Publication 4

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Interactive effects of temperature and radiation on polar macroalgae

Journal of Phycology (in preparation)

Interactive effects of temperature and radiation on the mycosporine-like amino acid contents in polar macroalgae

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ABSTRACT

INTRODUCTION

Temperature and solar radiation are two of the major factors controlling growth, reproduction, geographical and depth distribution of macroalgae (Aguilera et al. 1999; Bischof et al. 1998a; Bischoff and Wiencke 1995; Breeman 1988; Davison 1991; Dring et al. 1996, Franklin and Forster 1997; Hanelt et al. 1997; Wiencke and tom Dieck 1989, 1990; Wiencke et al. 1993, 1994, 2000). While temperature has a strong influence on almost all types of biochemical reactions, sunlight is directly used by macroalgae for photosynthesis and photomorphogenetic processes. These abiotic parameters undergo changes in response to seasonal variability and global change phenomena such as global warming, ozone depletion in the atmosphere and the resulting increase of damaging ultraviolet radiation (UVR). It is predicted that global air temperature might increase by about 0.3 °C per decade (Beardall et al. 1998 and references therein), this rise maybe more pronounced at higher latitudes (Barry et al. 1995, Beardall et al. 1998), and may affect the biosphere.

Endemic Antarctic macroalgae are well adapted to the polar habitat, showing low temperature requirements for growth and survival (Wiencke and tom Dieck 1989, 1990, Bischoff-Bäsmann and Wiencke 1996), as well as relatively low temperature optima for photosynthesis, compared to cold-temperate species (Wiencke and tom Dieck 1989, Wiencke et al. 1993, Eggert and Wiencke 2000). Some species even die when exposed to temperatures >5°C (Bischoff-Bäsmann and Wiencke 1996). In contrast to the usually cold and stable temperature conditions of the Southern Ocean, ranging from –1.7 to 2.0 °C (e.g. Schodlok et al. 2002), the water temperature of tidal rock pools (South Shetland Islands) may rise up to 14 °C (Klöser et al. 1994) during Antarctic summer and hence affect intertidal species.

Ozone depletion in the stratosphere, which particularly occurs in polar regions (Holm-Hansen *et al.* 1993, Groß *et al.* 2001), results in increases of biologically harmful UVB radiation (UVBR, 280 – 320 nm) reaching the Earth´s surface. UVBR also penetrates into the water column down to 10 - 30 m, depending on water transparency (Karentz 1989, Kirk 1994). Moreover, the seasonal variability of irradiance is also very marked

in polar regions where solar radiation reaches the extremes of 24 h daylength in summer and 24 h darkness in winter within the polar circles.

The daily, seasonal and environmental changes of UVR may have negative effects on macroalgae, especially when growing in the supra- and eulittoral. Therefore, the plants have developed protection and repair mechanisms such as the synthesis and accumulation of UV- absorbing compounds, in order to block or reduce harmful irradiance. One such group of sunscreen compounds, the mycosporine-like amino acids (MAAs), are conjugated cyclic, water-soluble molecules that absorb in the UVB and UVA (320 - 400 nm) range (Dunlap and Shick 1998, Shick et al. 2000, Karentz 2001). Among macroalgae, MAAs are predominantly synthesized in red algae and few green algal species (Hoyer et al. 2001 and references therein), most probably through several enzymatic steps of the shikimate pathway (Shick et al. 1999). The induction of MAA biosynthesis and subsequent accumulation might be species-specifically triggered by white light and/or UVR (Franklin et al. 1999, 2001; Hoyer et al. 2002; Karsten et al. 1998a,b; Karsten and Wiencke 1999; Kräbs et al. 2002).

The simultaneous action of temperature and irradiance on macroalgae has been investigated in several studies. Poll et al. (2002) investigated at the temperature dependence of UVR-induced DNA damage, whilst Pakker et al. (2000) additionally studied the DNA photo repair process. These authors demonstrated that the formation of DNA damage as monitored by the amount of cyclobutane-pyrimidine dimers (CPD) and of 6-4 photoproduct accumulation seemed to be almost independent of temperature in Arctic and temperate macroalgal isolates in the tested temperature range from 0 to 25 °C. However, the efficiency of photorepair of CPD increased with increasing temperature (optimal at 25 °C), whereas the repair of damage caused by 6-4 photoproduct showed an optimal efficiency at 12 °C in the cold-temperate red alga *Palmaria palmata*. Effects of temperature and irradiance on photosynthesis (Gómez et al. 2001) were also investigated. Photosynthesis was more inhibited by UVR at a temperature of 15 °C than at 25 °C in the temperate red algae *Gelidium pulchellum*. Furthermore, it has been indicated that low temperatures may enhance detrimental UVR effects in Antarctic cyanobacteria (Ross and Vincent 1998).

