Wavelength dependent induction and biosynthesis of UV-absorbing mycosporine-like amino acids in marine macroalgae

Wellenlängen-abhängige Induktion und Biosynthese von UV-absorbierenden Mykosporine-ähnlichen Aminosäuren in marinen Makroalgen

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Zusammenfassung

Mykosporin-ähnliche Aminosäuren (MAAs) sind eine Gruppe von UV absorbierenden Molekülen, die in Rotalgen synthetisiert werden. Ihre gemeinsame Grundstruktur ist ein Cyclohexenon-Ring, an den ein oder zwei Aminosäuren und/oder Amine gebunden sind. Aufgrund ihrer Absorptionsmaxima zwischen 309 und 360 nm wurde vorgeschlagen, dass sie als natürlicher Sonnenschutz wirken. Über den Biosyntheseweg und die Regulationsmechanismen der MAA Biosynthese ist nur wenig bekannt.

In der vorliegenden Arbeit wurde die wellenlängen-abhängige MAA Biosynthese in *Chondrus crispus* untersucht. Sowohl UV-A Strahlung als auch Blaulicht hat eine hohe Quantenwirksamkeit für die Biosynthese von MAAs. Diese deutet auf die Existenz von Photorezeptoren in den jeweiligen Wellenlängen-Bereichen hin. Unter UV-A exponierte Algen synthetisieren hauptsächlich Shinorin, während Algen, die unter Blaulicht exponiert wurden hauptsächlich Palythin bilden.

Des weiteren wurde ein guter Hinweis auf eine Umwandlung von Shinorin in Palythin gefunden. Die dafür notwendigen Enzyme sind wahrscheinlich Licht reguliert. Polychromatische Wirkungsspektren, die für die Akkumulation von MAAs berechnet wurden, deuten darauf hin, dass langwellige UV-A Strahlung und Blaulicht diese Umwandlung stimuliert, während UV-B Strahlung hemmend darauf wirkt. UV-B Strahlung hemmt darüber hinaus möglicherweise auch die gesamte Biosynthese von MAAs. Der genaue Angriffsort dieser allgemeinen Hemmung der MAA Biosynthese ist unbekannt, aber mehrere Ziele kommen dafür in Frage.

Für die MAA-Induktion wurde ein Aktionsspektrum unter monochromatischem Licht im Bereich von 280 bis 750 nm erstellt. Das berechnete Aktionsspektrum deutet darauf hin, dass der für die Shinorin Photoinduktion verantwortliche Photorezeptor, ein unbekannter UV-A Photorezeptor mit Absorptionsmaxima bei 320, 340 und 400 nm ist. Die Absorptionseigenschaften des vorgeschlagenen Blaulicht Photorezeptors konnten nicht identifiziert werden.

Neben der Photoinduktion von MAAs durch Photorezeptoren ist eine zusätzliche Stimulierung der Biosynthese von MAAs durch ansteigende Konzentrationen von reaktiven Sauerstoffverbindungen denkbar. Darüber hinaus können hohe Konzentrationen von reaktiven Sauerstoffverbindungen möglicherweise die Umwandlung von Shinorin und Porphyra-334 in Mycosporin-Glycin in

trockengefallenen Individuen der Gezeiten-Rotalgengattung *Porphyra* einleiten, wodurch zusätzliche Antioxidantien während der Austrocknung gebildet werden.

Die dargestellten Ergebnisse zeigen die hohe Flexibilität von Rotalgen, die Konzentration und Zusammensetzung der MAAs and die jeweiligen Lichtbedingungen anzupassen. Akkumulierte MAAs schützen - zumindest teilweise – vor UV Strahlung. Neben der primären Funktion als Sonnenschutz, können MAAs in einigen Arten möglicherweise auch als Antioxidantien wirken.

Summary

Mycosporine-like amino acids (MAAs) are a class of UV absorbing compounds synthesized in red algae. They have a cyclohexenone core in common, to which one or two amino acids and/or amines are conjugated. Because of their absorption spectrum between 309 and 360 nm it has been proposed that they act as nature's sunscreen. Information on both the biosynthetic pathway and the regulating mechanisms of MAA biosynthesis is limited.

In this study the wavelength dependent MAA biosynthesis in *Chondrus crispus* was examined. Both, UV-A radiation and blue light have a high quantum efficiency for MAA formation, indicating the existence of photoreceptor chromophores. Specimens exposed to UV-A synthesize shinorine as major MAA, while those exposed to blue light mainly accumulate palythine.

A strong indication for an interconversion of shinorine to palythine was found. The putative enzymes regulating the interconversion may be light regulated. Polychromatic response spectra calculated for MAA accumulation indicate that long wavelength UV-A and blue light stimulate the interconversion, while UV-B radiation inhibits it. Beside the inhibitory effect of UV-B on the interconversion of MAAs, UV-B may also inhibit MAA biosynthesis in general. The specific target of the latter is unclear, but multiple targets are possible.

An action spectrum for MAA induction was measured under monochromatic light in the range from 280 to 750 nm. The action spectrum indicates that the photoreceptor mediating shinorine photo-induction might be an as yet unidentified UV-A photoreceptor with absorption peaks at 320, 340 and 400 nm. The absorption characteristics of the putative blue light photoreceptor could not be identified.

Beside the photo-induction of MAAs mediated through the proposed photoreceptors, an additional stimulation of MAA biosynthesis through increased reactive oxygen species may be possible. In addition, high concentrations of reactive oxygen species may stimulate an interconversion of shinorine and porphyra-334 to mycosporine-glycine in the red algal genus *Porphyra*, providing an additional antioxidant during desiccation.

Data presented demonstrate the high flexibility of redalgae to adjust MAA concentration and composition to the given radiation conditions. Once accumulated, MAAs provide – at least to some extent – photo-protection against UV radiation. Beside the primary function as sunscreens MAAs may act as antioxidants in some species.

List of abbreviations

ATP	adenosine-triphosphate
CFCs	chlorofluorocarbons
D1	reaction center protein 1 of PS II
DAHP	3-deoxy-D-arabinoheptulosinate-7-phosphate
4-DG	4-deoxygadusol
DHQ	Dehydroquinate
DNA	desoxyribonucleic acid
DW	dry weight
e.g.	for example
ETR_{max}	maximal relative electron transport rate
FAD	flavin adenine dinucleotide
Fv/Fm	maximal quantum yield of photosynthesis
FW	fresh weight
HPLC	high performance liquid chromatography
MAA(s)	mycosporine-like amino acid(s)
MW	mean value
nm	nanometer
PAR	photosynthetic active radiation (400-700 nm)
PI-curve	photosynthesis versus irradiance curve
PS I	photosystem I
PS II	photosystem II
RNA	ribulosenucleic acid
ROS	reactive oxygen species
Rubisco	ribulose-1,5-bisphosphate carboxylase/oxygenase
SD	standard deviation
SE	standard error
UV	ultraviolet
UV-A	ultraviolet A radiation (315-400 nm)
UV-B	ultraviolet B radiation (280-315 nm)
UV-C	ultraviolet C radiation (190-280 nm)
W	Watt
λ	wavelength

1. Introduction

1.1 Marine macroalgae: their habitat and ecological importance

Macroalgae are important in terms of biodiversity (Norton et al. 1996) and play an important role in shallow water and coastal marine ecosystems (Klöser et al. 1994, Gómez et al. 1997). Although a global estimation of marine macroalgal biomass and production is difficult to conduct (Rasmus 1992), a contribution of 3 % of global primary production has been estimated (Lüning 1990). 5 % of total marine primary production is due to macroalgae (Mann 1973, Smith 1981). On an area basis, the productivity of macroalgal vegetations can rival or exceed that of terrestrial ecosystems (Mann 1973, Smith 1981, Thomas 2002).

Macroalgal vegetation provides important nursery areas and habitats for fish and invertebrates, substrates for various epiphytes and sessile animals; it buffers the water column against large changes in nutrient concentration and stabilizes sediments (Duarte 1995, Klöser et al. 1996). Macrophytes themselves as primary producers provide food for marine herbivores and detrivores (Dunton and Schell 1987, Iken 1996, Iken et al. 1997).

Macroalgae occupy habitats (especially on hard substrates) along global shores, strictly confined to the photic zone. Their vertical distribution can be divided into supralittoral, eulittoral/intertidal and sublittoral habitats. Depending on their vertical position on the shore macroalgae must cope with variations in several environmental factors. Specimens of the supralittoral must resist long, unpredictable periods of desiccation as well as considerable changes in salinity, temperature and irradiance. Eulittoral algae also must cope with these environmental factors during low tide. In contrast, sublittoral species are constantly submerged, and are only exposed to changing light conditions. All environmental factors show a predominant seasonal variation (Lüning 1990).

Seawater temperature and irradiance (especially UV radiation) influence survival, growth and reproduction (Bischoff-Bäsmann and Wiencke 1996, Franklin and Forster 1997, Gómez et al. 1998, 2001). Thus, both global warming and enhanced UV radiation due to stratospheric ozone depletion are likely to affect the biodiversity and productivity

of macroalgal communities. Because marine flora and fauna are closely linked, this may affect the entire coastal ecosystem.

1.2 UV radiation and its effect on marine macroalgae

A natural shield against harmful ultraviolet radiation is provided by the gas ozone, present in the stratosphere at altitudes of approximately 10 to 50 km (Solomon 1990). The ozone layer absorbs UV-C radiation (100-280 nm) and large portions of UV-B radiation (280-315 nm), while UV-A radiation (315-400 nm) and PAR (400-700 nm) passes almost unaffected.

After short-lived concerns in the early 1970s that anthropogenic emission might deplete stratospheric ozone (Day and Neale 2002), chlorofluorocarbons (CFCs) were proposed as catalysts of ozone depletion (Molina and Rowland 1974). A few years' later scientists agreed that ozone depletion by CFCs is a significant threat (National Academy of Science 1979). Since 1980, UV-B radiation increased from mid-latitudes to polar regions (4-7 % at mid-latitudes, 22 % in the Arctic and 130 % in the Antarctic; Madronich et al. 1998).

An ozone concentration depletion of 10 % results in an increase of 50 % in transmission at 297 nm, 25 % at 303 nm and 0 % at 325 nm (Roy et al. 1990). Harmful UV-B radiation will therefore increase without a proportional increase in UV-A and PAR (Halliwell and Gutteridge 1989).

Scientists predicted that ozone depletion will remain severe during the next decade. In the middle of the 21st century ozone density may return to pre-1980 levels (Shindell et al 1998, Montzka et al 1999).

Since the discovery of ozone depletion and the resulting increase in UV-B radiation, many researchers have investigated UV effects on organisms and ecosystems (reviewed in Franklin and Forster 1997, de Mora et al. 2000, Hester and Harrison 2000, Cockell and Blaustein 2001).

The main targets of UV radiation are bio molecules such as nucleic acid and proteins. These suffer structural damage that may result in an impairment or elimination of their physiological function (Halliwell and Gutteridge 1989, Vincent and Neale 2000). UV-B radiation results directly in the production of cyclobutane-pyrimidine dimers, while UV-A causes strand breaks and DNA cross links through photodynamic production of

hydroxyl radicals (Peak and Peak 1990). Both processes result in mutations during replication of DNA.

Proteins, which function as enzymes, hormones or structural components of cell organelles, can be damaged or their pool size can be reduced by UV radiation. Thus photosynthesis can be impaired through damage of the D1 protein of photosystem II (Vass 1997), a reduced activity of Rubisco, the CO_2 fixing enzyme (Bischof et al. 2000, 2002 a, b) and a loss of phycobiliproteins as well as other photosynthetic pigments (Lao and Glazer 1996, Bischof et al. 2000). Based on an impaired photosynthesis, growth may be affected by availability of fewer energy equivalents and reduced carbon fixation (Aguilera et al 1999, Kuhlenkamp et al. 2001).

During photosynthesis UV radiation enhances the production of reactive oxygen species, which are able to oxidize DNA, RNA, proteins and pigments (Halliwell and Gutteridge 1989). Thus, UV radiation and oxidative stress have a synergistic effect.

To counteract harmful effects of UV radiation, organisms – such as macroalgae – have evolved repair and mitigating mechanisms (Roy 2000). Thus, photolyase enzymes repair cyclobutane-pyrimidine dimers (Batschauer 1993, Nakajima et al. 1998) and reactive oxygen species are eliminated by a number of different enzymes and antioxidants (Collen and Davison 1999, Aguilera et al. 2002a). Beside different repair mechanisms the avoidance of UV induced damage is important. Plants block potential harmful wavelengths by using UV absorbing compounds such as coumarins, flavonoids, mycosporine-like amino acids, phenolic substances or scytonemin (Menzel et al. 1983, Garcia-Pichel and Castenholz 1991, Logemann et al. 2000, Schoenwaelder 2002, Shick and Dunlap 2002).

If repair and mitigating mechanisms are impaired or lacking, UV radiation affects the whole metabolism. A reduction of growth, reproduction and productivity (Dring et al. 1996, Aguilera et al. 1999, Wiencke et al. 2000, Makarov and Voskoboinikov 2001) combined with genetic damage (Kulunscics et al. 1999, Vincent and Neale 2000) and depressed photosynthetic activity (Bischof et al. 2000, 2002 a, b, Hanelt and Nultsch 2003) may finally alter biodiversity and thus the community structure of ecosystems (Madronich et al. 1995).

1.3 Mycosporine-like amino acids (MAAs)

MAAs are a class of small, colourless, highly water soluble, polar and at cellular pH uncharged or zwitterionic amino acid derivates (Carreto et al. 1990 b). They have a molecular weight of between 244 and 334 g mol^{-1} and a high molar absorptivity (ε = 28100-50000 mol^{-1} cm^{-1}) for UV-A and UV-B, resulting in narrow absorption spectra between 309 and 360 nm (Tab.1.1; Bandaranayake 1998, Dunlap and Shick 1998, Cockell and Knowland 1999).

The common core structure is a cyclohexenone or cyclohexenine ring conjugated with one or two amines, mostly amino acids and amino alcohols (Fig. 1.1). The absorption characteristic of these molecules is dependent on a system of double bounds in the core ring structure, which is altered through the conjugated amines (Banderanayake 1998, Cockell and Knowland 1999). Monosubstituted MAAs (oxo MAAs; e.g. mycosporine-glycine) with a cyclohexenone core have their absorption maximum in the UV-B, while bisubstituted MAAs (imino MAAs; e.g. shinorine) with a cyclohexenine core have their absorption maximum in the UV-A.

Since the discovery of the UV-absorbing substances "S-320" by Shibata (1969), later identified as MAAs (Dunlap and Chalker 1986), 19 different MAAs have been characterized (overview in Dunlap and Shick 1998). In most cases names are given after amines conjugated to the C_1 and/or C_3 atom of the core ring (e.g. mycosporine-glycine having a glycine conjugated to the C_3 atom), in other cases after the organism in which they were found (e.g. palythine, palythinol and palythene from the sea anemone *Palythoa tuberculosa*; Takano et al. 1978 a, b) or the location from which the organism was collected (e.g. shinorine from the red alga *Chondrus yendoi* collected near Shinori, Japan; Tsyjino et al. 1990).

Table 1.1: Molecular extinction coefficients and molecular weights for MAAs typically occurring in marine red algae.

| MAA	Extinction coefficient (mol^{-1} cm^{-1})	Molecular weight (g mol^{-1})	Reference
Mycosporine-glycine	28100	245.23	Ito and Hirata 1977
Shinorine	44668	332.31	Tsujino et al. 1980
Porphyra-334	42300	346.33	Takano et al. 1979
Palythine	36200	244.24	Takano et al. 1978 a
Asterina-330	43500	288.30	Gleason et al. 1993
Palythinol	43500	302.32	Takano et al. 1978 b
Palythene	50000	284.31	Takano et al. 1978 b
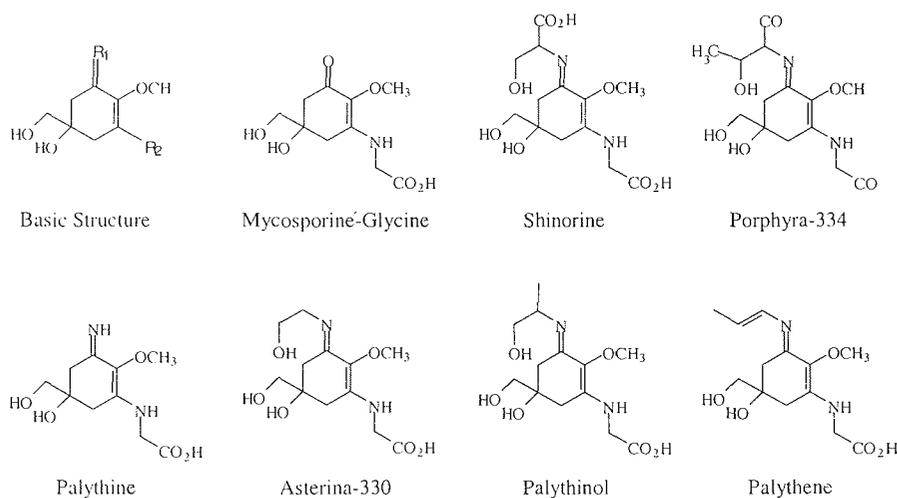
Figure 1.1: Structures of the most common MAAs in marine red algae; R_1 = amino acid or amine, R_2 = glycine, serine or taurine

Recently, a new type of MAAs has been discovered in dinoflagellates. Carreto et al. (2001) extracted MAAs with a-typical absorption spectra characterized by the presence of a maximum with a pronounced shoulder in the UV spectrum. Although a clear identification of these compounds is still missing, the authors believe that single MAAs are covalently linked to one another. Beside a broader UV screen, which can also be obtained by synthesizing different MAAs, the importance of these new MAAs might be the reduction of ionizeable groups. The authors regard this as a mechanism to counteract physiological limitations in accumulating MAAs as osmolytes (Oren 1997, Karsten 2002).

