). A similar trend was observed in the asterina-330 content (Figs 9, 10). A decrease in the mycosporine-glycine concentration was also observed in both gametophytes and tetrasporophytes. This decrease was noteworthy at PAB under both LL and HL irradiance treatments (Figs 11, 12). Even though initial concentration of each specific MAA was similar between gametophytes and tetrasporophytes (Figs 7, 9, 11), the proportion of shinorine and palythine relative to the total MAA concentration was different in gametophytes and tetrasporophytes (Figs 13, 14). After 14 days of cultivation, tetrasporophytes exhibited a higher proportion of palythine and asterina-330 (Figs 14, 15) while gametophytes increased the proportion of mycosporine -glycine mainly under PA and PAB treatments (Fig. 16).

Carrageenan yield

Initial carrageenan yield of gametophytes (33.9 ± 2.4) and tetrasporophytes (29.8 ± 7.9) was similar ($P = 0.311$). These yields varied after 14 d of culture, and this variation in yield was caused by the interaction among reproductive phase, radiation, and irradiance treatment ($F = 4.12$; $P = 0.029$). Whereas the carrageenan yield decreased in tetrasporophytes under all radiation treatments, but mainly under HL, gametophytes exhibited an increase in carrageenan yield in HL under PA and PAB treatments (Figs 17, 18).

Pearson correlation values obtained among dependent variables assessed in gametophytes and tetrasporophytes of *Mazzaella laminarioides* are shown in Table 2. Most variables were significantly correlated. GR was significantly and negatively correlated to the carrageenan yield in tetrasporophytes, whereas in gametophytes a positive correlation was observed ($P < 0.05$). Carrageenan yield was negatively correlated with light intensity in tetrasporophytes but positively correlated in gametophytes. It is important to note that carrageenan yield was negatively correlated with all types of MAAs in gametophytes, and positively correlated in the case of tetrasporophytes, although these values were not significant.

Principal component analysis

Results for PCA analysis are shown in Table 3 and in Fig. 19; the position of the 36 samples included in the analysis is shown. The three principle axes accounted for 90% of the variation, indicating that the remaining 10% was not explained by this representation. In the first component, 40% of the variation was accounted for by MAAs and carrageenan, with the former being positively and the latter negatively related. Along the second component, an additional 36% of the variation was accounted for by irradiance and GR (positive correlation) and by carrageenan yield (negative correlation). An additional 14% of the variation is accounted for by PCA axis 3, with irradiance and GR having the strongest correlations with this axis (–0.667 and 0.541, respectively).