The present study explores the influence of temperature on the biosynthesis of MAAs in polar macroalgae under simultaneously increased PAR and UVR conditions. Moreover, the capability of photosynthetic apparats to cope with changes in the interactions of

those abiotic parameters was investigated. We compared two red algae from the Antarctic (*Iridaea cordata*, *Palmaria decipiens*), and one from the Arctic (*Palmaria palmata*), usually occurring in the eulittoral to the sublittoral zone. Additionally one *Prasiola* taxa (Chlorophyta) from the Antarctica, that typically grow in the supralittoral zone and even in terrestrial locations such as in avian rookeries, exposed to full sunlight conditions, complete this study. Results were discussed in the context of a possible relationship of algal stress responses to seasonal and global change phenomena.

MATERIALS AND METHODS

Algal material

The macroalgal species *Iridaea cordata* (Turner) Bory de Saint Vincent, *Palmaria decipiens* (Reinsch) Ricker and *Prasiola crispa* ssp. *antarctica* (Kützing) Knebel from Potter Cove, King George Island, Antarctica (Jubany Station, Dallmann Laboratory, 62°14´S, 58°40´W) and *Palmaria palmata* (Linnaeus) Kuntze from Kongsfjord, Ny-Ålesund, Spitsbergen, Arctica (78°55´N, 11°55´E) were isolated for culture purposes. They were established as permanent growth cultures in the laboratory under dim light (PAR, 15 μmol photons $m^{-2}s^{-1}$), simulating fluctuating Antarctic daylengths (Wiencke 1990a, b), with Provasoli-enriched North Sea water (Starr and Zeikus 1987) at a salinity of 30-32 PSU, aerated with membrane filtered air (pore size 0.2 μm). All algal species were cultured at a temperature of 0 °C.

Experimental set up

Plants were transferred from preculture to the experimental temperatures of 5 and 10 °C, respectively, which realistically can be reached in the polar summer months (Winkler et al. 1998, Svendson et al. 2002). The light:dark cycles was 16:8 h and the PAR intensity 15 μmol photons $m^{-2}s^{-1}$. Red algae were trimmed with a razor blade into similar sized thalli pieces, and kept at those temperatures for 23 days to acclimate and to aid in wound healing, thereby avoiding additional stress at the start of the experiment. Afterwards algae were exposed to three different radiation conditions; PAR (400 to 700 nm, 36 μmol photons $m^{-2}s^{-1}$), PAR + UVA (295 to 400 nm, 5.0 W m^{-2}), and PAR + UVA + UVB (295 to 320 nm, 0.41 W m^{-2}) at 5 °C and 10 °C. Daylight fluorescent lamps (Lumilux Deluxe, Osram, Germany), in combination with Q-Panel UVA-340

fluorescent tubes, (Cleveland, USA) emitting a spectrum similar to solar radiation in the UVR range were used. Spectra emitted by these artificial radiation sources were measured with a Spectro 320 D spectroradiometer (Instrument Systems, Germany).

During the experiment, algae were kept in glass beakers filled with filtered nutrient-enriched sea water plus 2.1 mM sodium hydrogen carbonate, as an additional inorganic carbon source. Glass vessels were covered with specific filter foils to cut-off UVB + UVA radiation (400 nm cut-off: Folex PR, Folex, Dreieich, Germany), only UVBR (320 nm cut-off: Ultraphan URUV, Digefra, München, Germany), and with a filter with no transmission under 295 nm (Ultraphan UBT, Digefra, München, Germany).

The MAA induction kinetics were followed for 15 days of exposure to the artificial radiation harvesting the plants at day 2, 5, 8 and 15. The samples were oven-dried at 50 °C overnight, and then stored in Eppendorf tubes under dry and dark conditions prior to MAA analysis.

Determination of optimal quantum yield of PSII

Photosynthetic activity was determined by measuring the variable chlorophyll fluorescence of photosystem II with a pulse amplitude modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany). The optimum quantum yield was estimated as ratio of variable to maximum fluorescence (F_v/F_m) of dark-acclimated plants as described in detail by Bischof et al. (1998). Six replicates were measured for each radiation and temperature treatment at day 2, 6 and 11 after keeping the plants for 6 to 8 hours in darkness. The relative changes of F_v/F_m were calculated by setting the initial sample values to 100%. The statistically significant differences between the two temperatures were assessed by the Mann-Whitney-Test and significances are listed in Table 1.