MAAs have been found in autotrophic and heterotrophic organisms, from tropical through warm- and cold temperate regions to polar seas, mostly in marine, but also in freshwater and terrestrial habitats (Karentz et al. 1991, Banaszak et al. 1998, Karsten et al. 1998 b, c, Jeffrey et al. 1999, Shick and Dunlap 2002 and references therein).

The cellular localization of MAAs in algae is still unknown. Garcia-Pichel (1994) suggested that MAAs occur homogeneously within the cell, whereas Neale et al. (1998 a) suggest that they may not. Recently, Maruyama et al. (2003) were able to show that MAAs are localized in the cup-shaped cytoplasma of the tunic bladder cells of

symbiotic didemnid ascidians. Furthermore, this cell type was denser in the upper tunic above the colony's zooids.

1.3.1 Biosynthetic pathway of MAA synthesis and evolutionary considerations

Because of the phylogenetically widespread occurrence of MAAs, especially in marine taxa, it is assumed that they arose early in evolution (Dunlap and Shick 1998, Cockell and Knowland 1999). As life first developed in the absence of atmospheric O_2, these organisms had to cope with the full solar spectrum including the highly energetic wavelengths of UV-C. The simple structure of 4-deoxygadusol (4-DG), an early intermediate of the shikimate pathway with an absorption maximum at 294 nm, may have acted as a UV-C and UV-B sunscreen (Garcia-Pichel 1998). Later in evolution, amine condensation with 4-DG may have provided MAAs, with monosubstituated MAAs (λ_{max} = UV-B) occurring earlier than bisubstituated (λ_{max} = UV-A), reflecting the rising atmospheric oxygen levels and the resulting spectral composition of solar irradiance at the earth's surface (Garcia-Pichel 1998).

Evidence for MAA biosynthesis via the shikimate pathway is still scarce. 3-Dehydroquinate (DHQ), a shikimate pathway intermediate, is the precursor for the six-membered carbon ring common in mycosporines in terrestrial fungi (Favre-Bonvin et al. 1987), which are structurally similar to MAAs. Both fungal mycosporines and MAAs might be synthesized from DHQ via gadusol (see references in Bandaranayake 1998, Shick et al. 2000).

MAA synthesis is inhibited in the coral *Stylophora pistillata* by N-phosphonomethylglycine ("glyphosate"), a specific inhibitor of the shikimate pathway (Shick et al. 1999). Furthermore, externally administered tyrosine, a feedback inhibitor of the shikimate pathway in cyanobacteria, depressed MAA formation in the cyanobacterium *Chlorogloeopsis* (Portwich and Garcia-Pichel 2003). Both results point to MAA synthesis via the shikimate pathway.

In addition, Portwich and Garcia-Pichel (2003) could demonstrate that radiolabelled glycine and serine is incorporated into the respective side chains of mycosporine-glycine and shinorine. Based on their results, they proposed that 4-DG conjugated with glycine results in mycosporine-glycine. The latter incorporating serine forms shinorine. They suggest further that the high diversity of MAAs is produced by a variation in the amine conjugated to mycosporine-glycine. In contrast (or addition) to an individual biosynthesis based on mycosporine-glycine, many authors suggest an interconversion of

MAAs with shinorine and/or porphyra-334 as precursors (e.g. Carreto et al. 1990 b, Franklin et al. 1999, Shick et al. 2000, Whitehead et al. 2001).

Because the shikimate pathway is restricted to bacteria, algae, plants and fungi (Hermann and Weaver 1999, Shick et al. 1999), invertebrates lacking phototropic symbionts have to obtain MAAs from their diet (reviewed in Shick et al. 2000, Shick and Dunlap 2002). No data are available about the biosynthesis of MAAs in macroalgae.

1.3.2. Sunscreening function of MAAs

The observation that MAA concentration in algae and corals is correlated with the vertical distribution in which specimens were collected (e.g. Dunlap et al. 1986, Kuffner et al. 1995, Yakovleva et al. 1998, Karsten et al. 1998 b) lead to the assumption that MAAs act as photo-protective sunscreens against UV radiation (e.g. Sivalingam et al. 1976, Bandaranayake 1998, Dunlap and Shick 1998).

To be counted as UV sunscreens, MAAs have to comply three requirements (Cockell and Knowland 1999): they have to absorb UV radiation, they have to be UV induceable and they should enhance the physiological resistance against UV radiation. The first requirement is fulfilled, because MAAs have absorption spectra within the UV bands. Furthermore, they have no fluorescent capability (shinorine; Shick et al. 2000) or only very low quantum yield of fluorescence (porphyra-334; Conde et al. 2000). In addition, radical production was absent in shinorine solution irradiated with UV radiation (Shick et al. 2000). These characteristics are consistent with a high efficiency of thermally dissipation of absorbed UV-B energy, supporting the hypothesis of a photo protective function (Shick and Dunlap 2002). In this connection, the high photo stability of MAAs in vitro and vivo should be mentioned (Adams and Shick 1996, 2001, Conde et al. 2000).

The second requirement is fulfilled in most cases, but not in general (see Hoyer et al. 2002 and below). Because organisms containing MAAs are generally better adapted and less affected by UV radiation than those without (e.g. Neale et al. 1998 a, Bischof et al. 2000, Karsten et al 2001, Aquilera et al. 2002b, Goncalves et al. 2002, Litchman et al. 2002, Lesser and Barry 2003), the third requirement is also fulfilled. Nevertheless, the degree of protection varies (Lesser 1996, Neale et al. 1998 a, b, Franklin et al. 1999).

The accumulation of MAAs is only one of various strategies to minimize UV induced damage (Roy 2000). Therefore, it is hard to determine whether an increased resistance

to UV radiation is due to the formation of MAAs and/or other photo protective or repair processes (Neale et al. 1998 b). Detailed investigations will be necessary to clarify which part of the acclimation process to UV radiation is due to MAA accumulation.

Protection of photosynthesis due to MAA accumulation could be demonstrated (e.g. Karsten et al. 1999). Biological weighting functions clearly show that photosynthetic sensitivity to UV radiation in MAA-rich phytoplankton is lower at wavelengths strongly absorbed by MAAs (Neale et al. 1998 a, Litchman et al. 2002). In contrast, in the diatom *Thalassiosira weissflogii* the synthesis of MAAs proceeds only after photosynthesis recovers from UV stress, but MAAs protected against photo inhibition once accumulated (Zudaire and Roy 2001).

This suggests a close link between photosynthesis and MAA biosynthesis, which is supported by the inhibitory effect of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) on dinoflagellate MAA formation (Carreto et al. 1990 a). Presumably 60 ATP-equivalents or 600 moles of photons captured in photosynthesis are necessary for synthesis of one mole of MAAs (references in Shick and Dunlap 2002). Thus, the cost for MAA formation is considerable and may amount to 19 % of the cost of total cell production (Raven 1991). Therefore, synthesizing MAAs as photo protection might occur at the expense of other cellular processes such as growth (Hernando et al. 2002), resulting in strongly entangled relationships among different responses to UV radiation.

1.3.3 Other functions of MAAs

In addition to their primary role as UV sunscreens, MAAs also have other functions. One of these is the role as antioxidant. Based on the assumed biochemical evolution of MAAs via 4-DG, the enhancement of the UV sunscreen capacity to the UV-A band of the solar spectrum coincides with a loss in antioxidant properties. While 4-DG is a strong antioxidant (Dunlap and Shick 1998), monosubstituated MAAs have only moderate, concentration dependent antioxidant activities and imino MAAs are oxidatively robust (Dunlap and Yamamoto 1995). However, exceptions seem to exist: The imino MAA usujirene, extracted from the red alga *Porphyra yezoensis*, was proved to be a strong antioxidant (Nakayama et al. 1999).

In addition, Dunlap and Shick (1998) demonstrated that shinorine and porphyra-334 can be bioconverted into mycosporine-glycine and further into 4-DG through *Vibrio* bacteria. This is a good indication that the biosynthetic pathway of MAA can be

reversed, leading to MAAs as precursors of antioxidants. However, the generality of this process has to be proven and the enzymes controlling it have yet to be identified.

In some cyanobacteria MAAs act as osmolytes (Oren 1997, Karsten 2002). However, this is not a general feature. In the cyanobacterium *Chlorogloeopsis* the final MAA concentration is also positively correlated to salinity, but the accumulated MAA concentration represents only 5 % of the internal osmolyte concentration, which makes it unlikely that they contribute to the osmotic regulation (Portwich and Garcia-Pichel 1999). In contrast to the positive correlation between cyanobacterial MAAs and salinity, the MAA concentration of the red algae *Palmaria palmata* and *Devaleraea ramentacea* seemed not to be correlated to the surrounding salinity (Karsten et al. 2003).

Recently, Misonou et al. (2003) were able to demonstrate that MAAs protect thymine by a direct energy transfer process from molecule to molecule, quenching the excited thymine residue, which results in DNA protection.

1.3.4. Factors controlling MAA formation

Extensive sampling in different regions and water depths within some taxa revealed that the location of the habitat plays an important role. On the latitudinal scale MAAs occur most frequently and in high concentrations in tropical organisms. The concentration of MAAs declines over warm to cold temperate regions (Karsten et al. 1998 b, c, Karsten and West 2000, Shick and Dunlap 2002). The increase in UV radiation with decreasing latitudes may be an explanation for this trend (Frederick et al. 1989).

Furthermore, MAA concentration is positively correlated to the vertical distribution of specimens (bathymetric scale). Thus, organisms inhabiting shallow waters generally contain more MAAs than deep-sea species (Shick et al. 1996, Dunlap and Shick 1998, Karsten et al. 1998 b, 1999, Dunlap et al. 2000, Franklin et al. 1999, Karsten and Wiencke 1999).

In addition, the light climate in algal communities also influences MAA formation. Thus, thallus parts exposed to high irradiances exhibit higher MAA concentrations than basal parts (Wood 1989, Molina and Montecino 1996, Muszynski et al. 1998, Karsten and Wiencke 1999).

Even though temperature and PAR irradiance gradients parallel the gradient of UV radiation on the latitudinal and bathymetric scales and might therefore have an influence on MAA formation, UV radiation is regarded as the main influence on MAA synthesis (Shick and Dunlap 2002). For example, over 70 % of the variation in MAA

concentration exhibited in corals can be explained by differences in exposure to UV radiation (Lesser 2000).

Beside the location of the habitat, seasonal variation in irradiance has an influence on MAA formation. In general, higher MAA concentrations can be found in summer than in winter (Post and Larkum 1993, Michalek-Wagner 2001, Aguilera et al. 2002a, Dummermuth et al. 2003 a, b).

Other than irradiance, nutrient availability, especially nitrogen, influences the synthesis of MAAs. Dinoflagellates grown under nitrogen limitation and high irradiance exhibit similarly low MAA concentrations as specimens grown under low irradiance (Litchman et al. 2002).

1.3.5 Wavelength dependence of MAA formation

Because irradiance is an important factor controlling MAA synthesis, many authors have investigated the irradiance-dependent formation of MAAs. The experimental set-ups mostly distinguish between PAR, PAR plus UV-A and PAR plus UV-A and UV-B using different cut-off filters. The results have revealed a very diverse induction pattern. Hoyer et al. (2002) found different responses to PAR alone, PAR plus UV-A and PAR plus UV-A and UV-B. The first response type increased MAA concentration under both additional UV-A and UV-A plus UV-B, having the highest MAA concentration in the latter. The second response type had the highest MAA concentration under PAR plus UV-A, with no additional induction due to UV-B. The third response type contained the highest MAA concentration under PAR plus UV-A, but additional UV-B lead to a decrease in MAA concentration.

In recent years, many authors have investigated the wavelength dependent induction of MAA biosynthesis (Carreto et al. 1990 a, Riegger and Robinson 1997, Portwich and Garcia-Pichel 2000, Gröniger and Häder 2002, Kräbs et al. 2002, Sinha et al. 2002), resulting in a more detailed but still diverse picture. Using the same polychromatic experimental set-up, Riegger and Robinson (1997) demonstrated that wavelengths between 370 and 460 nm exhibit the highest quantum efficiency for MAA formation in two Antarctic diatoms, while the prymnesiophyte *Phaecystis antarctica* showed a response maximum at around 345 nm. UV-B radiation was most effective for MAA accumulation in two cyanobacteria and the green alga *Prasiola stipitata* (Portwich and Garcia-Pichel 2000, Gröniger and Häder 2002, Sinha et al. 2002). In addition to UV radiation, blue light induces MAA accumulation in the dinoflagellate *Alexandrium*

excavatum (Carreto et al. 1990 a) and the red alga *Chondrus crispus* (Franklin et al 2001). Thus, the information about the nature of the regulating mechanism is limited and rise the question of the general distribution of these characteristics within the algal classes.

1.4 Action spectrum versus Biological weighting function

Presumably since the discovery that sunlight is composed of different radiation colours, the question "Do these different components of irradiance result in differences of metabolic responses?" has emerged (Neale 2000). In the nineteenth century the first crude action spectra identified chlorophyll as chromophore responsible for plant growth (Daubeny 1836, Engelmann 1882, Draper 1884). In the 1940s the term "action spectrum" was coined (Kleczkowski 1972).

In recent decades, more sophisticated methods to study wavelength-dependent plant responses have been developed. The different approaches can be divided into two groups: the first using narrowband (monochromatic) and the second using polychromatic irradiance (Coohill 1990). Although both approaches have been called "action spectrum" in the past (e.g. Rundel 1983, Coohill 1990), a stricter use of this term is advisable to give an immediate idea of what kind of light source was used.

The term "action spectrum" means that monochromatic irradiance is used to study metabolic responses. The criteria for calculating an action spectrum are strict and include, for example, the transparency of samples at the wavelength of interest, an equal number of photons available for each chromophore, and the reciprocity of the measured response to the irradiation dose irrespective of the exposure time (for detailed description see Shropshire 1972, Coohill 1990). If carefully conducted, an action spectrum can determine the absorption characteristics of the target chromophore responsible for the investigated physiological process (Coohill 1990). Nevertheless, this approach is highly artificial and implies that each wavelength contributes independently to the measured effect (Neale 2000).

When polychromatic irradiance is used to investigate physiological responses, the term "biological weighting functions" should be used. In general, a set of cut-off filters is used to selectively eliminate shorter wavelengths from the given radiation spectrum. The wavelength dependent response is calculated through a simple equation (Rundel

1983) or an extensive model sometimes including the time course of the response and inhibiting effects (for detailed descriptions see Cullen and Neale 1997, Neale 2000). The resulting biological weighting function tends to mask the target chromophore, but it provides a more accurate description of the total biological response (Coohill 1989, 1992).

Thus, action spectra and biological weighting functions have their advantages and limitations and it is therefore necessary to provide detailed information on the experimental set up so that the reader understands the limitations of presented data (Coohill 1992). Nevertheless, both approaches are important to understand UV effects. While action spectra identify the absorption characteristics of the chromophore responsible for a specific biological response (Coohill 1990), biological weighting functions help to understand biological responses based on interacting wavelengths including repair and mitigating processes (Coohill 1992).

1.5 Photoreceptors

Solar radiation is the primary energy source for life on earth and it's availability controls growth and development of organisms. To acclimate (short term) and adapt (long term) to changes in environmental radiation conditions, organisms need a way to sense light as signal and to transduce this signal, thus controlling physiological processes (Rüdiger and Figueroa 1992). Molecules, which perform this role, are referred to as photoreceptors.

During evolution different photoreceptors (chromophores) evolved in different evolutionary branches of biochemical pathways (Hegemann et al. 2001). These chromophores can be divided into three different classes: tetrapyrolles (e.g. phytochromobilin in phytochrom), polyene (e.g. retinal in rhodopsin) and aromates (e.g. flavins and pterins in cryptochrom; Hellingwerf et al. 1996). With a combination of these chromophores, impinging irradiation can be sensed in respect to the available light quality and quantity. The transduction of light signals is achieved via photo reduction or photoisomerisation, transforming the light signal into a chemical signal (Häder and Tevini 1987).