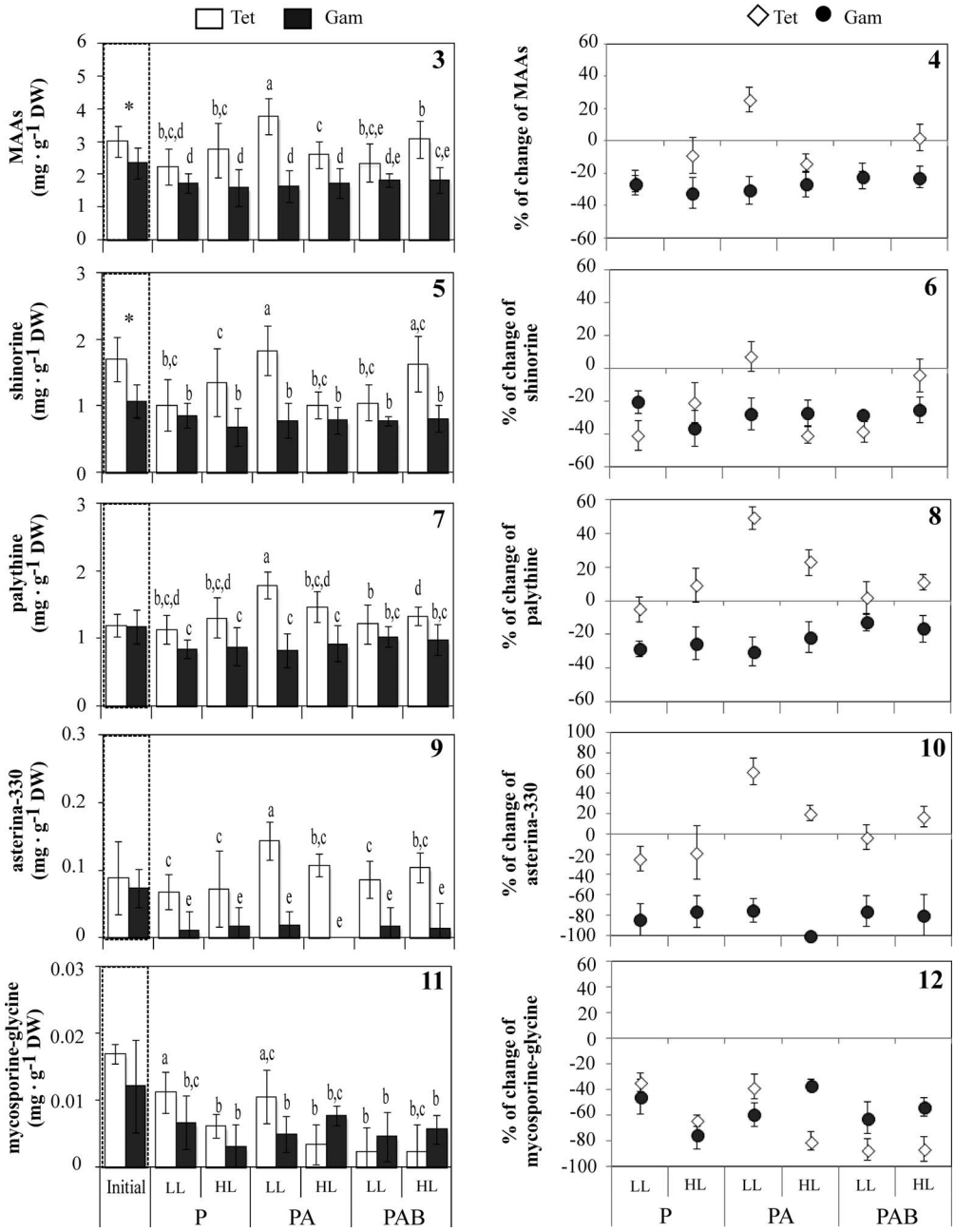
DISCUSSION

This study shows different physiological responses of gametophytes and tetrasporophytes of *Mazzaella laminarioides* to high irradiance, assessed as carrageenan yield and MAA content and composition, when exposed to different solar radiation treatments.

Gametophytes vs tetrasporophytes under UVR

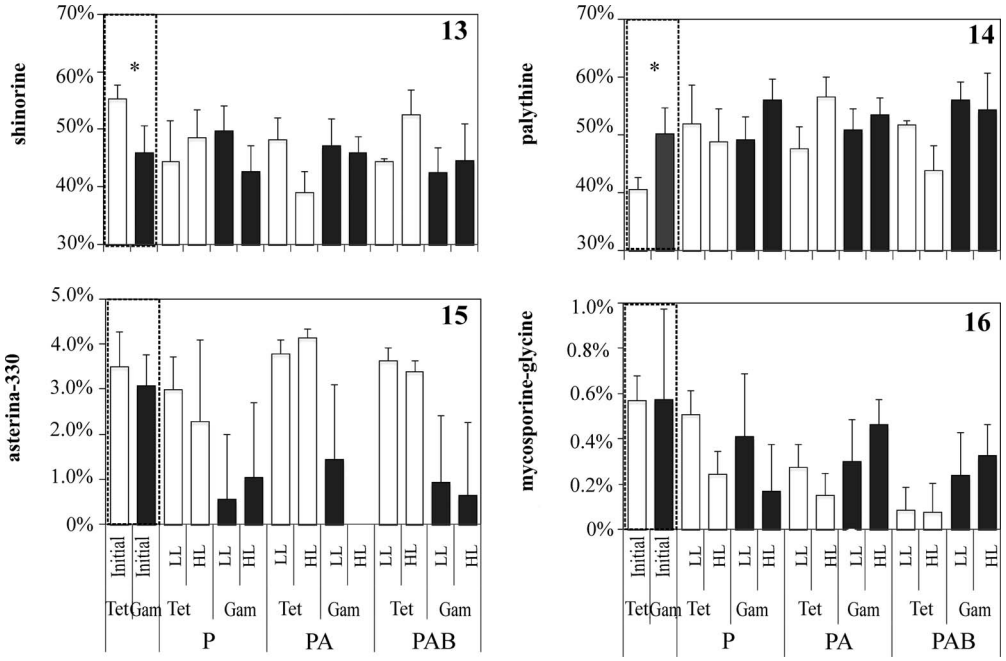
Differential responses of carrageenan yield and MAA content and composition between the reproductive phases of *Mazzaella laminarioides* were observed during the exposure to different light qualities of solar radiation.