MAA extraction and analysis

A 25 % aqueous methanol (v/v) extraction was made from 10 – 20 mg dry weight (DW) of the algal samples. After evaporating to dryness under vacuum (Speed Vac Concentrator SVC 100H) extracts were re-dissolved in 100% methanol for partial purification, evaporated again to dryness and then re-dissolved in 2.5 % methanol (v/v). Samples were analysed with a Waters high-performance liquid chromatography (HPLC)

system using a mobile phase of 5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water according to Hoyer *et al.* (2002). Quantification was made using the molar extinction coefficients listed in Karsten et al. (1998c). Unless otherwise indicated, all MAA concentrations are given as mean values of 4 - 5 replicates (±SD), expressed as concentration on a dry weight basis (mg MAA g^{-1} DW).

Table 1: Statistical significances between the two temperature regimes (5 and 10°C) in the absolute values of the optimum quantum yield (Fv/Fm) measured at day two, six and 11. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, -- : not significant.

		Prasiola crispa	*Palmaria palmata*	*Palmaria decipiens*	*Iridaea cordata*
	initial sample	--	*	**	**
Day 2	PAR	**	--	--	--
	PAR+A	**	--	--	*
	PAR+A+B	--	--	--	*
Day 6	PAR	**	--	--	*
	PAR+A	*	--	--	--
	PAR+A+B	--	*	--	--
Day 11	PAR	*	--	--	--
	PAR+A	--	--	--	*
	PAR+A+B	--	--	--	--

Differences in the MAA content under the distinct filter treatments and at the different temperatures were statistically verifyed by using a two-way ANOVA followed by a multiple comparison test (Tukey-Kramer HSD - test). When no homogeneity of variances could be obtained a Kruskal-Wallis test was assessed. Significances occurred when the probability were at $p \leq 0.05$.

RESULTS

MAA inventory

Eight different MAAs were detected in the three red algae investigated. Shinorine, porphyra-334 (P-334), palythine, asterina-330 and palythinol occurred as main MAAs. Inconsistent traces of mycosporine-glycine and usujirene were found in *Iridaea cordata* and *Palmaria palmata*, respectively. *Palmaria decipiens* exhibited traces of palythene/usujirene in some samples, and in the 5 °C treatment, traces of mycosporine-glycine. The green algal species *Prasiola crispa* ssp. *antarctica* contained a chemically unknown UV-absorbing substance with an absorbing maximum at 324 nm (unknown-324) as the main compound (Hoyer et al. 2001).

Temperature and radiation effects on total MAA concentrations

No obvious effect of enhanced temperature under the different radiation conditions was detected in all algal samples. In the Antarctic subspecies *Prasiola crispa* ssp. *antarctica*, in which all samples contained almost the same concentrations of the substance unknown-324, except for the samples at 10 °C, which exhibited significant higher concentrations of the substance unknown-324 after exposure to PAR+UVA for 5 days (Fig. 1).

A more inconsistent pattern of temperature effects was found in the red algae. Generally, excluding the initial samples, the 5 °C-experiment samples exhibited higher MAA concentrations than those of the 10 °C-experiment. However, the MAAs and their ratios/patterns within species were often variable. The most pronounced temperature effect was detected in the Antarctic algae *Iridaea cordata* and *Palmaria decipiens* (Fig. 1). In *I. cordata,* all samples at 5 °C contained higher total MAA concentrations than the isolates at 10 °C. After 5 days, the statistically significant differences in concentrations were found in the samples exposed to PAR- and PAR+UVA+UVB ($p=0.02$ and $p=0.011$, respectively). Similar responses were found in isolates harvested after 8 and 15 days, the latter exhibiting statistically significantly higher MAA contents under all radiation treatments at 5 °C (PAR: $p<0.007$; PAR+UVA: $p=0.0002$; PAR+UVA+UVB: $p<0.002$; data not shown).

In the Antarctic red algae *Palmaria decipiens*, the pattern of temperature effects on the MAA synthesis/accumulation was quite similar to that of *I. cordata*. After 5 days, all samples cultured at 5 °C exhibited higher total MAA concentrations than those at 10 °C showing statistically significant differences in the isolates after exposure to the PAR ($p<0.04$) and the full radiation spectrum ($p=0.005$). Similar results were found after 8 and 15 days (data not shown). In the red algal species *Palmaria palmata* from the Arctic, the total MAA concentrations were not, or only slightly higher at 5 °C than at 10 °C (Fig. 1). Only the samples exposed to the full radiation spectrum ($p<0.0002$) and harvested after 8 days showed statistically significant results at 5 °C (data not shown).