The following main photoreceptors have been identified (Hegemann et al. 2001, Watanabe 2004 and references therein): 1) Rhodopsin, with the chromophore retinal,

sensing light between 360 and 635 nm, 2) Phytochromes, with a phytochromobilin as chromophore, sensing red light, 3) Photoactive yellow proteins, with a 4-hydroxycinnenate chromophore, 4) Cryptochrome, with flavins and/or pterins as chromophore sensing UV-A and blue light and 5) Phototropin with a flavin chromophore sensing UV-A and blue light.

One or more photoreceptors from one or more photoreceptor-classes can be involved in the regulation of a physiological process. Casal (2000) pointed out that the interaction between signals perceived by different photoreceptors can occur at three different levels: the control of a photoreceptor by other photoreceptors, the physical interaction between photoreceptors and the interaction downstream from the photoreceptor molecules. The advantage of photoreceptor interactions may be a broader screening of the radiation spectrum received by plants (Casal 2000) and a network of interacting signal pathways, which are predicted to have emergent properties that are not obvious for each pathway in isolation (Weng et al. 1999). These include the integration of signals across multiple time scales, the generation of distinct outputs depending on input strength and duration and self-sustained feedback loops (Bhalla and Iyengar 1999).

With such a complex system of screening capacity and light signal transmission plants are able to respond very efficiently and flexibly to changes in spectral distribution and light intensity.

1.6 Introduction to research questions

Based on a great number of induction experiments it is a standing assumption that MAAs are photo-inducible. Experimental set-ups mostly distinguished between PAR, PAR plus UV-A and PAR plus UV-A and UV-B. The reported results indicate that algae accumulating MAAs can be divided into three different types: 1. species with increased MAA concentration under both additional UV-A and UV-A plus UV-B and the highest MAA concentration under the latter radiation conditions; 2. species with highest MAA concentration under PAR plus UV-A and a neutral effect of additional UV-B radiation and 3. species with highest MAA concentration under PAR plus UV-A, but with lower MAA concentrations when also exposed under UV-B (Hoyer et al. 2002).

The proportionally greater inductive effect of UV radiation compared with PAR and, in type 1 algae, of UV-B over UV-A, may also be explained by a specific effect of UV radiation resulting in an transcriptional upregulation of enzymes involved in MAA biosynthesis (Shick et al. 2000). In this case MAA accumulation in type 2 and 3 algae may be explained by an UV-B induced depression of photosynthesis, which results in fewer energy equivalents for metabolic processes including MAA biosynthesis.

A second possibility of UV stimulated MAA synthesis, which does not necessarily involve specific UV-receptors of direct UV effects on genes coding for MAA biosynthesis enzymes, may be an indirect stimulation caused by higher concentrations of ROS (Shick et al. 2000). In recent years, it has become evident that ROS are key regulators of plant metabolism (Foyer and Noctor 2003, Mahalingam and Fedoroff 2003). Thus, ROS can regulate both transcriptional and post-transcriptional processes (Sauer et al. 2001, Droge 2002, Ermark and Davies 2002).

Based on the – not entirely proven – assumption that MAA biosynthesis proceeds via an early branch of the shikimate pathway (Shick et al. 1999, Portwich and Garcia-Pichel 2003), it is interesting to note that genes coding for the first enzyme in the shikimate pathway are upregulated by UV radiation (Logemann et al. 2000). The signalling mechanism in this case is not known, but stimulation by ROS is discussed (Shick et al. 2000 and references therein).

Because a dose-dependent accumulation of MAAs in the dinoflagellate *Alexandrium excavatum* was reported for blue, but not for red light (Carreto et al. 1990 a), photoreceptor(s) for specific wavebands of PAR can not be ruled out (Shick et al. 2000). In this connection, photoreceptor(s) absorbing UV radiation may also be possible. Thus far, photoreceptors for UV-B (Portwich and Garcia-Pichel 2000), UV-A (Franklin et al. 2001) and blue light (Carreto et al. 1990a, Franklin et al. 2001) have been proposed.

In this respect, the main purpose of the present study was to give a more detailed picture of wavelength-dependent MAA accumulation in the red alga *Chondrus crispus*. The main research questions were:

- Are MAAs photo-inducible?

and if they are

- What are the absorption characteristics of the relevant photoreceptor(s)?

1.7. Thesis outline

In recent years, many authors have investigated wavelength-dependent MAA biosynthesis (Carreto 1990a, Riegger and Robinson 1997, Portwich and Garcia-Pichel 2000, Gröniger and Häder 2002, Kräbs et al. 2002, Sinha et al. 2002), which resulted in a more detailed but very diverse picture of wavelengths responsible for MAA formation. In *C. crispus* UV-B, UV-A and blue light have been reported to lead to an accumulation of MAAs (Karsten et al. 1998 a, Franklin et al. 2001, Kräbs et al. 2002). Because of this, *C. crispus* was used in this study to obtain more information on the wavelength-dependent accumulation of MAAs in both field and laboratory experiments. In some of the conducted experiments other physiological parameters were included to put the accumulation of MAA into context of other acclimation processes. In addition, variations in MAA concentration and composition in the red algal genus *Porphyra* was investigated during desiccation.

Publication I describes variations in the MAA content in the red alga genus *Porphyra* during tidal cycles. The relationship between MAA concentration, irradiance and desiccation was investigated in both field and laboratory experiments.

Publication II focuses on the photo acclimation of *C. crispus* and *Mastocarpus stellatus* to different light qualities. Chlorophyll fluorescence and activity of ribulose-1,5-bisphosphate carboxylase/oxygenase was measured and the concentration and composition of MAAs analysed, to set MAA accumulation into the context of other acclimation processes.

The effect of different wavebands on MAA formation in *C. crispus* was investigated in publication III, IV and V. In publication III different band-pass filters were used to test, whether UV radiation, blue, green or red light stimulates MAA formation. In contrast, cut-off filters were used to conduct polychromatic response spectra under both natural radiation and laboratory condition in publication IV and V, respectively.

In Publication III the effect of different wavebands on MAA formation was tested. In addition, UV stress tests were conducted to investigate the photo protective value of MAAs.

Publication IV describes the acclimation of *C. crispus* to different light qualities. Photosynthesis and the concentration of photosynthetic pigments and MAAs were

investigated. Polychromatic response spectra for MAA accumulation were calculated. With this, the impact of MAA accumulation during acclimation should be estimated. Publication V focuses on polychromatic response spectra for MAA accumulation under both the full light spectrum and UV radiation alone.

In Publication VI an action spectrum for monochromatic light in the range from 280 to 750 nm was described for shinorine induction in *C. crispus*.

Finally, results will be summarized and discussed and a model for MAA biosynthesis will be proposed.

2. Material and Methods

2.1 Okazaki Large Spectrograph

The Okazaki Large Spectrograph (OLS; Watanabe et al. 1982) at the National Institute for Basic Biology (NIBB), Okazaki, Japan provides monochromatic light by a large spectrograph equipped with a 30 kW xenon arc lamp (Ushio Electric Co., Tokyo, Japan). The light beam is reflected first by a plane mirror and than by a condensing mirror. After reflection by a diffraction grating, it passes through an intercepting plate window of different optical filters into the irradiation room (Fig. 3.1).
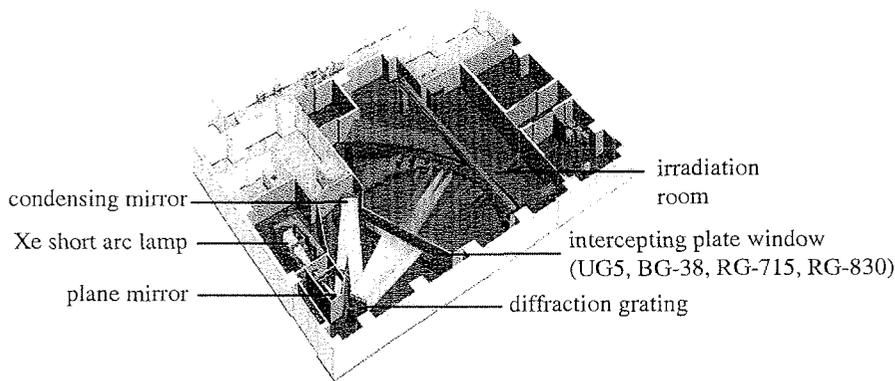
Figure 3.1: Okazaki Large Spectrogra

2.2 Polychromatic response spectra

Polychromatic response spectra were calculated from the respective MAA concentrations accumulated in *C. crispus* grown under different filter conditions. First, second order polynomial (shinorine) and linear (palythine, asterina-330) equations were fitted through the measured data points for the respective MAA concentration accumulated in algae exposed under each filter. Using the equations obtained a

"theoretical" concentration of the respective MAA was calculated for a specific day of the experiment. A mean value was calculated and used for a group of filter treatments with no statistically significant difference in MAA concentration, while the individually calculated value was used directly if the pattern in MAA accumulation under the filter in question was significantly different from neighbouring filter treatments. To calculate the polychromatic response spectrum for the total MAA concentration, the mean value for the steady state was calculated. Statistically significant differences between different filter treatments were taken into consideration as described above.

Response spectra were calculated with this statistically adjusted data set: the difference in MAA concentration in algae grown under two sequentially numbered cut-off filters was divided by the difference in total irradiance between the two light fields beneath the filters according to Rundel (1983). All calculations of polychromatic response spectra are based on the median wavelength of the difference in total irradiance obtained by subtraction of two sequential light fields as described by Riegger and Robinson (1997).

2.3 Action spectrum

The reciprocal of the fluence rate required for formation of a certain amount of shinorine was calculated from the linear part of the fluence response curves and plotted against wavelength (for overview see Shropshire 1972, Schäfer and Fukshansky 1984, Holmes 1997).

2.4 Biological variables

Maximal quantum yield of photosystem II electron transport of dark adapted algae (4 min) was determined by the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) with a PAM 2000 chlorophyll fluorometer (Walz, Effeltrich, Germany), following the protocol described in detail by Hanelt (1998). Photosynthesis versus irradiance curves were recorded with the same fluorometer as described by Bischof et al. (1999).

Changes in the pigment composition and content were analysed as described by Bischof et al. (2002).

Changes in MAA composition and content were analysed as described by Kräbs et al. (2002) with the modification of the mobile phase to 5 %, 15 % or 25 % aqueous methanol (v/v) plus 0,1 % acetic acid (v/v) in water.

2.5 Data treatment

Statistical significance (p<0,05) of difference in MAA and pigment content accumulated under each cut-off filter and for different times as well as their combined effect was tested by two way analysis of variance (ANOVA) followed by Least Significant Difference-Test (LSD). Calculations were done using the program Statistica Kernel-Version 5.5A (StatSoft, Inc., Tulsa, OK, USA). When requirements for ANOVA were not fulfilled, a Mann & Whitney Test (p<0,05; Lozán & Kausch 1998) was conducted.

3. Publications

Publ. I: Effects of desiccation and irradiance during tidal cycles on the content of the mycosporine-like amino acids (MAAs) in *Porphyra umbilicalis* (L.) J. Ag. and *Porphyra purpurea* (Roth) C. Ag.

Gudrun Kräbs and Christian Wiencke

Publ. II   Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline?

Kai Bischof, Gudrun Kräbs, Dieter Hanelt & Christian Wiencke
Helgoland Marine Research (2000) 54, 47-52

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Publ. III   Blue light and UV-A radiation control the synthesis of mycosporine-like amino acides in *Chondrus crispus* (Florideophyceae)

Linda A. Franklin, Gudrun Kräbs & Ralph Kuhlenkamp
Journal of Phycology (2001) 37, 257-270

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Publ. IV   Photosynthesis, photosynthetic pigments and mycosporine-like amino acids after exposure of the marine red alga *Chondrus crispus* (Gigartinales, Rhodophyta) to different light qualities

Gudrun Kräbs & Christian Wiencke
Submitted to Phycologia

Publ. V Polychromatic response spectra for the accumulation of UV-absorbing mycosporine-like amino acids in the red alga *Chondrus crispus* Stackh.

Gudrun Kräbs and Christian Wiencke
In preparation, to be submitted to Journal of Phycology

Publ.VI A monochromatic action spectrum for the photoinduction of the UV-absorbing mycosporine-like amino acid shinorine in the red alga *Chondrus crispus* Stackh.

Gudrun Kräbs, Masakatsu Watanabe & Christian Wiencke
Revised manuscript in press by Photochemistry and Photobiology (2004) 79 (6)

Explanation of my part of each publication:

Publication I, IV and V

I created the experimental set-up, conducted the experiment and wrote the first version of the manuscript, which was then improved in collaboration with the co-author.

Publication II and III

I performed the MAA analysis and contributed to the discussion.

Publication VI

I created the experimental set-up together with my first co-author, conducted the experiment and wrote the first version of the manuscript, which was then improved in collaboration with the co-authors.

Effects of desiccation and irradiance during tidal cycles on the content of the mycosporine-like amino acids (MAAs) in *Porphyra umbilicalis* (L.) J. Ag. and *Porphyra purpurea* (Roth) C. Ag.

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Abstract

The effect of irradiance and desiccation during tidal cycles on mycosporine-like amino acids (MAAs) of the red algal genus *Porphyra* was examined on two sunny days in early summer on Helgoland and in the laboratory. During morning and evening high tide algae collected in the field exhibited high concentration of imino MAAs, while markedly lower concentrations could be found in desiccated thalli at noon. A counter-rotating pattern of mycosporine-glycine could be observed in May, while the pattern of mycosporine-glycine concentration coincided with the pattern of imino MAAs in June. Algae collected in June had a four-fold higher MAA concentration than algae collected in May and the decrease/increase in MAA content occurred faster, but the difference in MAA content between submerged and desiccated thalli remained the same. Thalli kept submerged throughout the day in June exhibited no changes in MAA content. The trend measured in May could be confirmed through a shortened tidal cycle in the laboratory, although the difference in MAA content between submerged and desiccated thalli was lower than in the field. This might be due to the lower PAR and the absence of UV radiation. Simulating high tide at noon, similar results could be found, but with reversed premises. We could show that the MAA content is dependent on the water content of the algae and the incident irradiance. The possible role of this irradiance and water content dependent cycle of MAAs might be their function as antioxidants and as their precursors.

Key words: desiccation, mycosporine-like amino acids, *Porphyra*

Introduction

Species of the red algal genus *Porphyra* abundantly occupy the intertidal and upper sublittoral zone of rocky shorelines (Lüning 1990), and have to cope with extreme periodical changes of various environmental factors such as irradiance, desiccation, temperature, salinity and nutrient availability. Of these stress factors, Maegawa et al. (1993) regard solar UV radiation as one of the most important factors controlling the depth distribution of red algae, which is supported by other studies of macroalgal zonation patterns from polar to temperate regions (Dring et al. 1996; Hanelt et al. 1997; Bischof et al. 1998, 2000).

Intertidal and upper sublittoral red algae contain high amounts of mycosporine-like amino acids (Hoyer et al. 2001). Due to their absorption spectra between 310 and 360 nm, it is assumed that MAAs act as UV sunscreens (Dunlap and Shick 1998, for overview see Bandaranayake 1998; Shick et al. 2000). Comparisons of physiological processes in algae with accumulated MAAs to those without demonstrated that the former are generally more resistant to UV induced damage, although the degree of protection varies (Lesser 1996; Neale et al. 1998; Franklin et al. 1999).

Beside this UV screening role, several MAAs have demonstrated antioxidant properties (Dunlap and Yamamoto 1995; Dunlap and Shick 1998; Nakayama et al. 1999; Shick and Dunlap 2002) and were thought to have an osmotic function (Oren 1997; Karsten 2002, but see Portwich and Garcia-Pichel 1999). Furthermore, they might protect the DNA molecules by quenching the excitation state of thymine molecules (Misonou et al. 2003).

Studies on the accumulation of MAAs in species exposed to different radiation conditions were usually performed for periods of several days or weeks (Franklin et al. 1999; Karsten et al. 2001). To our knowledge there has been no study examining the variation of MAAs during tidal cycles at different times of day. In the present study we examined the MAA content of *Porphyra umbilicalis* and *P. pupurea* on different levels of the Helgoland shoreline in May and June to examine the effect of desiccation and natural solar irradiance on the MAA concentration within the algae. Furthermore, we simulated two different reduced tidal cycles in the laboratory, exposing algae to a course of PAR irradiance to obtain a better understanding of the relationship between desiccation, impinging irradiance and MAA content. Our results give an improved

insight into the physiological function of MAAs under changing environmental conditions in the intertidal zone.

Materials and Methods

Plant material and culture conditions

For the outdoor experiment, samples of *Porphyra umbilicalis* growing on the high tide water line were taken every hour at the east pier of the northeast harbour of Helgoland (German Bight, North Sea; 54°11'N, 7°53'E) on 3 and 4 May 1997. On the 7 June 1999 thalli of *Porphyra* spp. were collected from two different levels of the shoreline of the north-west harbour of Helgoland, (1) *Porphyra umbilicalis* approximately 50 cm below the high tide water line and (2) *Porphyra pupurea* circa 150 cm below it. Additionally, some thalli from the upper shore location were placed into a small water basin placed close to their original growth site. Every hour the water was exchanged to minimize changes in temperature and nutrient levels. Samples were taken every half hour. On both dates low tide was around noon.