An increase of MAAs under full solar radiation, mainly under high PAR + UV, was expected; however, the total MAA content tended to be reduced in both gametophytes and tetrasporophytes under almost all treatments, with the exception of low PAR + UV-A. Under the last treatment, tetrasporophytes exhibited an increase in MAAs as observed in *Porphyra columbina* Montagne cultivated under PAR + UV-A solar radiation (Korbee-Peinado *et al.* 2004). The total MAA increase in *Mazzaella laminarioides* was a result of the increase of shinorine, palythine, and asterina-330. The two latter MAAs could be increased as a photoregulated response as a consequence of light stress because these MAAs have high



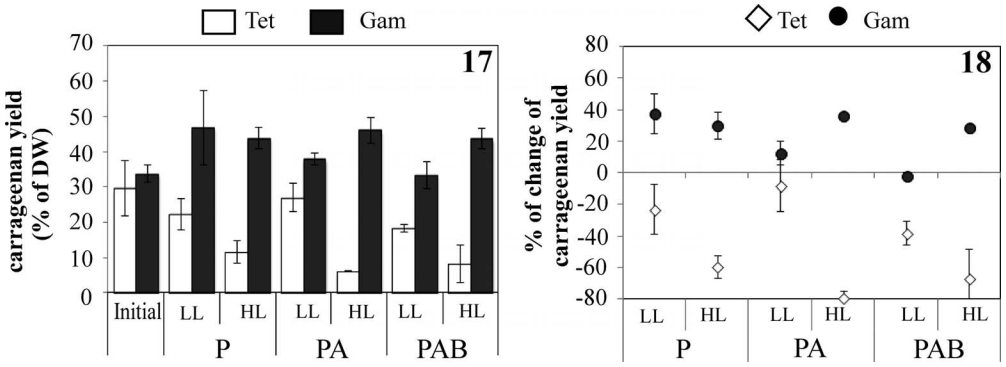
Figs 3–12. Total MAA content, MAA composition, and changes in the percentage (in relation to initial values) of total MAAs in tetrasporophytes (Tet) and gametophytes (Gam) of *Mazzaella laminarioides* cultivated under different solar radiation treatments: PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB) and under two irradiances (HL: high light, and LL: low light) during 14 d. Data are expressed as mean values ± standard error of the mean ($n = 3$). * indicates differences between initial mean values (Student's t test; $P < 0.05$), whereas different letters indicate differences among treatments during 14 d [Fisher least significant difference test: $P < 0.05$].