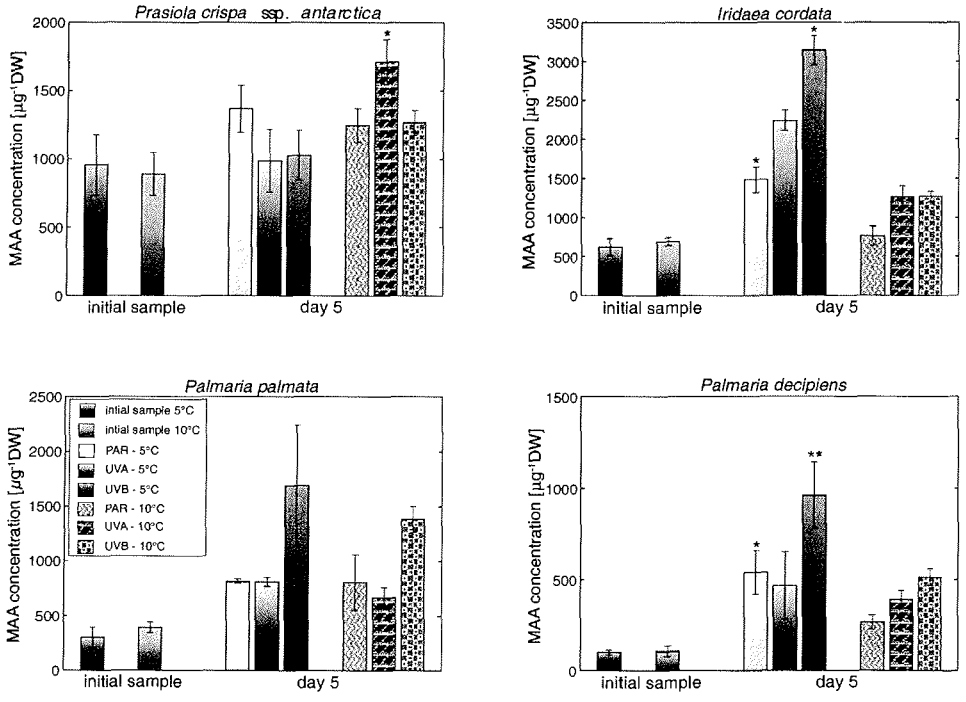

Figure 1: Total concentration of MAAs of the initial samples and after five days of exposure to different radiation conditions (PAR, PAR+UVA, PAR+UVA+UVB) at 5 and 10°C. *Prasiola crispa* ssp. *antarctica* from King George Island (Antarctica). *Iridaea cordata* (at 5 °C the PAR+UVA sample and at 10 °C the PAR+UVA+UVB sample: $n=3$). *Palmaria decipiens* (at 5 °C the PAR samples: $n=2$; the PAR+UVA samples: $n=3$). *Palmaria palmata* (at 5 °C the PAR+UVA+UVB samples: $n=3$); Asteriks indicate significant differences in the concentrations of MAAs between the two temperature regimes (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$). Bars are means (± SD). Note the different MAA concentration range.

Temperature and radiation effects on individual MAAs

In *Iridaea cordata*, the main MAA showing temperature-dependent differences in patterns of synthesis/accumulation was shinorine, followed by palythine. After 5 days in all 5 °C-samples, palythine was present in significantly higher concentrations than in the corresponding 10 °C-samples ($p<0.0003$), the content of shinorine were also significantly higher in the PAR- and PAR+UVA+UVB-treated samples ($p=0.03$ and $p=0.05$, respectively). After 8 days, a similar situation was found as was seen after 15 days, where the temperature effect on shinorine synthesis/accumulation was very obvious, resulting in significanes in all samples at 5 °C (PAR: $p<0.025$; PAR+UVA: $p<0.002$; PAR+UVA+UVB: $p<0.0002$). The same situation was observed in the palythine synthesis of the PAR ($p<0.006$) and PAR+UVA-treated samples ($p<0.0002$; data not shown).