For the laboratory experiments, thalli of *Porphyra umbilicalis* were collected from the high tide water line of the east pier of the northeast harbour of Helgoland in September 1997. They were cultured for two months at 15°C, 100 μmol m^{-2} s^{-1} PAR and a light dark cycle of 16:8 hours in Provasoli enriched North Sea water (McLachlan 1973), before being transferred to the experimental set up.

The experimental set up consisted of a slide projector and a mirror reflecting the light beam onto a table. Samples were exposed for eight hours in small, shallow, transparent boxes to one hour of each 150 (1st and 8th hour), 300 (2nd and 7th hour), 500 (3rd and 7th hour) and 800 μmol m^{-2} s^{-1} PAR (4th and 5th hour), obtained through the use of the neutral density filters T=0.2, T=0.4, T=0.6 and T=0.8 (Schott, Mainz, Germany), respectively.

In the first experiment, morning and evening high tide was simulated. Therefore, samples were submerged in seawater for the first and eighth hour of the experiment. In the second experiment noon high tide was simulated keeping specimens submerged in seawater during the fourth and fifth hour of the experiment. In this case algae were kept over night within moist tissue paper. In both experiments samples were taken every hour.

The state of desiccation was calculated using the following equation:

$$\text{Waterloss (\%)} = 100 - (100 * (FW - FW_{rel}))/(FW-DW)$$

with the initial fresh weight FW, the relative fresh weight FW_{rel} at the sampling time and the dry weight DW.

Light measurements

Radiation conditions at Helgoland during the experiment were continuously monitored by a PUV-500 radiometer (Biospherical Instruments, San Diego, USA) mounted on the roof of the Biologische Anstalt Helgoland.

Analysis and identification of MAAs

The samples were silica dried and MAAs were subsequently extracted for 2 hours in 25 % aqueous methanol (v/v) at 40°C. The extracts were evaporated to dryness under reduced pressure (Speed Vac Concentrator SVC 100 H, Savant Instruments. Inc., Holbrook, USA) and redissolved in the initial volume of 100 % methanol. The extracts were separated on a Waters HPLC system fitted with a Knauer Spherisorb RP-8 column. The mobile phase was 15 % or 25 % aqueous methanol (v/v) plus 0.1 % acetic acid (v/v), run isocratically with a flow rate of 0.7 ml min^{-1}. Peaks were detected at 330 nm and absorption spectra were recorded from 290 to 400 nm. The MAAs were identified by absorption spectra, retention time, and in the case of shinorine by co-chromatography with extracts of the red alga *Mastocarpus stellatus*. Quantification was made using published extinction coefficients (Takano et al. 1978a,b; Tsujino et al. 1980; Dunlap et al. 1986; Gleason et al. 1993). Because of their absorption spectra concentrations of both substances unknown 1 and 2 (Fig. 1) were calculated as for porphyra-334 to give a rough quantification. Results are expressed in mg g^{-1} DW.
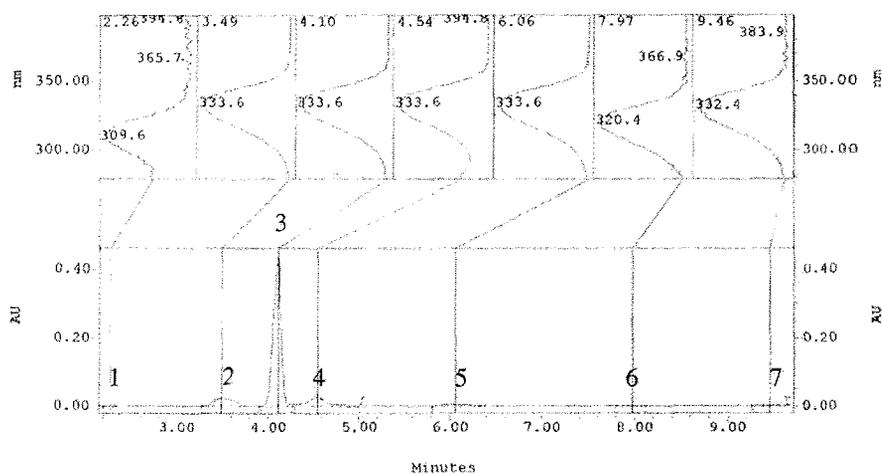
Figure 1: HPLC chromatogram with peak absorption spectra of a 25 % aqueous methanol (v/v) extract; a mobile phase of 15 % aqueous methanol (v/v) and 0.1 % acetic acid (v/v) was run isocratically over a Knauer Spherisorb RP-8 column; AU: absorption units at 330 nm. 1 = mycosporine-glycine, 2 = shinorine, 3 = porphyra-334 = 4. unknown 1, 5 = unknown 2, 6 = palythine, 7 = palythinol.

Data treatment

Statistical significance in MAA contents on 3 and 4 May 1997 and in the laboratory experiment was calculated using the Mann and Whitney Test ($p < 0.05$) and the Mediantest ($p < 0.1$) (Lozán and Kausch 1998). Statistical significance ($p < 0.05$) of differences in MAA content in *Porphyra* spp. (7 June 1999) between different sampling times and between desiccated and submerged thalli at a specific time were tested by one and two way analysis of variance (ANOVA) followed by Least Significant Difference-Test (LSD). Calculations were done using the program Statistica Kernel-Version 5.5A (StatSoft, Inc., Tulsa, OK, USA)

Results

The first experiment was conducted on 4 May 1997. It was mostly sunny during the experiment (Fig. 2A and Tab. 1). The temperature was between 7.3 and 10.8 °C, the relative atmospheric humidity between 64 and 99 %, and the wind speed between 7.3 and 10.8 m s^{-1} (Deutscher Wetterdienst, Hamburg, Germany). High tide was at 9:27 h and 21:52 h, low tide at 16:15 h.
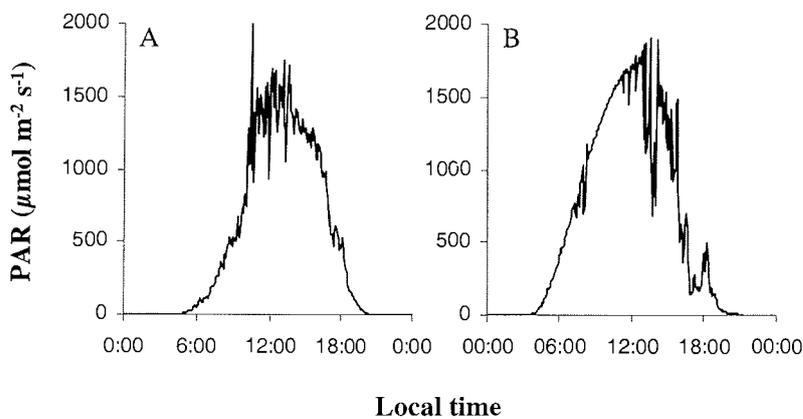

Figure 2: Course of solar PAR, as recorded on 4 May 1997 (A) and 7 June 1999 (B).

Table 1: Daily dose of solar radiation

Date	305 nm	320 nm	340 nm	380 nm	400-700 nm
		kJ m^{-2}			mol m^{-2}
03.05.1997	0,477	5,747	11,620	17,139	40,418
07.06.1999	0,407	6,479	13,049	20,313	49,103

The content of various MAAs in *Porphyra umbilicalis* showed a considerable variation dependent on the time of day and the tidal cycle. The MAA content of the imino MAAs porphyra-334, palythine, palythinol and palythene was reduced half an hour after morning high tide, and within the next hour during further desiccation to values close to zero. In contrast, the amount of mycosporine-glycine increased in desiccated thalli at low tide (Fig. 3). Two hours before the evening high tide an increase in the imino
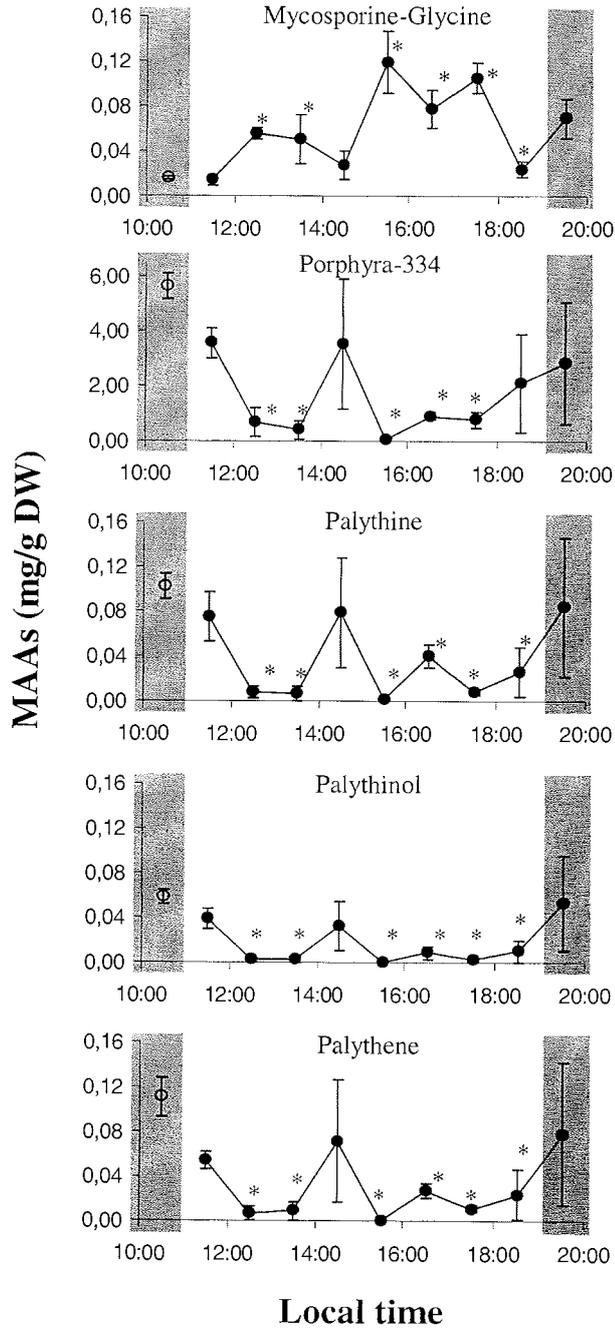
- 27 -

Figure 3: Daily cycle of MAA content in *Porphyra umbilicalis*, as recorded on 3 (●) and 4 (○) May 1997; MV±SD, n = 3; statistical significant differences (p<0,05) from start value at 11:30 h of 4 May are marked (*); shaded areas represent water coverage of sampling site.

MAAs was observed with a parallel decline of mycosporine-glycine. At the evening high tide all algae exhibited similar MAA concentrations and distributions to those observed during the morning high tide. Throughout the daily cycle, there was an exponential correlation between the accumulated amount of imino MAAs and mycosporine-glycine (Fig. 4).

Figure 4: Ratio between mycosporine-glycine and porphyra-334, as recorded on 3 and 4 May 1997; values from 19:30 h (O) are left out of the applied exponential fitting.

$y = 0,0553x^{-0,9571}$
$R^2 = 0,5051$

Imino MAAs (mg/g DW)

Mycosporine-Glycine (mg/g DW)
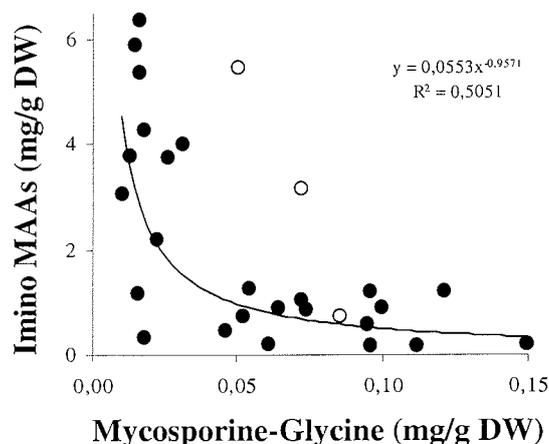
The second experiment was conducted on 7 June 1999. During the experiment it was sunny (Fig. 2B and Tab. 1), the temperature was between 12.7 and 16 °C, the relative atmospheric humidity between 77 and 88 % and the wind speed between 2.3 and 6.7 m s^{-1} (Deutscher Wetterdienst, Hamburg, Germany). High tide was at 6:50 h and 19:08 h, low tide at 13:22 h.

In *P. umbilicalis* collected approximately 50 cm below the high tide water line, the daily cycle of shinorine and porphyra-334 concentration was similar to that found in May (Fig. 5). The concentration of palythine, asterina-330 and palythinol remained stable throughout the experiment. The amount of imino MAAs was four times higher than in May and the reduction of these MAAs happened faster (within 30 minutes), but to approximately the same extent (circa 10 mg g^{-1} DW), down to an imino MAA amount of roughly 15 mg g^{-1} DW.

In contrast to the pattern of mycosporine-glycine concentration with high values during low tide in May, the content of mycosporine-glycine in thalli sampled in June showed the same pattern as the imino MAA concentration.

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Mycosporine-Glycine

Shinorine

Porphyra-334

Σ (Palythine, Asterina-330, Palythinol)

Local time

Figure 5: Daily cycle of MAA content in *Porphyra umbilicalis* growing approximately 50 cm below high tide waterline, as recorded on 7 June 1999; ● = specimens undergoing natural desiccation, ○ = specimens artificially covered under water; MV±SD, n = 5-15; shaded areas represent water coverage of sampling side; for statistical data see Table 2.
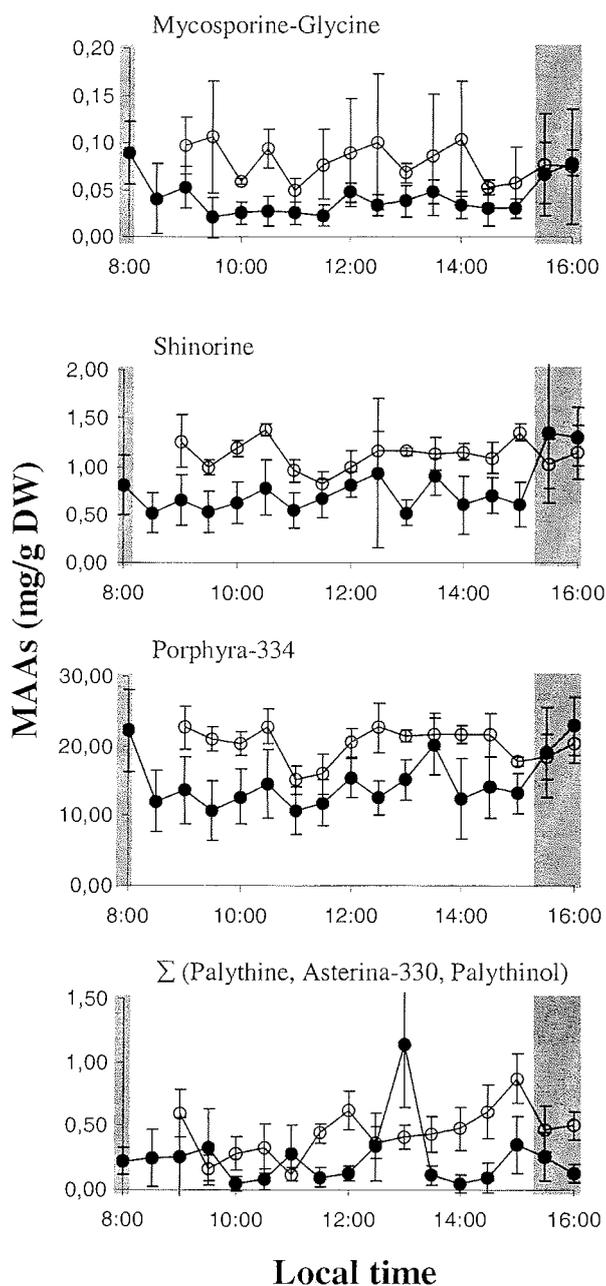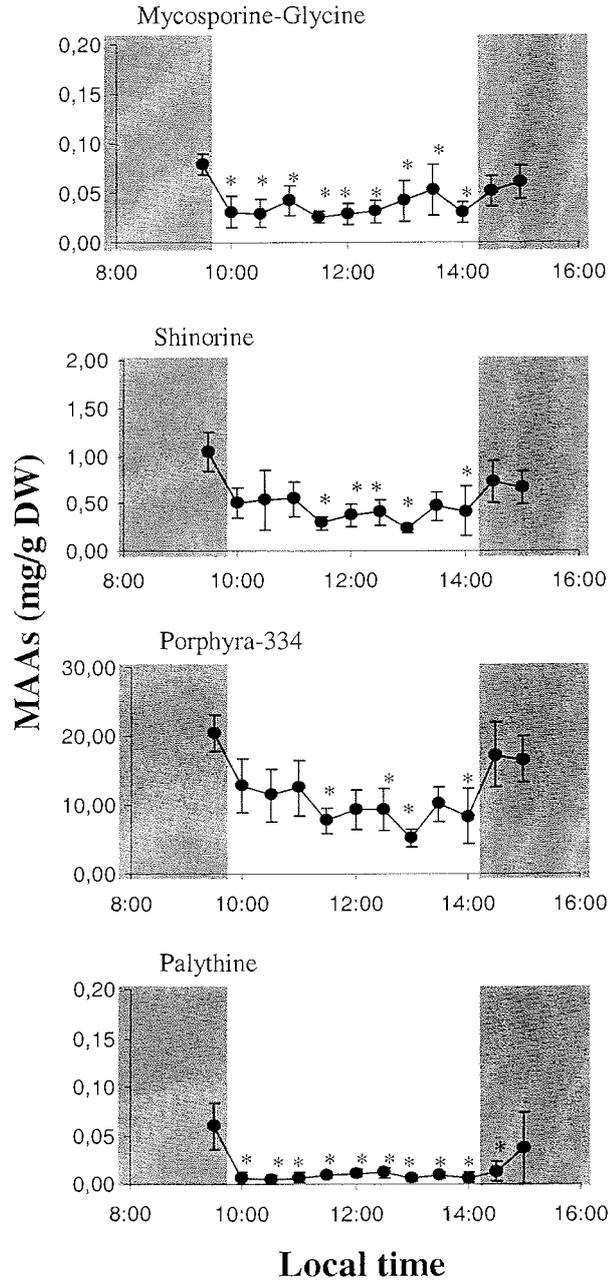
Figure 6: Daily cycle of MAA content in *Porphyra purpurea* growing approximately 150 cm below high tide waterline, as recorded on 7 June 1999; MV±SD, n = 10-15; statistical significant differences (p<0,05) from start value at 9:30 h are marked (*); shaded areas represent water coverage of sampling side.