antioxidant activity (De la Coba *et al.* 2009). By using polychromatic light, Kräbs *et al.* (2002) reported that the wavelengths that are effective for the induction of MAAs vary within MAAs. In fact, wavelengths between 350 and 490 nm (UV-A and blue light) promoted the accumulation of shinorine in *Chondrus crispus* Stackhouse (Kräbs *et al.* 2002), and palythine and asterina-330 in *P. leucosticta* Thuret (Korbee *et al.* 2005a). The results of the present study are partially consistent with those reported by Navarro *et al.* (2016) for gametophytes and tetrasporophytes of *M. laminarioides* grown under different levels of solar radiation. Those authors reported a spontaneous response of exposure to different radiation treatments: shinorine accumulation in tetrasporophytes under PA and PAB at 6 d, and asterina-330 accumulation in tetrasporophytes under P and in gametophytes under PAB. In addition, synthesis of mycosporine–glycine (UV-B-absorbing MAA) was observed in gametophytes and tetrasporophytes as a cumulative response to the solar radiation exposure. Even though not all of our results matched those reported by Navarro *et al.* (2016), the



Figs 13–16. Relative MAA composition (mean, $n = 3$) in tetrasporophytes (Tet) and gametophytes (Gam) of *Mazzaella laminarioides* cultivated under different solar radiation treatments: PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB) and under two irradiances (HL: high light, and LL: low light) during 14 d. Data are expressed as mean values ± standard error of the mean ($n = 3$).

ability of the reproductive phases to change their internal MAA concentrations under highly variable environmental radiation was demonstrated. Furthermore, Navarro *et al.* (2016 and here) showed differential responses of gametophytes and tetrasporophytes after exposure to solar radiation. Our work also demonstrates that the reproductive phases respond differently not only under high UV, including PA and PAB treatments, but also under low UVR. Moreover, the most notable differences between gametophytes and tetrasporophytes appear mainly under PA and PAB at high irradiance (e.g. % asterina-330 and carrageenan yield). This result could suggest that both gametophytes and tetrasporophytes have different strategies regarding MAAs and carrageenan content accumulation to cope with different UV light stress. Tetrasporophytes exhibited a higher MAA content when compared with gametophytes, independently of irradiance and radiation treatments. In contrast, gametophytes exhibited an increase in carrageenan yield when exposed to high UV (PA and PAB treatments) solar radiation compared with tetrasporophytes. This last result supports our hypothesis about the effect of UVR on polysaccharide synthesis under high solar radiation in gametophytes but not in tetrasporophytes. The increase of the polysaccharide yield has been thought of as part of a protection mechanism, preventing or reducing the penetration of UVR into cells by scattering, absorption, and dispersion of radiation, as suggested for *Kappaphycus alvarezii* (Schmidt *et al.* 2009, 2010), *Iridaea cordata* (Navarro *et al.* 2010), and *Chondracanthus teedei* (Mertens ex Roth) Kützing (Schmidt *et al.* 2012). Schmidt *et al.* (2012) and Navarro *et al.* (2010) reported an increase in the density and thickness of cell walls, and this increase implies an intense polysaccharide production (Pue-



Figs 17, 18. Carrageenan yield and percentage of change of carrageenan yield in relation to the initial values in tetrasporphytes (Tet) and gametophytes (Gam) of *Mazzaella laminarioides* cultivated under different solar radiation treatments: PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB) and under two irradiances (HL: high light, and LL: low light) during 14 d. Data are expressed as mean values ± standard error of the mean ($n = 3$).

Table 2. Pearson correlation values obtained among dependent variables after cultivating of *Mazzaella laminarioides* under three radiation treatments: PAR (P), PAR + UV-A (PA), and PAR + UV-A + UV-B (PAB), and under two intensities of light (HL: high irradiance, and LL: low irradiance) during 14 d. Values range between −1 and 1. Bold values are significantly correlated at $P < 0.05$. Dependent variables: MAAs, mycosporine-like amino acids; myc–glyc, mycosporine–glycine; shinorine; palythine; asterina-330; GR, growth rate; and carrageenan yield ($n = 18$).