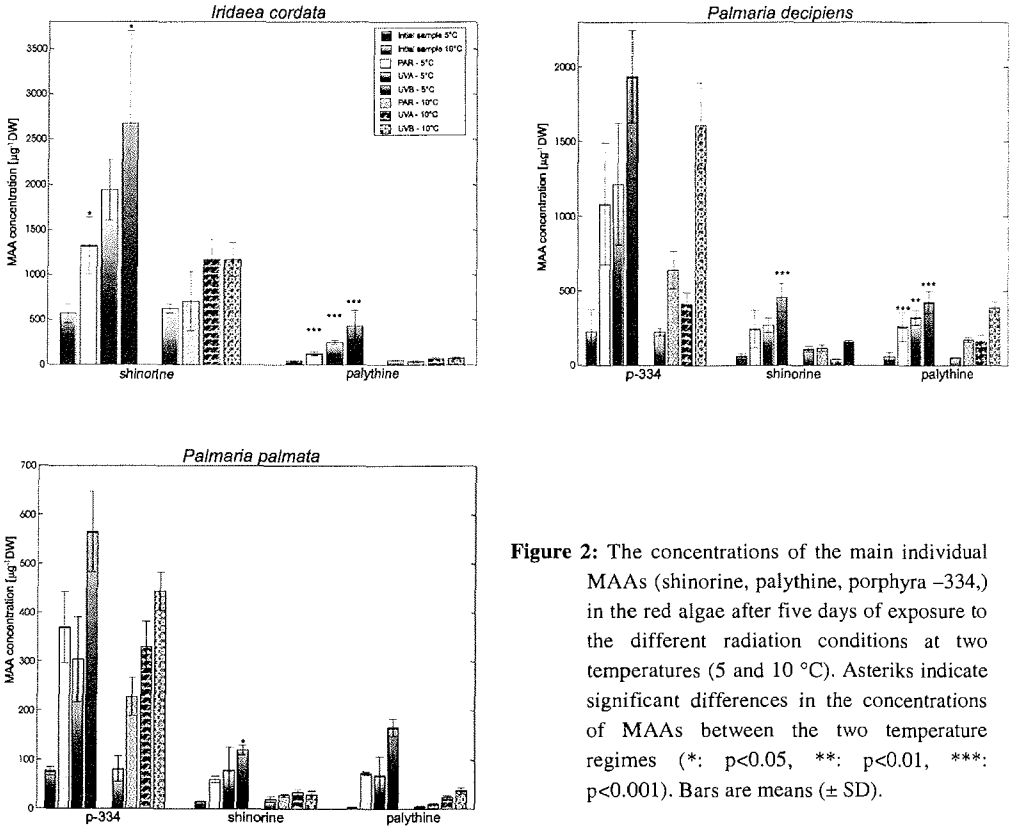

Figure 2: The concentrations of the main individual MAAs (shinorine, palythine, porphyra –334,) in the red algae after five days of exposure to the different radiation conditions at two temperatures (5 and 10 °C). Asteriks indicate significant differences in the concentrations of MAAs between the two temperature regimes (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$). Bars are means (± SD).

The main MAA in *P. decipiens* was P-334 followed by shinorine and palythine in similar concentrations. Temperature effects on P-334 were not so clear, resulting in mostly equimolar concentrations. The only significant difference was found in the PAR-treated samples after 8 and 15 days ($p=0.005$; $p<0.01$, respectively). In contrast, shinorine and palythine showed clear effects when exposed to different temperature regimes, exhibiting higher concentrations at 5 °C than at 10 °C. The shinorine content was statistically significantly higher in samples exposed to the full radiation spectrum ($p<0.0002$) after 5 days, under all radiation treatments after 8 days (PAR: $p<0.003$; PAR+UVA: $p<0.02$; PAR+UVA+UVB: $p<0.005$) and in the UVR-treated isolates (PAR+UVA: $p<0.001$; PAR+UVA+UVB: $p<0.02$) after 15 days. The most pronounced temperature effect on the palythine synthesis was found after 5 days (Fig. 2), resulting in significances of all samples (PAR: $p<0.0002$; PAR+UVA+UVB: $p<0.005$; PAR+UVA+UVB: $p<0.0002$).

In *Palmaria palmata*, the same MAA combination as in *P. decipiens* was found. The concentrations of P-334 remained quite constant whereas shinorine was the most temperature affected MAA showing significantly higher concentrations exposed to the full radiation spectrum ($p<0.04$) after 5 days (Fig. 2). After 8 days, under the same treatment, this significance was found for each individual MAA (P-334: $p<0.0002$; shinorine: $p<0.0002$; palythine: $p<0.05$; data not shown).

Spectral radiation effects

In the green algae *P. crispa* ssp. *antarctica*, radiation did not obviously influence the synthesis/accumulation of the UV-absorbing substance-324. In contrast, in the red algae cultured at 5°C the highest MAA concentrations occurred under the full radiation spectrum. The induction pattern showed in *Iridaea cordata* cultured at 5 °C and in *P. decipiens* cultured at 5 °C and 10 °C an additional increase in MAA concentrations after exposure to the different radiation conditions whereas in *Palmaria palmata* only the full radiation spectrum led to an increase of MAAs.

Effects on photosynthesis (optimal quantum yield)

In the initial samples of all algae, the optimal quantum yield of PS II (F_v/F_m) was significantly higher in the 5 °C conditions compared to the 10 °C samples after 23 days

of acclimation time before the start of the experiment (Table 1). After two days of exposure to the different radiation conditions, almost all samples at 5 °C exhibited a lower F_v/F_m than the initial samples. The 10 °C-samples of the green algae and *Iridaea cordata* still had F_v/F_m data in the range of the initial values after 2 days, followed by a decrease after 6 days. An acclimation of photosynthesis was detected at day 11, where a recovery to 90-100% took place (Figure 3), except the UVR-treated samples of *P. crispa* ssp. *antarctica*. In the red algae, the relative changes in F_v/F_m were obviously lower in the 10 °C samples than in the 5 °C-samples indicating that they are less affected by changes in irradiance. However, in these species, the absolute values do not differ significantly between the two temperature ranges. Furthermore, after 11 days, there are also no obvious differences found in the green algal values (for the exceptions see Table 1).