Table 2: Statistical significance (p<0.05) of difference in MAA between concentrations at 8:00 h and different sample times (●, ○) and between thalli undergoing natural desiccation (●) and submerged thalli (○) at a specific sampling time (one and two way ANOVA followed by LSD).

		8:30 h	9:00 h	9:30 h	10:00 h	10:30 h	11:00 h	11:30 h	12:00 h	12:30 h	13:00 h	13:30 h	14:00 h	14:30 h	15:00 h	15:30 h	16:00 h
●	Mycosporine-Glycine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	Shinorine															+	+
	Porphyra-334	+	+	+	+	+	+	+	+	+	+		+	+	+		
	Palythine		+							+	+						
	Asterina-330			+					+	+					+	+	
	Palythinol		+		+	+		+	+		+	+	+	+		+	+
○	Mycosporine-Glycine																
	Shinorine	+			+	+											
	Porphyra-334	+			+	+									+		
	Palythine	+			+					+					+		
	Asterina-330	+	+	+	+	+									+		
	Palythinol		+	+	+	+						+					
○ versus ●	Mycosporine-Glycine				+	+	+		+	+	+	+	+	+			
	Shinorine				+	+					+		+	+	+	+	
	Porphyra-334			+	+	+			+	+	+		+	+	+		
	Palythine			+	+	+		+	+		+	+	+	+	+	+	
	Asterina-330			+	+	+		+	+		+	+	+	+	+		
	Palythinol						+		+		+		+	+		+	

Table 3: Statistical significant differences between the two artificial tidal cycles at a specific time: Mann and Whitney Test (p<0.05)/Mediantest (p<0.1)

	0 hour	1 hour	2 hour	3 hour	4 hour	5 hour	6hour	7 hour	8hour
Mycosporine-Glycine	+/+	-/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+
Shinorine	+/+	-/-	+/+	+/+	+/+	-/-	-/-	-/-	+/+
Porphyra-334	+/+	-/-	+/+	-/+	-/-	-/+	-/-	-/-	+/+
Unknown 1	-/-	-/+	+/+	+/+	+/+	+/+	-/+	+/+	+/+
Unknown 2	+/+	+/+	-/-	+/+	+/+	-/+	-/-	-/+	+/+
Palythine	+/+	+/+	-/+	+/+	-/+	-/+	-/-	+/+	-/+

Specimens of *P. purpurea* collected approximately 150 cm below the high tide water line exhibited the same pattern of MAA accumulation as described for algae collected 50 cm below it.

Irrespective of the shoreline-level, thalli exposed to desiccation stress exhibited the same pattern in MAA content, while thalli kept artificially submerged showed no changes in the amount of accumulated MAAs (Fig. 5 and 6).

Laboratory experiments showed that a tidal cycle with a period reduced to 8 h, with morning and evening high tide, exhibited the same pattern in the concentration of imino MAAs and mycosporine-glycine as in algae collected in May (Fig.7). The only difference was, that the reduction of imino MAAs is approximately one fourth of the reduction found in thalli exposed to natural solar irradiance.

Time (h)

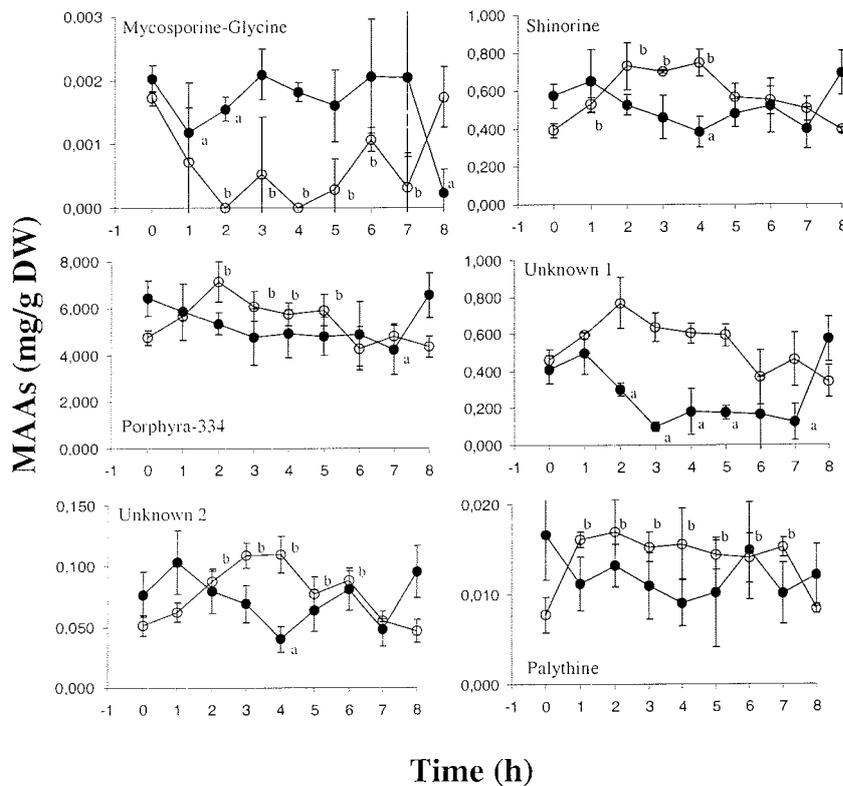Figure 7: Cycle of MAA content in *Porphyra umbilicalis* during a shortened, artificial tide cycle; specimens submerged during first and last hour (●) and specimens submerged during fourth and fifth hour (O) of the experiment; MV±SD, n = 3; statistical significant differences (p<0,05) from start value are marked (a and b, respectively), for statistical differences between the tide cycle see Table 3.

Simulating high tide at noon resulted in a relationship between the accumulated imino MAA content and mycosporine-glycine, similar to the experiment with morning and evening high tide, but with reversed premises. Thus, high mycosporine-glycine concentrations were found in the beginning and at the end of the experiment after 8 h, while high imino MAA concentrations were found after about (1) 2 to 4 (7) h.

The relationship between mycosporine-glycine and imino MAAs was not so clear as in the field experiment in May, but a linear correlation between them, might exist irrespective of the simulated tidal cycle (Fig. 8A). The highest correlation was found between mycosporine-glycine and the MAA unknown1 (Fig. 8B).

Plotting the water loss against the amount of imino MAAs it becomes obvious, that the content of imino MAAs decreases at a water loss of approximately 10 % of the internal water content and stays constant at water losses up to 60 % (Fig. 9).
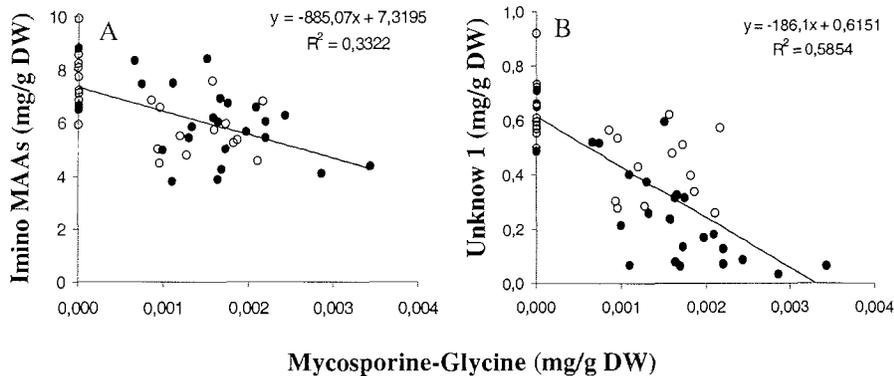

Mycosporine-Glycine (mg/g DW)

Figure 8 (↑): Ratio between mycosporine-glycine and the total concentration of imino MAAs (A) and unknown 1 (B), respectively in *Porphyra umbilicalis* during a shortened, artificial tide cycle; specimens submerged during first and last hour (●) and specimens submerged during fourth and fifth hour (O) of the experiment.

Figure 9 (→): Ratio between desiccation status and imino MAA content in *Porphyra umbilicalis* during a shortened, artificial tide cycle; specimens submerged during first and last hour (●) and specimens submerged during

Internal water loss (%)
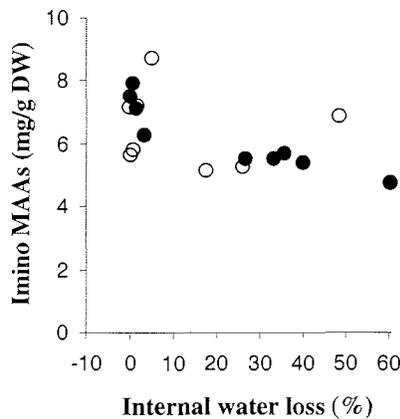
Discussion

Our results show for the first time that the MAA content in a red alga changes with the tide. Until now the MAA concentration in the genus *Porphyra* was regarded as constantly high without room for short-term induction (Gröniger et al. 1999; Hoyer et al. 2001). In our experiment, the MAA content, however, is not directly related to the tide as it remains stable when algae are artificially submerged during low tide (Fig. 5) and during rainy days (data not shown). Furthermore, the "tide-dependent" changes can be simulated by exposing long term submerged algae to artificial tidal cycles (Fig. 7). Thus, the changes in MAA content are due to a desiccation of the thalli and are therefore dependent on the water content. This was demonstrated also in the laboratory experiment. Irrespective of the simulated tidal cycle, the decrease in the imino MAAs is associated with a loss of about 10 % loss of the thallus water content (Fig. 9).

As solar irradiance, temperature, wind and atmospheric humidity affect the rate of desiccation (Bell 1995), this would also explain the faster decrease of the MAA content in June. Thus, higher temperatures and irradiance measured in June would increase the desiccation rate of the thalli and the critical threshold of 10 % loss of water content would be reached sooner. To make a precise prediction, temperature and atmospheric humidity as well as wind speed should be measured near the *Porphyra* canopy.

Theoretically, an explanation for the water dependent changes in MAA content would be a possible function of the MAAs as organic osmolytes. Because MAAs are highly water soluble, polar and - at cellular pH - uncharged or zwitterionic amino acid derivates (Carreto et al. 1990), they exhibit the typical characteristics of an organic osmolyte (Kirst and Bisson 1979). Furthermore, high concentrations of MAAs do not have harmful effects on the cellular metabolism, another feature of "compatible solutes" (Brown and Simpson 1972). While some authors described an osmotic function of MAAs in cyanobacteria (Oren 1997; Karsten 2002), this is not the case here, because the imino MAAs decrease under hypersaline conditions in desiccating thalli (Fig. 5-7) and stay stable in hyposaline conditions when exposed to rain. This is not the pattern characteristic for osmolytes, which should increase/decrease during hyperosmotic/hypoosmotic stress (Kirst 1990).

The content of mycosporine-glycine detected in algae collected in May and in thalli exposed in the laboratory experiment, however, would fit into this pattern. However, this pattern was not found in the experiment conducted in June. Furthermore, compared

with the concentration of other osmolytes such as (iso) floridoside, which have been found at concentrations of between 50 and 220 μmoles per g fresh weight in *P. umbilicalis* (Wiencke and Läuchli 1981; for *P. purpurea* see Reed et al. 1980) the changes in mycosporine-glycine content (1 mg g^{-1} DW ~ 4 μmol g^{-1} DW) would only play a minor role. Therefore we conclude that, as in *Palmaria palmata* and *Devaleraea ramentacea* (Karsten et al 2003), MAAs do not have a function as osmolytes in *Porphyra* spp.. Similar results were reported for the cyanobacterium *Chlorogloeopsis*, in which the final MAA concentration is positively correlated to salinity, but represents less than 5 % of the internal osmolytes and it is therefore unlikely that they contribute to osmotic regulation (Portwich and Garcia-Pichel 1999).

A more probable explanation for the variation in MAA content may be their function as an antioxidant-system to protect algae from reactive oxygen species (ROS).

Singlet oxygen ($^1O_2$), super oxide (O_2^-), peroxides (e.g. H_2O_2) and hydroxyl radicals (OH$^-$) are generated both by respiration and photosynthesis and damage essential components of cells such as proteins, membrane lipids and nucleic acids (Asada and Takahashi 1987; Bowler et al. 1992). Desiccation, which can disrupt respiration and photosynthesis, can lead to an increased formation of ROS (Bowler et al. 1992; Smirnoff 1993; Alscher et al. 1997; Yordanov et al. 2000). Thus, Davison and Pearson (1996) regard protection against ROS as an important mechanism in stress tolerance of intertidal algae. Beside enzymes (e.g. ascorbate peroxidase, gluthation reductase, super oxide dismutase), plants use antioxidants (e.g. ascorbic acid, gluthation, α-tocopherol) to scavenge ROS (Bowler et al. 1992; Ingram and Bartels 1996). This strategy has also been detected in macroalgae (Aguilera et al. 2002; Bischof et al. 2003; Dummermuth et al. 2003).

There is good evidence that some MAAs (e.g. mycosporine-glycine) also have antioxidant properties (Dunlap and Yamamoto 1995; Nakayama et al. 1999; Shick and Dunlap 2002). Furthermore, Dunlap and Shick (1998) demonstrated that shinorine and porphyra-334 can be bioconverted into mycosporine-glycine and further into the strong antioxidant 4-deoxygadusol through *Vibrio* bacteria. Although data on enzymes regulating this kind of bioconversion in algae are still missing, we would like to suggest that the responsible enzymes exists and hypothesise the following scenario:

After emersion of *Porphyra* thalli ROS concentration increases due to photosynthetic activity, which is fully functional until thalli are desiccated to approximately 60 % relative water content (Stocker and Holdheide 1938; Lipkin et al. 1993). The

presupposed enzymes converting shinorine and porphyra-334 into mycosporine-glycine are presumably activated through desiccation and/or higher concentrations of ROS. The ten-fold lower increase in mycosporine-glycine compared to the decrease in shinorine and porphyra-334 content of algae collected under natural conditions is explained through a decomposition of oxidised mycosporine-glycine (Dunlap and Yamamoto 1995). With rehydration the presupposed enzymes are inactivated and the pool of shinorine and porphyra-334 is refilled. Presumably, a steady state between synthesis of shinorine and porphyra-334 and the interconversion to mycosporine-glycine is reached at some point during emersion. As we do not have any information about 4-deoxygadusol concentrations during tidal cycles, it is yet unclear whether the bioconversion stops at the mycosporine-glycine level or is continuous to 4-deoxygadusol.

Based on the results of the laboratory experiment, unknown 1 and 2 (maybe isomers of shinorine and/or porphyra-334) might be important steps within the interconversion (especially unknown 1). It is likely that they also exist in field samples, but remained undetected because the HPLC analysis was performed with a higher methanol concentration. Therefore, it is important to study isomers, their function and properties, as recently done for the cis-trans isomers palythene and usujirene (Conde et al. 2003).