	Myc–glyc	Shinorine	Palythine	Asterina	MAAs	Carragenan	GR
Gametophytes							
Irradiance	−0.021	−0.118	0.021	−0.162	−0.048	**0.546**	0.433
Radiation treatment	−0.043	−0.075	0.252	0.009	0.085	−0.380	0.280
Myc–glyc		0.111	0.170	−0.006	0.163	0.226	−0.021
Shinorine			**0.672**	−0.134	*0.855*	−0.011	−0.163
Palythine				0.100	*0.932*	−0.137	−0.148
Asterina-330					0.117	−0.296	0.106
MAAs total						−0.104	−0.179
Carragenan							0.259
Tetrasporophytes							
Irradiance	−0.432	0.086	−0.016	0.021	0.086	**−0.868**	**0.518**
Radiation	**−0.526**	0.167	0.072	0.190	0.111	−0.210	−0.170
Myc–glyc		0.187	0.260	0.181	0.241	**0.627**	−0.197
Shinorine			**0.702**	**0.764**	*0.942*	0.315	−0.276
Palythine				**0.960**	*0.863*	0.249	−0.100
Asterina-330					*0.905*	0.236	−0.066
MAAs total						0.336	−0.197
Carragenan							**−0.624**

schel 1979; Tsekos 1981, 1985) from intense Golgi and endoplasmic reticulum activity (Pueschel 1979; Tsekos 1981, 1985; Delivopoulus *et al.* 1999). However, in the case of *K. alvarezii* and *C. teedei*, the increase in cell wall thickness was a result of neutral polysaccharide (possibly cellulose) synthesis (Schmidt *et al.* 2009, 2012) but not necessarily carrageenan accumulation. In fact, a decrease in carrageenan yield and in gel strength was reported in *K. alvarezii* exposed to UV-B radiation (Eswaran *et al.* 2001). Data from the latter study support our results only for tetrasporophytes of *M. laminarioides*, in which a decrease in carrageenan yield took place after 14 d of exposure to solar radiation. Furthermore, Navarro *et al.* (2014) reported that tetrasporophytes of *M. laminarioides* did not increase the carrageenan yield when compared with the initial value after 18 d of culture under full solar radiation. Thus, our data could suggest that *M. laminarioides* has a different dynamic accumulation of carrageenan, which depends on the reproductive phase, with the gametophyte increasing its carrageenan content under solar radiation stress. This increase in carrageenan content may contribute to diminish the negative effects of UVR in gametophytes. Even though synthesis of extracellular polysaccharides with its UV-absorbing compounds was suggested as a passive UV screen against long-time exposure in the cyanobacterium *Nostoc commune* Vaucher ex Bornet & Flahault (Ehling-Schulz *et al.* 1997), further investigation is needed to assess the relevance of carrageenan content as a protection mechanism against UVR in macroalgae.

Table 3. Results of the principal component analysis. Thirty-six cases were included in the analysis considering the MAA composition, carrageenan yield, and GR of gametophytes and tetrasporophytes cultivated under high and low irradiance. Only the loading factors for the three first components are shown. Bold values are significantly correlated at $P < 0.05$.

Eigenvalues			
	Eigenvalues	%Variation	Cumulative%variation
PC1	1.61	40.3	40.3
PC2	1.44	36.0	76.3
PC3	0.567	14.2	90.5
Eigenvectors (coefficients in the linear combinations of variables making up PCs)			
Variable	PC1	PC2	PC3
Irradiance	−0.052	**0.709**	**−0.667**
MAAs	**0.701**	0.084	−0.190
Carrageenan	**−0.615**	−0.324	−0.476
GR	−0.358	**0.620**	**0.541**