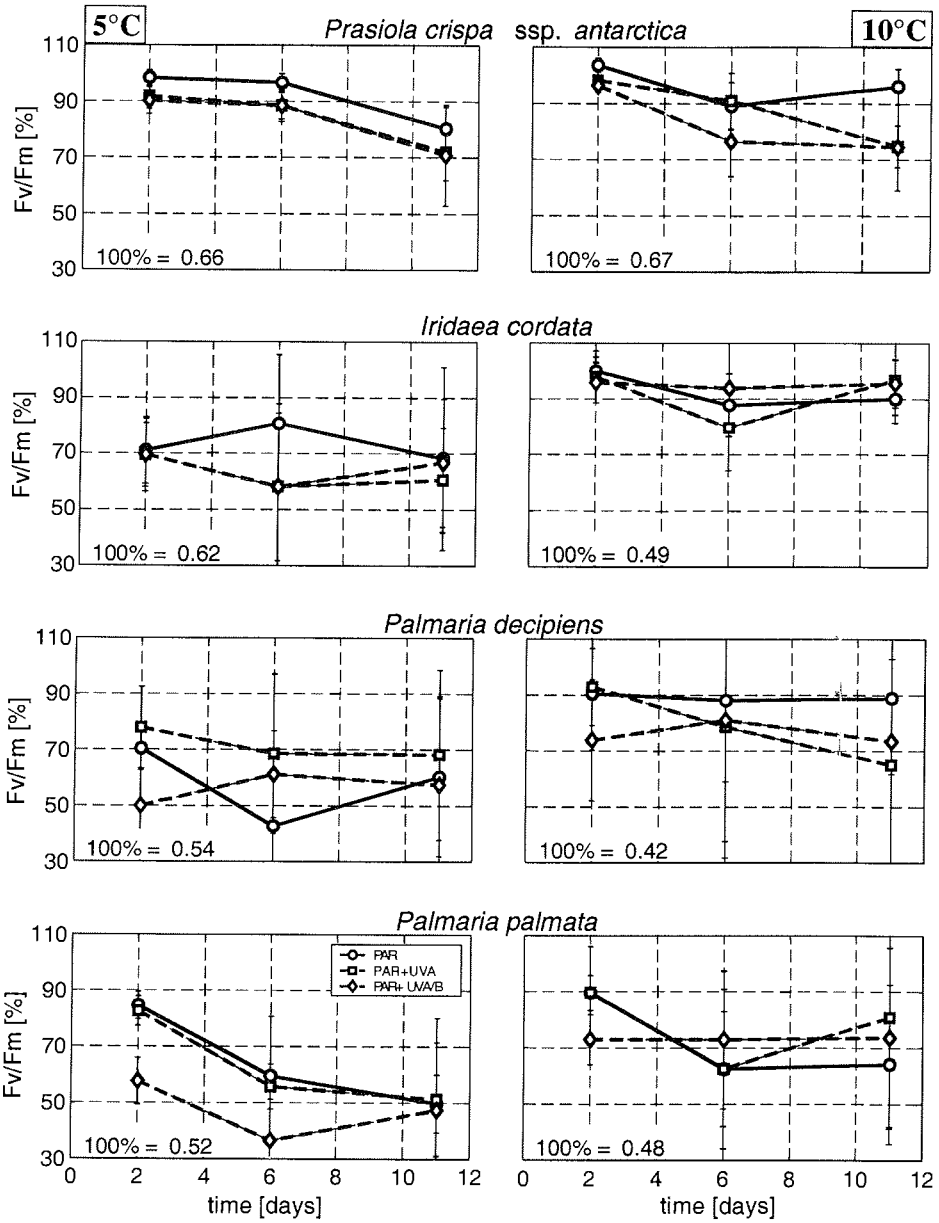

Figure 3: Relative differences in optimal quantum yield of PS II (Fv/Fm) of algae exposed to different radiation conditions PAR; PAR+UVA; PAR+UVA+UVB) at two temperatures (5 and 10 °C). Initial samples were standardized to 100 %. Fv/Fm was measured after a recovery period of 6-8 h darkness at day 2, 6 and 11.Values are means (± SD).

DISCUSSION

This study highlights that temperature (5 and 10 °C) alone has no effect on MAA occurrence in polar algae. However, the interaction between temperature and different radiation treatments generally resulted in significantly higher MAA concentrations at 5 °C than at 10 °C in both Antarctic red algal species. In most samples of the Arctic *Palmaria palmata* and Antarctic *Prasiola crispa* ssp. *antarctica* there were no significant differences found. The recovery after photosynthetic inhibition was better at 10 °C than at 5 °C in all samples, irrespective of the MAA level. The temperature and radiation effects on MAA occurrence and photosynthesis are discussed together with their interaction.