The different correlation between mycosporine-glycine and imino MAA concentrations (Fig. 3 and 8) suggest that the light climate during desiccation plays an important role for the course of this process. Thus, the exponential correlation between mycosporine-glycine and imino MAA concentration found in algae collected in May under natural solar radiation might reflects the two fold higher light intensities and inclusion of UV radiation compared to the PAR intensities in the laboratory experiment. As UV radiation and high light intensities increase the formation of ROS (Aguilera et al. 2002; Smirnoff 1993; Yordanov et al. 2000), this might also reflect the necessity of a higher efficiency of this process.

In this respect the seasonal differences in the course of mycosporine-glycine concentration during emersion might be explained. Higher PAR and UV intensities may make it necessary for a pool of mycosporine-glycine already exists prior to emersion and that the oxidation/decomposition of mycosporine-glycine to be faster due to a higher level of ROS. It is therefore possible that these enzymes are activated and deactivated in spring and fall, while they remain activated in summer.

The seasonal differences also indicate that beside the function of MAAs as sunscreens (e.g. Dunlap et al. 1986; Dunlap and Shick 1998; Conde et al. 2000) a major role of MAAs in *Porphyra* spp. might be their role as antioxidant and as precursors of antioxidants.

Although this scenario is highly hypothetical and needs to be proved, we suggest that an antioxidant system like this exists in *Porphyra* spp.. Whether it is a common protection mechanism existing in all intertidal algae exhibiting MAAs must also be ascertained.

Our data indicate that MAAs contribute to desiccation tolerance, adding a further substance/mechanism enhancing the ability to withstand emersion. They might not play a major role, but since many mechanisms interact and act at different stages of desiccation and as sensitive compartments can be protected in more than one way, they may play an essential role in desiccation tolerance (Davison and Pearson 1996; Hoekstra et al. 2001 and references therein).

References

Aguilera J, Dummermuth A, Karsten U Schriek R, Wiencke C (2002) Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Artic marine macroalgae. Polar Biol 25:432-441.

Alscher RG, Donahue JL, Cramer CL (1997) Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100:224-233

Asada K, Takahashi M (1987) Production and scavenging of active oxygen in photosynthesis. In: Kyle DJ, Osmonde CB, Arntzen CJ (eds) Photoinhibion. Elvesier, New York, p. 227-287

Bandaranayake WM (1998) Mycosporines: are they nature's sunscreens? Nat Prod Rep 15:159-172

Bell EC (1995) Environmental and morphological influences on thallus temperature and desiccation of the intertidal alga *Mastocarpus papillatus* Kützing. J Exp Mar Biol Ecol 191:29-55

Bischof K, Hanelt D, Wiencke C (1998) UV-radiation can affect depth-zonation of Antarctic macroalgae. Mar Biol 131:597-605

Bischof K, Kräbs G, Hanelt D, Wiencke C (2000) Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline? Helgol Mar Res 54:47-52

Bischof K, Janknegt PJ, Buma AGJ, Rijstenbil JW, Peralta G, Breeman A (2003) Oxidative stress and enzymatic scavenging of superoxide radiacals induced by solar UV-B radiation in *Ulva* canopies from southern Spain. Sci. Mar. 67:353-359

Bowler C, Montagu M, Inzé D (1992) Superoxide dismutase and stress tolerance. Annu Rev Physiol Plant Mol Biol 43:83-116

Brown AD, Simpson JR (1972) Water relations of sugar-tolerant yeast: the role of intercellular polyols. J Gen Microbiol 72:589-591

Carreto JI, Carigan MO, Daleo G, de Marco SG (1990) Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-protective compounds? J Plankton Res 12:909-9121.

Conde FR, Churio MS, Previtali CM (2000) The photoprotector mechanism of mycosporine-like amino acids: Excited-states properties and photostability of phorphyra-334 in aqueous solution. J. Photochem. Photobiol. B: Biol. 56:139-144

Conde FR, Carignan MO, Churio MS, Carreto JI (2003) In vitro cis-trans photoisomerization of palythene and usjirene. Implications on the *inVivo* transformation of mycosporine-like amino acids. Photochem. Photobiol. 77:146-150

Davison IR, Pearson GA (1996) Stress tolerance in intertidal seaweeds. J Phycol 32:197-211

Dring MJ, Wagner A, Boeskov J, Lüning K (1996) Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements: influence of collection depth and season, and length of irradiation. Eur J Phycol 31:293-302

Dummermuth AL, Karsten U, Fisch KM, König GM, Wiencke C (2003) Responses of marine macroalgae to hydrogen-peroxide stress. J Exp Mar Biol Ecol 289:103-121

Dunlap WC, Chalker BE, Oliver JK (1986) Bathymetric adaptations of reefbuilding corals at Davies Reef, Great Barrier Reef, Australia: III. UV-B absorbing compounds. J Exp Mar Biol Ecol 104:239-248

Dunlap WC, Shick JM (1998) Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: A biochemical and environmental perspective. J Phycol 34:418-430

Dunlap WC, Yamamoto Y (1995) Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. Comp Biochem Physiol 112B:105-114

Franklin LA, Yakovleva I, Karsten U, Lüning K (1999) Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. J Phycol 35:682-693

Gleason DF (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astroides*. Limnol Oceanogr 38:1542-1463

Gröniger A, Hallier C, Häder DP (1999) Influence of UV radiation and visible light on *Porphyra umbilicalis*: Photoinhibition and MAA concentration. J Appl Phycol 11:437-445

Hanelt D, Melchersmann B, Wiencke C, Nultsch, W (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255-266

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. TRENDS Plant Science 6:431-438

Hoyer K, Karsten U, Sawall T, Wiencke C (2001) Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and development stages. Mar Ecol Prog Ser 211:117-129

Ingram J, Bartels D (1996) The molecular basis of dehydration tolerance in plants. Annu Rev Plant Physiol Plant Mol Biol 47:377-403

Karsten U (2002) Effects on salinity and ultraviolet radiation on the concentration of mycosporine-like amino acids in various isolates of the benthic cyanobacterium *Microcoleus chthonoplastes*. Pycol Res 50:129-134

Karsten U, Bischof K, Wiencke C (2001) Photosynthetic performance of Artic macroalgae after transplantation from deep to shallow waters. Oceologia 127:11-20

Karsten U, Dummermuth A, Hoyer K, Wiencke C (2003) Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Artic red algae from shallo waters. Polar Biol 26:249-258

Kirst GO, Bisson MA (1979) Regulation of turgor pressure in marine algae: ions and low-molecular-weight organic compounds. Aust J Plant Physiol 6:539-556

Kirst GO (1990) Salinity tolerance of eukaryotic marine algae. Annu Rev Plant Physiol Plant Mol Biol 41:21-53

Lesser MP (1996) Elevated temperatures an ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol Oceanogr 41:271-283

Lipkin Y, Beer S, Eshel A (1993) The ability of *Porphyra linearis* (Rhodophyta) to tolerate prolonged periods of desiccation. Bot Mar 36:517-523

Lozán JL, Kausch H (1998) Angewandte Statistik für Naturwissenschaftler. Parey, Berlin

Lüning K (1990) Seaweeds. Wiley, New York

Maegawa M, Kunieda M, Kida W (1993) The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jpn J Phycol 41:207-214

Misonou T, Saitoh J, Oshiba S, Tokitomo Y, Maegawa M, Inoue Y, Hori H, Sakurai T (2003) UV-absorbing substance in the red alga *Porphyra yezoensis* (Bangiales, Rhodophyta) Block Thymine Photodimer Production. Mar Biotechnol 5:194-200.

Nakayama R, Tamura Y, Kikuzaki H, Nakatani N (1999) Antioxidant effect of the constituents of susabinori *(Porphyra yezoensis)*. JAOCS 76:649-653

Neale PJ, Banazak AT, Jarriel CR (1998) Ultraviolet sunscreens in *Gymnodium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. J Phycol 34:928-938

McLachlan J (1973) Growth media – marine. In: Stein JR (ed) Handbook of Phycological Methods. Cambridge University Press, Cambridge, pp 25-51

Oren A (1997) Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. Geomicrobiology 14:231-240

Portwich A, Garcia-Pichel F (1999) Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912. Arch Microbiol 172:187-192

Reed RH, Collins JC, Russell G (1980) The effects of salinity upon galactosyl-glycerol content and concentration of the marine red alga *Porphyra purpurea* (Roth) C.Ag.. J Exp Bot 31:1539-1554

Shick JM, Dunlap WC, Buettner GR (2000) Ultraviolet (UV) protection in marine organisms II. Biosynthesis,accumulation, and sunscreening function of mycosporine-like amino acids. In: Yoshikawa T, Toyokuni S, Yamamoto Y, Naito Y (eds) Free Radicals in Chemistry, Biology and Medicine. OICA International, London, pp 215-228

Shick JM, Dunlap WC (2002) Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. Annu Rev Physiol 64:223-262

Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27-58

Stocker A, Holdheide W (1938) Die Assimilation Helgoländer Gezeitenalgen während der Ebbezeit. Z Bot 32:1-59

Takano S, Uemura D, Hirata Y (1978a) Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tubercolosa*. Tetrahedron Lett 26:2299-2300

Takano S, Uemura D, Hirata Y (1978b) Isolation and structure of two new amino acids, palythinol and palythene, from the zoanthid *Palythoa tuberculosa*. Tetrahedron Lett 49:4909-4912

Tsujino I, Yabe K, Sekikawa I (1980) Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendoi* Yamada et Mikami. Bot Mar 23:65-68

Wiencke C, Läuchli A (1981) Inorganic ions and floridoside as osmotic solutes in *Porphyra umbilicalis*. Z Pflanzenphysiol 103:247-258

Yordanov I, Velikova V, Tsonev T (2000) Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38:171-186

ORIGINAL ARTICLE

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Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline?

Abstract *Chondrus crispus* and *Mastocarpus stellatus* both inhabit the intertidal and upper sublittoral zone of Helgoland, but with *C. crispus* generally taking a lower position. Measurements of chlorophyll fluorescence, activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO), and content and composition of UV absorbing mycosporine-like amino acids (MAAs) were conducted in the laboratory, to test whether susceptibility to UV radiation may play a role in the vertical distribution of these two species. Effective and maximal quantum yield of photochemistry as well as maximal electron transport rate (ETR_{max}) in *C. crispus* were more strongly affected by UV-B radiation than in *M. stellatus*. In both species, no negative effects of the respective radiation conditions were found on total activity of RubisCO. Total MAA content in *M. stellatus* was up to 6-fold higher than in *C. crispus* and the composition of MAAs in the two species was different. The results indicate that, among others, UV-B sensitivity may be a factor restricting *C. crispus* to the lower intertidal and upper sublittoral zone, whereas *M. stellatus* is better adapted to UV radiation and is therefore more competitive in the upper intertidal zone.

Key words *Chondrus crispus* · Chlorophyll fluorescence · *Mastocarpus stellatus* · Mycosporine-like amino acids · UV radiation

Introduction

Both *Chondrus crispus* and *Mastocarpus stellatus* are abundant species of red algae along the coasts of the North Atlantic and inhabit the intertidal and upper sublit-

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toral zone of rocky shorelines (Lüning 1990). However, *M. stellatus* was not recorded from the island of Helgoland before 1983 when a few sporophytes were introduced during a scientific campaign (Kornmann and Sahling 1994). In the following years, *M. stellatus* established and dispersed all over the island on hard substrates in the lower intertidal zone. *C. crispus* has always been regarded as an abundant species on Helgoland within its main distribution in the upper sublittoral and below the understorey of the intertidal zone (Kornmann and Sahling 1977). After the introduction of *M. stellatus*, competition between the two species may have been initiated at locations where their habitats overlap, leading to the presently observed zonation pattern. Previous comparative studies on temperature, desiccation and freezing resistance revealed a generally higher stress tolerance of *M. stellatus* (Mathieson and Burns 1971; Dudgeon et al. 1989, 1995), which may favour growth in variable environments, such as the intertidal and the upper sublittoral zone.

Recent studies stress the potential role of solar and especially UV radiation in determining macroalgal zonation patterns from polar to temperate regions (Dring et al. 1996a; Hanelt et al. 1997; Bischof et al. 1998a). Moreover, Maegawa et al. (1993) regard solar UV radiation as one of the most important factors controlling the depth distribution of red algae on the shore. In the study presented here, it was tested whether UV radiation may be a factor contributing to the distribution of populations of *M. stellatus* and *C. crispus* on the shores of Helgoland. Therefore, different photosynthetic parameters, such as effective quantum yield, maximal quantum yield of dark acclimated samples, and maximal electron transport rate (ETR_{max}) as studied by pulse amplitude modulated fluorescence (PAM) measurements, as well as activity of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) were determined during exposure of experimental individuals to artificial UV radiation. Additionally, the content of UV screening mycosporine-like amino acids (MAAs) was measured to test whether the species have different capabilities for protection against UV radiation.

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Materials and methods

Young thalli of *Mastocarpus stellatus* (Stackh. in With.) Guiry and *Chondrus crispus* Stackh. were collected from the same shore level in the NE Harbour of Helgoland (German Bight, North Sea; 54° 11′N, 7° 53′E) on 2 June 1999 during low tide and transferred to the laboratory. There, specimens were cleaned of epiphytes, and thallus branches about 2 cm long were removed to be cultivated in nutrient-enriched seawater (Provasoli 1986) and dim light for 2 days.

Samples were then grown at 10°C in 0.5 l glass dishes 18 cm in diameter and exposed to a 16:8 h light:dark cycle, supplemented with 6 h of UV in the middle of the light phase, from 1000 to 1600 hours. Cultures, each containing up to 80 thallus branches per species and treatment, were stirred regularly to guarantee equal light exposure. Every alternate day, the medium in the dishes was replaced. The experimental material was exposed to artificial UV radiation generated by three UVA-340 (Q-Panel, Cleveland, USA) fluorescent tubes at irradiances of 10 W m^{-2} UV-A (320–400 nm) and 1 W m^{-2} UV-B (280–320 nm). Photosynthetically-active radiation (PAR) produced by daylight fluorescent tubes was 25 μmol m^{-2} s^{-1}. Experimental irradiance of PAR was measured with a Licor Li-189 radiometer (Quantum, USA), for the UV range, a RM-21 (Dr. Gröbel, Germany) bandpass radiometer equipped with broadband 2p UV-A (320–400 nm) and UV-B (280–320 nm) sensors was used. Glass dishes were covered with different filter foils to cut off different wavelength ranges selectively from the spectrum emitted by the fluorescent tubes: samples receiving PAR + UV-A + UV-B were covered with an Ultraphan URT 300 foil (Digefra, Germany). To cut off only the UV-B range, a Folex PR foil (Dr. Schleussner, Germany) was used; to exclude the whole UV range (UV-A + UV-B) from the treatment, samples were covered with an Ultraphan URUV foil (Digefra). A comparison of the spectral properties of the different filter foils used in the experiment is given by Pérez-Rodríguez et al. (1998). For all parameters tested, sampling or measuring was conducted on the 1st, 2nd and 5th day of the experiment, immediately before the start of UV exposure, after 2, 4, and 6 h of UV irradiation and after 2 and 4 h after UV exposure had ceased.

During exposure in the climate chamber, measurements of effective quantum yield ($\Delta F/Fm'$; cf. Schreiber et al. 1994) under the different radiation conditions were carried out with a Diving PAM (Walz, Germany). Saturating pulse length was adjusted to 0.6 s with an intensity of approx. 5000 μmol m^{-2} s^{-1}. Variation of effective quantum yield was high due to the differences among the light fields so that 20–40 yield determinations were carried out at each measuring point, and mean values and standard deviations were calculated. Maximal quantum yield of photochemistry was determined by the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) with a PAM 2000 chlorophyll fluorometer (Walz), following the protocol described in detail by Hanelt (1998). Subsequently, photosynthesis versus irradiance curves were recorded with the fluorometer as described by Bischof et al. (1999). From this, ETR$_{max}$ values were extracted by regression analysis. Measurements of Fv/Fm and ETR$_{max}$ were conducted in triplicate from randomly collected samples.

For enzyme analysis, at each measuring point, subsamples of approx. 0.8 g fresh weight were frozen in liquid nitrogen and stored until assay. Total activity of RubisCO in crude extracts was tested with a coupled photometric test generally following the method described by Gerard and Driscoll (1996). Crude extracts were prepared by grinding frozen algal material to a fine powder and transferring it into ice cold extraction buffer (0.1 M Tris-Cl, 2 mM EDTA, 10 mM MgCl$_2$, 20% glycerol, 1% Triton X-100, 50 mM DTT, 100 mM Na ascorbate, and 10 mM NaHCO$_3$, at pH 7.6). 0.5 g fresh weight of tissue was mixed with 1 ml of extraction buffer. The assay mixture contained 50 mM HEPES, 10 mM NaHCO$_3$, 20 mM MgCl$_2$, 0.2 mM NADH, 5 mM ATP, 5 mM phosphocreatine, 5 units of creatine phosphokinase and 5 units of glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase. Sample loads contained 25 μl of extract, the reaction was started by adding ribulose-1,5-bisphosphate with a final concen-

tration of 2 mM in the cuvette. The time course of NADH oxidation was recorded by the decrease in absorbance at 340 nm. Activity was expressed as declining absorbance per mg protein and second (mAbs mg protein^{-1} s^{-1}). Overall content of soluble proteins in crude extracts was determined using a commercial protein assay (Bio Rad, USA). Protein content was determined by measuring extinction at 595 nm and calculating the concentration of proteins according to a calibration curve prepared with bovine serum albumin.