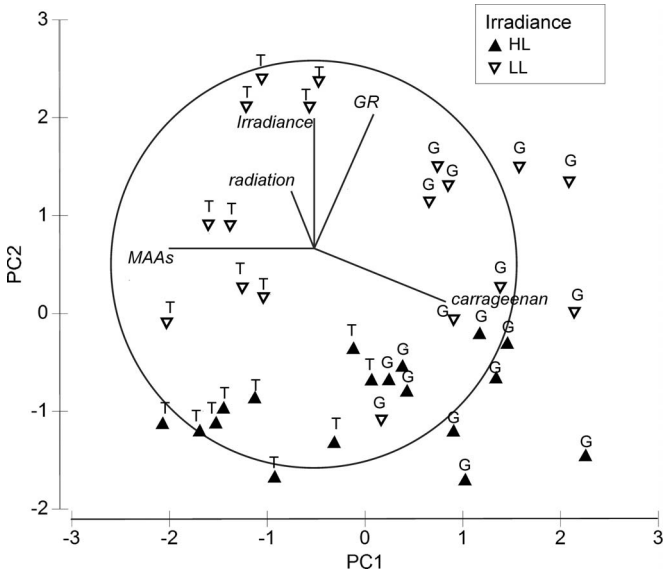


Fig. 19. Principal component analysis for tetrasporophytes (T) and gametophytes (G) of *Mazzaella laminarioides* cultivated under two irradiances (HL: high light, and LL: low light) during 14 d. Plots of axis PC1 vs axis PC2 representing the position of the 36 samples are included in the analysis.

The first axis in the PCA is determined, not by the irradiance to which the alga have been exposed, but by the differences between the carrageenan values and the MAAs, independently of the lighting conditions. On the PCA, it is important to indicate that a high variance explained with the first three axes, using a much lower number of variables than the samples, is precisely a guarantee that a few variables are really responsible for the variability of the data. Furthermore, the use of categorical or binary variables is perfectly accepted in the use of multivariate analysis. In our case, the clear differentiation between both levels along axis two could be explained by the use of the binary variables (HL-LL). However, it is import to emphasize that axis two is also determined by GR.

Ecological considerations

The fact that both gametophytes and tetrasporophytes grew under high solar radiation reflects that *Mazzaella laminarioides* is tolerant to high PAR and UVR. *Mazzaella laminarioides* inhabits the high and medium intertidal zone, where it is exposed to a highly variable light environment (UVR, temperature, salinity, and so on). Under these conditions, several mechanisms of acclimation could be used by this species, such as dynamic photoinhibition (Gómez *et al.* 2004; Marquardt *et al.* 2010) and even greater antioxidant enzymatic activity than that exhibited by species from the low intertidal and subtidal zones (Flores-Molina *et al.* 2014). Intertidal species can also avoid or minimize the damage caused by high solar radiation including UVR by accumulating MAA compounds (Dunlap & Shick 1998; Franklin *et al.* 2001; Korbee-Peinado *et al.* 2004; Bischof *et al.* 2006). In the case of *M. laminarioides*, both gametophytes and tetrasporophytes had MAAs, but in different concentrations. The difference between the MAA concentrations in each phase could result from the vertical distribution in the intertidal zone, with gametophytes distributed mainly in the upper to mid-intertidal and tetrasporophytes in the mid- to lower intertidal zones (Navarro *et al.* 2016), and could even result from the intrinsic differences in ploidy level. The differences in photosynthetic performance and physiological and biochemical responses under stress conditions that were reported by Varela *et al.* (2006) and Navarro *et al.* (2016) might have been caused by the distribution of the reproductive phases of *M. laminarioides* in the intertidal zone.

The change in the proportion of each MAA in relation to the total MAA concentration could suggest interconversions among different MAAs (toward asterina-330 and mycosporine–glycine in tetrasporophytes and gametophytes, respectively). The increase in these two types of MAAs could help the organisms cope with light stress on account of the UV absorption and antioxidant properties of both asterina-330 and mycosporine–glycine (Dunlap & Yamamoto 1995, De la Coba *et al.* 2009). The different distribution in the shore could also contribute to the difference in carrageenan content observed in the reproductive phases. Overall, changes in carrageenan yield and MAA composition and proportion could reflect an interaction between different stress factors leading to adjustments in the response to the conditions in which each reproductive phase is submitted in the natural environment.

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