Temperature effects

The hypothesis that MAAs are synthesized via the shikimate pathway (Shick et al. 1999) may involve several temperature dependent steps for individual MAAs. In addition, the accumulation or the conversion from one MAA to another might also be differentially affected by temperature. In this study, the MAA concentrations in low-light acclimated *Iridaea cordata*, *Palmaria decipiens*, *Palmaria palmata* and *Prasiola crispa* ssp. *antarctica* samples did not differ at 5 and 10 °C (Fig. 1). It is suggested that the enzyme activity may be higher at lower temperatures, although generally speaking, metabolism rates are lower at low temperatures. But similar exceptions have also been seen in some species of Antarctic diatoms, which show a maximum substrate affinity of ribulose-1,5-bisphosphate carboxylase at low temperatures (4.5 °C), compared to their temperate counterparts with a maximum at 20 °C (Descolas-Gros and Billy 1987).

In addition, a temperature effect on photosynthesis was found in low-light acclimated algae, in which the Fv/Fm values were higher at 5 °C than at 10 °C (Table 1, initial samples). This may indicate a successful cold adaptation to the polar environment. This assumption is supported by Hanelt et al. (1994) who found that the kinetics of photoinhibition and recovery were much faster in polar species than in tropical species suggesting that the enzymes of the photosystem II repair cycle may be adapted to low temperatures.

Radiation effects

General radiation effects on MAA synthesis in polar macroalgae have been investigated in a previous study (Hoyer et al. 2002). Those experiments were performed with three

different radiation conditions at 0°C, and resulted in three different response types relating to MAA concentrations (I; highest MAA concentration under PAR+UVA+UVB, II; highest MAA concentration under PAR+UVA, III; MAA decrease under PAR+UVR). In the present study, the red algae belonged to the first response type, as almost all samples at both 5 and 10 °C showed the highest MAA concentration under the full radiation spectrum (PAR+UVA+UVB; Fig. 1). The *Prasiola* species exhibited no clear induction pattern, which might be due to its more terrestrial growth habitat. Growing mainly out of the water there is generally no attenuation of solar radiation, and therefore the alga requires steady sunscreen protection.

Furthermore, this species has been characterized by Wiencke and tom Dieck (1999) as a mainly eurythermal species due to its wide growth temperature range from 0 to 20 °C, with a growth optimum at 5 °C. This may explain its enhanced ability to withstand temperature fluctuations with no change in UV-absorbing substances, and this may also be ecologically related to its more terrestrial growth habitat, where temperature changes are very marked and the solar radiation not attenuated.

Interactive effects

Elevated temperatures and a higher PAR, together with the additional UV radiation, resulted in very obvious differences in total MAA concentrations in *Iridaea cordata* and *Palmaria decipiens* (Fig. 1a, b). All samples produced significantly higher MAA concentrations at 5 °C than at 10 °C. These results may indicate that when MAA synthesis / accumulation is triggered by PAR and/or UVR, the necessary enzymatic processes may be temperature dependent and hence, successfully cold adapted. Cold adaptation with respect to growth in Antarctic macroalgae has been well documented (Wiencke and tom Dieck 1989, 1990, Bischoff-Bäsmann and Wiencke 1996, Eggert and Wiencke 2000). In these growth experiments, a growth optimum was found at 0 °C for *I. cordata* and at 5 °C for *P. decipiens* (Wiencke and tom Dieck 1989, 1990), in agreement with the higher MAA production seen at lower temperature in this study.

Slight or even no interactive effects of temperature and UVR were detected in *Palmaria palmata* from the Arctic (Fig. 1). This agrees well with a recent investigation by Poll et al. (2002) on temperature dependence of UV effects on; growth, optimum quantum yield of photosystem II and cyclobutane-pyrimidine dimers accumulation. This study concluded that the contribution of temperature to UV effects was small within the tested

temperature range from 6 to 18 °C. Nevertheless, Arctic cold temperate red algal species are less stenothermal than Antarctic ones exhibiting a broader temperature range for growth (up to maximal 25 °C) and survival at higher maximum temperatures between 17 and 25 °C (Wiencke et al. 1994, Bischoff-Bäsmann and Wiencke 1996). In the Arctic endemic species *Devaleraea ramentacea,* temperature requirements are only slightly elevated (upper thermal limits between 18 and 20 °C, Novaczek et al. 1990), compared to endemic Antarctic species (16 to 17 °C for *Palmaria decipiens*, Wiencke et al. 1994).