For analysis of content and composition of MAAs, samples of about 10 mg dry weight were prepared for HPLC analysis generally following the protocol of Karsten et al. (1998a) modified as follows: the mobile phase within the HPLC column was 10% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water; the flow rate was adjusted to 0.7 ml min^{-1} at 20°C. The MAAs were detected at 330 nm and absorption spectra (290–400 nm) were recorded at 1 s intervals directly on HPLC-separated peaks. Substances were identified according to spectra and retention time.

Results

Both species exhibited a differential response to the exposure to artificial UV radiation. In *C. crispus*, initial values of effective quantum yield ($\Delta F/Fm'$) were lower (0.455) than in *M. stellatus* (0.543; Fig. 1). In both species, the changes in $\Delta F/Fm'$ due to exposure to PAR or
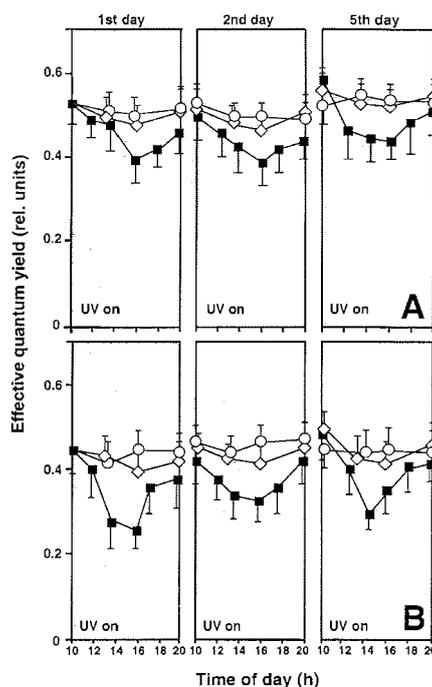

Fig. 1 Effective quantum yield of photosynthesis in *Mastocarpus stellatus* (**A**) and *Chondrus crispus* (**B**) during exposure (*marked grey*) to PAR (O), PAR + UV-A (◇), and PAR + UV-A + UV-B (■) and subsequent recovery in PAR only, for the 1st, 2nd and 5th day of treatment; n=20–40, mean values±SD

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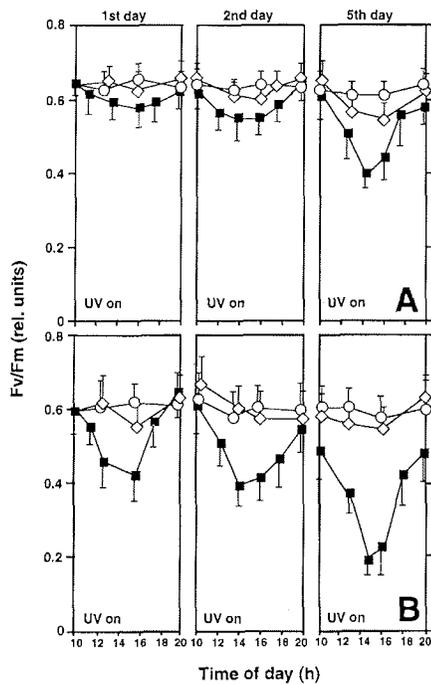
Fig. 2 Maximal quantum yield of photosynthesis (Fv/Fm) in *Mastocarpus stellatus* (**A**) and *Chondrus crispus* (**B**) during exposure (*marked grey*) to PAR (O), PAR + UV-A (◊), and PAR + UV-A + UV-B (■) and subsequent recovery in PAR only, for the 1st, 2nd and 5th day of treatment; $n=3$, mean values±SD

Fig. 3 Maximal electron transport rate (ETR_{max}) of photosynthesis in *Mastocarpus stellatus* (**A**) and *Chondrus crispus* (**B**) during exposure (*marked grey*) to PAR (O), PAR + UV-A (◊), and PAR + UV-A + UV-B (■) and subsequent recovery in PAR only, for the 1st, 2nd and 5th day of treatment; $n=3$, mean values±SD
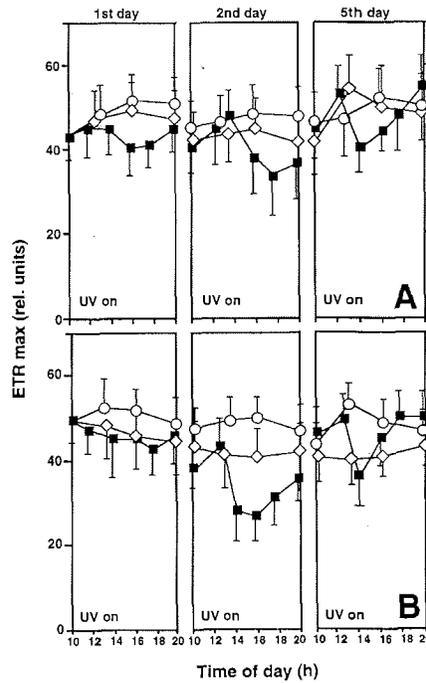
PAR + UV-A were very small in the course of the experiment. In contrast, additional UV-B radiation resulted in a marked reduction of effective quantum yield (Fig. 1A, B): throughout the experiment, UV-B irradiated samples of *M. stellatus* exhibited a reduction of approx. 20% in $\Delta F/Fm'$ after 6 h of exposure, while *C. crispus* showed a reduction to 60% of initial values on the first day. However, during the following days, there was a trend to a lesser degree of inhibition of $\Delta F/Fm'$ in this species.

Exposure to UV-B radiation resulted in a strong reduction of maximal quantum yield (Fv/Fm) in *C. crispus* (Fig. 2B), while samples under PAR and PAR + UV-A radiation remained almost unaffected. The UV-B induced reduction in Fv/Fm became more prominent in the course of the experiment: while during 6 h of exposure on the first day Fv/Fm dropped by 30% of initial values; a 70% decline was observed during exposure on the 5th day. Moreover, before the beginning of the fifth exposure, samples had not completely recovered overnight from the previous exposure. In contrast, in *M. stellatus*, maximal quantum yield was generally less affected by UV-B, with only a 15% reduction during exposure on the first day. However, also in this species, Fv/Fm values further declined in the course of the experiment, and were finally

reduced to 60% of the initial values (Fig. 2A). Also in *M. stellatus*, no adverse effects on maximal quantum yield were observed in samples shielded from UV-B.

ETR_{max} values in samples of *M. stellatus* were moderately promoted throughout the experiment under the PAR and PAR + UV-A treatment, as compared to the initial values (Fig. 3A), while no significant changes were observed due to additional UV-B irradiation during the first day. In contrast, during the 2nd day, a reduction in ETR_{max} was observed in UV-B exposed samples; however, this was only obvious after 6 h of exposure and during the recovery phase after UV irradiation had already ceased. Samples of *C. crispus* exposed to PAR + UV-A showed a trend to slightly lower ETR_{max} values in the course of the experiment, while values in PAR-irradiated samples remained unaffected (Fig. 3B). In contrast to the reactions of UV-B exposed samples on the first and 5th day of the experiment, ETR_{max} dropped to 50% of initial values after 6 h of UV-B exposure on the 2nd day.

Generally, under all experimental radiation conditions, RubisCO activities in samples of *M. stellatus* were higher than the initial values. Results obtained from UV-B irradiated samples were inconsistent and erratic but, especially in these samples, enzyme activity seemed to
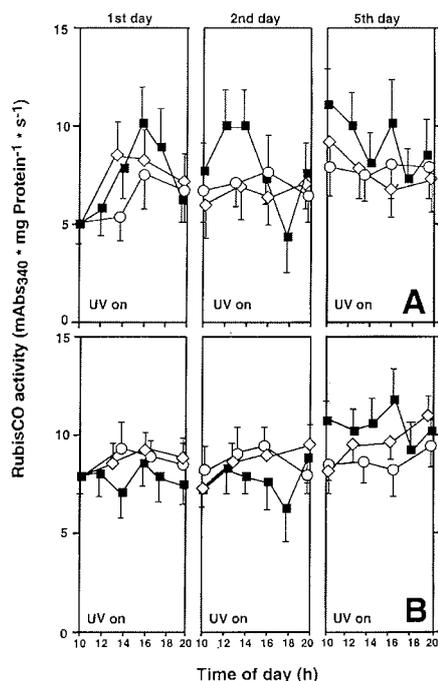
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Fig. 4 Total activity of RubisCO in *Mastocarpus stellatus* (A) and *Chondrus crispus* (B) during exposure (*marked grey*) to PAR (O), PAR + UV-A (◊), and PAR + UV-A + UV-B (■) and subsequent recovery in PAR only, for the 1st, 2nd and 5th day of treatment; *n*=3, mean values±SD

be promoted during exposure (Fig. 4A). In *C. crispus*, overall RubisCO activity was not significantly altered during the first 2 days of the experiment (Fig. 4B). However, throughout the fifth exposure, samples exposed to UV-B generally exhibited a 50% increase in activity compared with the initial values.

There were major differences in the content and composition of MAAs between the two species (Table 1).

Throughout the treatment, overall content in *M. stellatus* was up to nearly 6-fold higher than in *C. crispus*. Shinorine was the only MAA detected in *M. stellatus*. During the experiment, its content increased strongly to almost the double its initial value. In contrast, there was no trace of this compound in initial samples of *C. crispus*. However, in the course of the experiment, the *de novo* synthesis of shinorine contributed strongly to the almost 50% increase in total MAA content. The contents of asterina and palythene doubled, while the concentration of palythine did not change significantly during the treatment.

Discussion

Our results show that photosynthesis in *M. stellatus* was less sensitive to the experimental UV-B irradiances than *C. crispus*. Previous comparisons of the physiological responses of these two species revealed a generally higher tolerance of *M. stellatus* to different kinds of abiotic stress (Dudgeon et al. 1989, 1995). The authors showed that the fronds of both species are very similar in terms of ecophysiological characteristics (e.g. high photosynthetic capacity), but differences in stress tolerance characteristics, such as lower net photosynthetic rates during desiccation and freezing and slower recovery in *C. crispus*, may be important factors in terms of competition and occupied niches. Freezing is suggested to be especially important in controlling the distribution of these species on the shore (Dudgeon et al. 1989). For Helgoland, this may be an important factor as freezing periods over several weeks are frequently observed during winter (A. Wagner, personal communication). Generally, the higher stress tolerance of *M. stellatus* results in this species prevailing on exposed locations in the lower intertidal zone. In contrast, lower light compensation points of *C. crispus* permit optimal light utilisation and high growth rates at greater water depth or below canopies (Dudgeon et al. 1995).

Differences between the species in effective quantum yields under UV-B exposure were small (Fig. 1), but maximal quantum yields exhibited a much higher UV-B tolerance in *M. stellatus* (Fig. 2) indicating that energy

Table 1 Composition and content of mycosporine-like amino acids (*MAAs*) in *Chondrus crispus* and *Mastocarpus stellatus*, in samples taken before the start of the experiment and after the end of the fifth exposure to UV-B, mean values (*n*=3)±SD

Compound	Absorption maximum (nm)	*C. crispus* Initial content	(mg*g dry weight[-1]) Final content	*M. stellatus* Initial content	(mg*g dry weight[-1]) Final content
Shinorine	334	no trace	0.118 (±0.025)	2.650 (±0.500)	4.440 (±1.090)
Palythine	320	0.294 (±0.087)	0.285 (±0.107)	No trace	No trace
Asterina	330	0.089 (±0.023)	0.167 (±0.079)	No trace	No trace
Palythene	360	0.042 (±0.003)	0.092 (±0.019)	No trace	No trace
Σ MAAs		0.430 (±0.113)	0.670 (±0.221)	2.650 (±0.500)	4.440 (±1.090)

transfer between light harvesting complex and reaction centre should be more efficient (Krause and Weis 1991). In both species, UV-B induced impairment of maximal quantum yield increased during the treatment. These findings suggest that adverse effects on photosynthetic antenna systems may become more and more prominent in the course of repeated UV exposures and result in chronic damage to the antennae (Demmig-Adams and Adams 1992; Hanelt 1996). Different findings were reported in a study on the brown alga *Alaria esculenta* (Bischof et al. 1999). In this species, inhibition of maximal quantum yield becomes smaller and recovery faster after a few repeated exposures to UV. However, the *A. esculenta* samples were kept in dim light for 2 days between each repeated exposure, allowing complete recovery and acclimation to UV radiation.

It is important to note that in both species studied, the impairment of maximal as well as effective quantum yield was exclusively due to UV-B radiation; this is in contrast to a similar study on UV effects on several other red algal species from Helgoland (Dring et al. 1996a), where the UV-A range also strongly contributed to the reduction of Fv/Fm values. However, as experimental conditions were different between the studies, a comparison might be difficult. In the study cited, algae were exposed to UV-A and UV-B irradiances which were up to 50% higher than those in our study, but with the same low background of PAR. The strong effect of UV-A was predominantly found in red algae from the deep sublittoral zone, which are probably adapted to low irradiances. The samples used in our study were collected at the beginning of June and therefore might be acclimated to high irradiances which, in turn, might also reduce their sensitivity towards UV (Cen and Bornman 1990; Bischof et al. 1999). It was also shown by Dring et al. (1996a) that sensitivity towards UV exposure changes with the season. Due to the unnaturally enhanced UV-B:PAR ratio in laboratory experiments the detrimental effects of UV-B exposure might be overestimated compared to field conditions (Teramura 1986). Nevertheless, the higher sensitivity to UV-B radiation in *C. crispus*, as shown in our experiments, is likely to reflect higher susceptibility under natural radiation conditions, compared to *M. stellatus*; however, this remains to be confirmed in further studies.

The different composition and higher concentration of MAAs in *M. stellatus* might be one reason for its generally lower sensitivity of photosynthesis towards UV radiation compared to *C. crispus* [see also Karsten et al. (1998b), Dunlap and Shick (1998) and Sinha et al. (1998) for reviews on MAAs]. The potential of MAAs to protect the photosynthetic apparatus against UV radiation was shown by Karsten et al. (1999) in the Arctic endemic red alga *Devaleraea ramentacea*. A detailed study of the formation of MAAs in *C. crispus* was performed by Karsten et al. (1998a) who showed that shinorine is synthesised under UV radiation, while synthesis of palythine is mainly induced by PAR. This is in line with our results as PAR was low and concentration of palythine remained unchanged during the treatment. A high initial

content of shinorine in *M. stellatus* may favour its occurrence in sun-exposed locations, while the capability of *C. crispus* to synthesise shinorine certainly shows the potential to acclimate to changes in light climate.

After the release of *M. stellatus* in 1983, it first established in the intertidal zone at locations with a hard substrate, a position which is not inhabited by *C. crispus*. Generally, the type of substrate plays a major role in the settlement of *M. stellatus* (I. Bartsch, personal communication). At our study site in the NE harbour, the typical habitats of *C. crispus* and *M. stellatus* apparently overlap, as both species seem to find favourable conditions for establishment and growth.

For a physiological comparison it was important to collect samples at the same time from the same shore level, as these factors may strongly influence the UV sensitivity of macroalgae (Dring et al. 1996a; Bischof et al. 1998b), and acclimation to changing radiation conditions can proceed rapidly (Bischof et al. 1999). For *C. crispus*, increasing UV sensitivity with increasing growth depth and its acclimation to the respective *in situ* light climate was described in detail by Sagert et al. (1997). In a study of MAA formation in the red alga, *Devaleraea ramentacea*, the strong correlation between MAA content and sampling depth was also shown (Karsten et al. 1999). Additionally, all plants used in the experiments were of similar size and morphology, which are also important factors in UV tolerance (Dring et al. 1996b). As all environmental conditions were identical until harvest, acclimation to different conditions can be excluded as a reason for the differential response towards experimental UV exposure. Thus, genetic adaptation in the investigated species might be responsible for the different UV sensitivity in our experiments. The lower sensitivity of *M. stellatus* to UV-B radiation, which was shown in our study, might, in turn, be one aspect enabling this species to occur in more sun-exposed areas in the field. In summary, at sun-exposed locations, different composition of MAAs and a higher resistance of photosynthetic reactions may represent a competitive advantage of *M. stellatus* over *C. crispus*, which is restricted to more shaded habitats.