When total MAA concentrations change, this infers that the individual MAAs may show different induction patterns and responses to the interaction of temperature and radiation. Shinorine and palythine are the most significantly affected MAAs. In the two Antarctic red algae, their concentrations were higher at 5 °C than at 10 °C, and highest under the full radiation spectrum (Fig. 2). Shinorine and palythine are frequently detected together in algae having a broad depth distribution ranging from the upper to the lower sublittoral and also in tide pools (Karsten et al. 1998c, Hoyer et al. 2001). These algae are able to flexibly adjust their MAA concentrations depending on the prevailing environmental radiation conditions, and may sensitively react to the temperature changes when triggered by irradiance, as shown for some algae in this study.

The main MAA of *P. decipiens* and *P. palmata* is P-334, which seems to be one of the MAAs least affected by temperature and irradiance (Fig. 2). The latter is demonstrated by the absence of MAA induction in *Porphyra umbilicalis* under different radiation conditions containing P-334 as its main MAA (Gröniger et al. 1999). Similarly, *Porphyra endiviifolium*, which also primarily contains P-334 at a high level, only shows a slight but significant induction in MAAs under artificial exposure to PAR+UVA after 12 days (Hoyer et al. 2002). However, P-334 is often the main MAA in red macroalgae found in the (upper) eulittoral, therefore strongly exposed to UVR (Karsten et al. 1998b, Hoyer et al. 2001). It is postulated that these algae need high and steady MAA concentrations as sunscreen protection, in order to survive in such habitats. In addition, P-334 was also found to occur in sublittoral algae, where a similar flexibility in MAA formation was seen, relating to different radiation conditions. This may be ecologically important at specific times of the year, during episodes of seasonally high water

transparencies when UVBR can penetrate the water column down to 30 m (Karentz and Lutze 1990).

The UV-absorbing substance-324 in the *Prasiola* species did not show an obvious interaction effect with UVR and temperature on the synthesis / accumulation (Fig. 1). However, Gröniger and Häder (2002) suggested a clear induction of biosynthesis in the UVBR range (300 nm) for another species of the *Prasiola, P. stipitata* from cold-temperate Helgoland, Germany. Generally, *Prasiola* species need to cope with very extreme environmental conditions and can be subjected to ice-melt or rainwater pools, salt spray zones of the supralittoral and are even common in avian rookeries (Jakob et al. 1991). Therefore, these species must have developed morphological, physiological and biochemical protective mechanisms, such as thick cell walls as a measure against dehydration, temperature-tolerant photosynthetic activity, and the capacity of osmotic acclimation by using sucrose and sorbitol as osmolytes (Jakob et al. 1991, Jackson & Seppelt 1995). The chemically unknown UV-absorbing substance-324 is considered to be acting as a sunscreen against UV stress due to its absorbance maximum at 324 nm.

In the recovery stage, the relative changes in Fv/Fm were generally lower at 5 °C than at 10 °C in the red algae, although in the two Antarctic red algal species the MAA concentrations were higher at 5 °C, leading us to question its photoprotective role. MAA levels in Arctic *P. palmata* were more or less equal at both temperature treatments. Hence, it might be an interactive effect that under enhanced temperature, Fv/Fm is not protected by MAAs. However, in the two *Palmaria* species, one group with low MAA concentrations, after exposure to UVR stress showed, that the measured Fv/Fm in the recovery stage was faster and better in those samples with higher MAA levels (Hoyer et al., unpublished data). However, Ross and Vincent (1998) have suggested that UVR is more detrimental at lower temperatures, possibly indicating that the enzymatic repair mechanisms are too slow to compensate the damaging UVR effect. In contrast, Hanelt et al. (1994) found Antarctic field macroalgae, which were photosynthetically well adapted to their cold environment had no prejudice in regulation of their photosynthesis. But Hanelt et al. (1997) also reported that in laboratory experiments the temperature showed a pronounced effect on the reaction kinetics, and suggested that higher temperatures may be beneficial for the photoprotective process, which support the results from this study. The relative changes in *Prasiola crispa* ssp. *antarctica* do not differ markedly, suggesting a temperature-tolerant photosynthetic

activity. That agrees well with Hanelt et al. (1997) who suggested that most of the polar green algae are probably adapted to higher light and UV conditions as well as to changes in temperature due to their exposed habitat.

This investigation shows that the *Prasiola* species is less affected by seasonal and climate changes, in relation to temperature and UVR, indicating that it probably has additional protection mechanisms against enhanced UVBR and will withstand global change. Taking into consideration that UVR affects the depth distribution of algae (Bischof et al. 1998), the red algal species tested here will probably survive if global change phenomena become worse, mainly by inhabiting deeper areas where the UVR is more attenuated. Generally, the algal responses to the different temperatures and radiation conditions are very variable and seem to be species-specific.

Acknowledgements

This project was financially supported by the Deutsche Forschungsgemeinschaft (Ka 899/3-1/-2/-3). The authors like to thank M. Schoenwaelder for discussion and reading the manuscript.

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