References

Bischof K, Hanelt D, Wiencke C (1998a) UV-radiation can affect depth-zonation of Antarctic macroalgae. Mar Biol 131: 597–605
Bischof K, Hanelt D, Tüg H, Karsten U, Brouwer P, Wiencke C (1998b) Acclimation of brown algal photosynthesis to UV-radiation in Arctic coastal waters. Polar Biol 20:388–395
Bischof K, Hanelt D, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. Plant Biol 1: 435–444

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Cen YP, Bornman JF (1990) The response of bean plants to UV-B radiation under different irradiances of background visible light. J Exp Bot 41:1489–1495

Demmig-Adams B, Adams WW III (1992) Photoprotection and other responses of plants to high light stress. Annu Rev Plant Physiol Plant Mol Biol 43:599–626

Dring MJ, Wagner A, Boeskov J, Lüning K (1996a) Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements: influence of collection depth and season, and length of irradiation. Eur J Phycol 31:293–302

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K (1996b) Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life history stages of three species of Laminaria (Phaeophyta). Mar Biol 126:183–191

Dudgeon SR, Davison IR, Vadas RL (1989) Effect of freezing on photosynthesis of intertidal macroalgae: relative tolerance of Chondrus crispus and Mastocarpus stellatus (Rhodophyta). Mar Biol 101:107–114

Dudgeon SR, Kübler JE, Vadas RL, Davison IR (1995) Physiological responses to environmental variation in intertidal red algae: does thallus morphology matter? Mar Ecol Prog Ser 117:193–206

Dunlap WC, Shick JM (1998) Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. J Phycol 34:418–430

Gerard VA, Driscoll T (1996) A spectrophotometric assay for RubisCO activity: application to the kelp Laminaria saccharina and implications for radiometric assays. J Phycol 32:880–884

Hanelt D (1996) Photoinhibition of photosynthesis in marine macroalgae. Sci Mar 60:243–248

Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar Biol 131:361–369

Hanelt D, Melchersmann B, Wiencke C, Nultsch W (1997) Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255–266

Karsten U, Franklin LA, Lüning K, Wiencke C (1998a) Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga Chondrus crispus (Rhodophyta). Planta 205:257–262

Karsten U, Sawall T, Hanelt D, Bischof K, Figueroa FL, Flores-Moya A, Wiencke C (1998b) An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. Bot Mar 41:443–453

Karsten U, Bischof K, Hanelt D, Tüg H, Wiencke C (1999) The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga Devaleraea ramentacea (Rhodophyta). Physiol Plant 105:58–66

Kornmann P, Sahling PH (1977) Meeresalgen von Helgoland. Helgol Wiss Meeresunters 29:1–289

Kornmann P, Sahling PH (1994) Meeresalgen von Helgoland. Zweite Ergänzung. Helgol Meeresunters 48:365–406

Krause GH, Weis E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42:313–349

Lüning K (1990) Seaweeds. Wiley, New York

Maegawa M, Kunieda M, Kida W (1993) The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depths. Jpn J Phycol 41:207–214

Mathieson AC, Burns RL (1971) Ecological studies of economic red algae. I. Photosynthesis and respiration of Chondrus crispus Stackhouse and Gigartina stellata (Stackhouse) Batters. J Exp Mar Biol Ecol 7:197–206

Pérez-Rodríguez E, Gómez I, Karsten U, Figueroa FL (1998) Effects of UV radiation on photosynthesis and excretion of UV-absorbing compounds of Dasycladus vermicularis (Dasycladales, Chlorophyta) from southern Spain. Phycologia 37:379–387

Provasoli L (1986) Media and prospects for the cultivation of marine algae. In: Watanabe A, Hattori A (eds) Cultures and collections of algae. In: Proceedings of the Japanese Conference Hakone. Japanese Society of Plant Physiology, Tokyo, pp 63–75

Sagert S, Forster RM, Feuerpfeil P, Schubert H (1997) Daily course of photosynthesis and photoinhibition in Chondrus crispus (Rhodophyta) from different shore levels. Eur J Phycol 32:363–371

Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. Ecol Stud 100:49–70

Sinha RP, Klisch M, Gröninger A, Häder DP (1998) Ultraviolet-absorbing/screening substances in cyanobacteria, phytoplankton and macroalgae. J Photochem Photobiol B: Biol 47:83–94

Teramura AH (1986) Interaction between UV-B radiation and other stresses in plants. In: Worrest RC, Caldwell MM (eds) Stratospheric ozone reduction, solar ultraviolet radiation and plant life. NATO ASI series G8, Springer, Berlin Heidelberg New York, pp 327–343

BLUE LIGHT AND UV-A RADIATION CONTROL THE SYNTHESIS OF MYCOSPORINE-LIKE AMINO ACIDS IN *CHONDRUS CRISPUS* (FLORIDEOPHYCEAE)[1]

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The induction of UV-absorbing compounds known as mycosporine-like amino acids (MAAs) by red, green, blue, and white light (43% ambient radiation greater than 390 nm) was examined in sublittoral *Chondrus crispus* Stackh. Fresh collections or long-term cultures of sublittoral thalli, collected from Helgoland, North Sea, Germany, and containing no measurable amounts of MAAs, were exposed to filtered natural radiation for up to 40 days. The MAA palythine (λ_{max} 320 nm) was synthesized in thalli in blue light to the same extent observed in control samples in white light. In contrast, thalli in green or red light contained only trace amounts of MAAs. After the growth and synthesis period, the photosynthetic performance of thalli in each treatment, measured as pulse amplitude modulated chlorophyll fluorescence, was assessed after a defined UV dose in the laboratory. Thalli with MAAs were more resistant to UV than those without, and exposure to UV-A+B was more damaging than UV-A in that optimal (F_v/F_m) and effective (ϕ_{II}) quantum yields were lower and a greater proportion of the primary electron acceptor of PSII, Q, became reduced at saturating irradiance. However, blue light-grown thalli were generally more sensitive than white light control samples to UV-A despite having similar amounts of MAAs. The most sensitive thalli were those grown in red light, which had significantly greater reductions in F_v/F_m and ϕ_{II} and greater Q reduction under UV radiation. Growth under UV radiation alone had been shown previously to lead to the synthesis of the MAA shinorine (λ_{max} 334 nm) rather than palythine. In further experiments, we found that preexposure to blue light followed by growth in natural UV-A led to a 7-fold increase in the synthesis of shinorine, compared with growth in UV-A or UV-A+B without blue light pretreatment. We hypothesize that there are two photoreceptors for MAA synthesis in *C. crispus*, one for blue light and one for UV-A, which can act synergistically. This system would predispose *C. crispus* to efficiently synthesize UV protective compounds when radiation levels are rising, for example, on a seasonal basis. However, because the UV-B increase associated with artificial ozone reduction will not be accompanied by an increase in blue light, this triggering mechanism will have little additional adaptive value in the face of global change unless a global UV-B increase positively affects water column clarity.

Key index words: blue light; chlorophyll fluorescence; *Chondrus crispus*; MAA; macroalgae; mycosporine-like amino acids; photosynthesis; UV-B

The mycosporine-like amino acids (MAAs), a class of at least 19 compounds with absorbance between 310 nm and 360 nm (Shibata 1969, Tsujino et al. 1978, 1980, Nakamura et al. 1982, Karentz et al. 1991), have long been proposed to protect organisms from damage by UV radiation, particularly UV-B. Occurring in a number of taxonomically diverse organisms, particularly corals (Dunlap et al. 1986, Dunlap and Shick 1998), microalgae (Jeffrey et al. 1999), and rhodophyte algae (Sivalingam et al. 1974, Karentz et al. 1991, Karsten et al. 1998b, Hoyer et al. 2001), the concentration of MAAs in tissues is often correlated with irradiance, with variation observed at scales from the level of irradiance microclimate in algal turfs to the

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water column depth (e.g. Sivalingam et al. 1974, Beach and Smith 1996, Karsten et al. 1999, Karsten and Wiencke 1999, Hoyer et al. 2001). Interest in the biochemistry and physiology of MAAs and MAA-containing organisms has grown recently as reductions in the stratospheric ozone concentration and a correlated increase in the amount of UV-B at the Earth's surface have been reported.

MAAs are chemical derivatives of a mycosporine cyclohexenone or cyclohexenimine chromophore conjugated with one or two of several different amino acids. The MAA biosynthetic pathway is not conclusively known, but a recent study of the synthesis of MAAs in coral indicates that synthesis proceeds via early steps in the shikimic acid pathway (Shick et al. 1999), the process by which precursors of mycosporine are synthesized in fungi (Favre-Bonvin et al. 1987), and the general pathway for synthesis of aromatic amino acids. The basis of the diversity of amino acids substitutions is not clear, but selective hydrolysis of two MAAs, shinorine and phorphyra-334, to yield a third, mycosporine-glycine, by the marine bacterium *Vibrio harveyi* (Johnson and Shunk) Baumann et al. has been demonstrated (Dunlap and Shick 1998).

Macroalgae containing MAAs normally grow in shallow or eulittoral environments where changes in water clarity are stochastic. Additionally, these environments can impose multiple stresses on organisms in addition to UV radiation (UVR), for example high PAR, desiccation, and nutrient limitation. Comparison of the integrity of cellular components and physiological processes in algae that contain MAAs with those without shows that the former are generally more resistant to UV-B–induced damage, though the degree of protection varies (Garcia-Pichel et al. 1993, Lesser 1996, Neale et al. 1998, Franklin et al. 1999). Besides having a role in UV screening, several MAAs also demonstrate antioxidant properties (Dunlap and Shick 1998), and MAAs in blue green algae may act as compatible solutes (Oren 1997, but see Portwich and Garcia-Pichel 1999), suggesting that MAAs may be important compounds for resistance to more than just UV stress.

Perhaps it is not surprising that MAA absorption in the UV range does not necessarily mean that the presence of UV-B in the environment is a prerequisite for their synthesis. Although UV-A and particularly UV-B are required for MAA synthesis in some organisms, other species contain high levels of MAAs without ever having been exposed to UVR (c.f. Carreto et al. 1990, Hannach and Sigleo 1998, Karsten et al. 1998a, Franklin et al. 1999, Jeffrey et al. 1999, Karsten et al. 1999, Karsten and Wiencke 1999, Portwich and Garcia-Pichel 1999, Shick et al. 1999). In other cases, MAAs are not synthesized when the alga is exposed to either higher PAR or UV (Hoyer et al. 2001). These diverse responses raise questions about the underlying biochemical trigger for induction, the nature of the receptor, and the taxonomic commonality of the induction process.

Using natural radiation and filters differentially transparent to UV and PAR, we recently showed that identical patterns of MAA synthesis in the rhodophyte *C. crispus* can be induced by PAR alone or PAR containing either UV-A or UV-A plus UV-B (Franklin et al. 1999). Exposure to surface levels of UVR without PAR can also induce MAA synthesis in *C. crispus* (Karsten et al. 1998a), but these two conditions differ markedly in the relative proportion of the various MAAs produced. Only when PAR is present are MAAs produced that are quantitatively and qualitatively similar to those normally seen in eulittoral *C. crispus* populations. Induction by PAR follows a distinct pattern, with initial synthesis of the MAA shinorine (λ_{max} 334 nm), followed by synthesis of predominantly palythine (λ_{max} 320 nm) with a concomitant decline in shinorine content, leading to the proposal that interconversion is a key step in the synthesis of the main *C. crispus* MAA, palythine. In contrast, exposure to UVR alone led primarily to the synthesis of shinorine and lower amounts of palythine, perhaps representing the presence of two distinct photoreceptors. Results were more difficult to interpret because the PAR cut-off filter used in both experiments transmitted significant long-wave UV-A (50% transmittance at 380 nm).

We continued to investigate induction of MAA synthesis in *C. crispus* by PAR, by exposing sublittoral and laboratory-cultured thalli to white, red, green, and blue light filtered from natural irradiance, using filters with less than 5% transmittance at 380 nm. We also investigated the possible relationship between induction by PAR and UV, using sequential treatments. We show that synthesis of MAAs, particularly palythine, is preferentially induced by blue light, and blue light and UV-A interact to boost synthesis of shinorine. In a controlled laboratory test, photosynthesis in thalli with the highest levels of MAAs was more protected from UV-A than in thalli without MAAs, but there were unexpected differences in the photosynthetic response of MAA-containing samples to UV-B. This difference depended on the wavelength of light used to induce MAA synthesis. Of all conditions tested, prior exposure to white light led to the most effective protection and recovery from UV stress.

MATERIALS AND METHODS

Plant material and treatment conditions. Chondrus crispus was collected from the sublittoral zone (6 m below mean low water of spring tides) at Helgoland, Germany, a rocky island in the southeastern North Sea. Unialgal cultures were established from fertile gametophytes and grown in Provosoli's Enriched Seawater medium (Starr and Zeikus 1993) for four generations at 50 µmol photons·m⁻²·s⁻¹ (Daylight Delux, Osram, Germany), 16:8 day:night cycle, 18° C. One experiment was performed with 2–3 cm tetrasporophytes from these cultures. Other experiments were performed with fresh sublittoral (6 m) field material collected from the same location. Fresh material was cleaned of epiphytes and held overnight indoors in running seawater, before exposure to the treatment conditions.

The spectral response of MAA induction was tested using natural irradiance filtered to obtain four light conditions (Fig. 1): red (Lee, Andover, England), green (Kodak, Rochester, NY), blue (Lee), and white light (PAR reduced to 43% of surface ambient by neutral density screening, UV removed by Ultraphan 395 foil, Digefra GmbH, Munich, Germany). The fil-
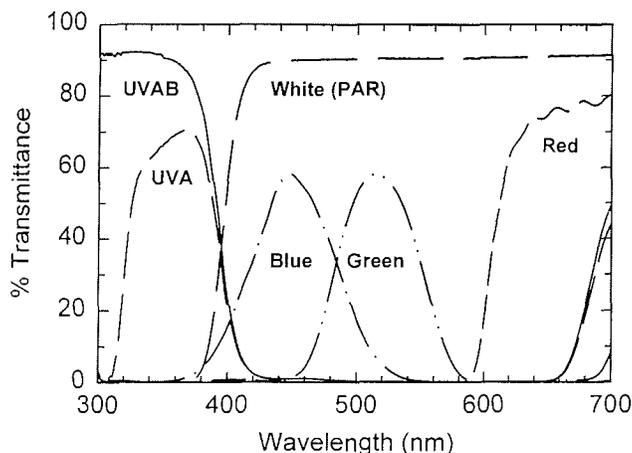
FIG. 1. Transmittance spectra of the colored foils and total UVR-blocking (white), PAR-blocking (UVAB), and UV-B-blocking (UVA) filters used during induction of MAAs.

ters and screens were selected to give as equal an energy distribution over the specific part of the spectrum as possible (Table 1). At midday on a clear day in June, the total natural energy transmitted in the 350- to 700-nm range was nearly equal among the blue, green, and red treatments. Both blue and white filters had small tails in the long UV-A range, 4% and 3% transmittance at 380 nm, respectively. The red and blue filters also transmitted equal amounts of far red (700–800 nm). Although the white and red filters transmitted equal amounts of red light (590–700 nm), the white treatment had 50% more blue light (350–500 nm) than the blue treatment, and the total energy transmitted in the white treatment (350–700 nm) was three times greater than any of the other filters. Natural light spectra were measured with an LI-1800UW spectroradiometer (Li-Cor, Lincoln, NE).

The filters and screens were wrapped around 65 × 20 cm diameter acrylic tubes (Plexiglas XT, Röhm GmbH, Darmstadt, Germany), open at each end for water flow. Thalli were attached in rows by fine silicone tubing to clear acrylic plates suspended lengthwise in the Plexiglas tubes. The tubes were suspended horizontally 2 cm below the water surface in a tank 1 × 1.5 × 0.5 m deep, containing 750 L seawater at 17.5° C (± 1.5° C). Fresh seawater was directed into the end of each tube at approximately 5 L·min⁻¹. Plants were put into and collected from the treatments in the evening. Results of the experiment with cultured thalli were confirmed by a replicate experiment using

TABLE 1. Comparison of the energy transmittance of the colored filters and the white light (PAR reduced with two layers of neutral density screening) treatments to the incident surface irradiance.

Filter color	Irradiance, 12⁰⁰ clear day, W·m⁻²			
	350–500 nm	425–600 nm	590–700 nm	350–700 nm
Blue	52.7			52.7
Green		55.1		55.1
Red			50.1	50.1
White	74	90	51	145
Incident surface	172	208	118	337

UV radiation was removed from the white light treatment with a UV-blocking filter. Measurements were made on a clear day at midday, the day before the start of the first experiment, using a LI-1800UW spectroradiometer.

fresh sublittoral field material. Results from the cultured material are presented.

In early autumn, the possible interaction of blue light and UV was tested by applying sequential light treatments. Sublittoral thalli were treated for 2 weeks in the blue tubes and then moved to small polyvinyl chloride holders covered with a UG5 glass filter (Schott Glaswerke, Mainz, Germany) that blocked all wavelengths greater than 400 nm (Fig. 1). In addition, a piece of 320-nm-long pass foil (Folex, Dr. Schleusner GmbH, Dreireich, Germany) was added to remove UV-B from the UVR spectrum (UV-A–treated samples), or a piece of 295-nm-long pass foil (Ultraphan 295, Lonza-Folien, Weil am Rhein, Germany) was added for a full UV-A+B treatment. Further details on these filter and polyvinyl chloride holder combinations are given in Karsten et al. (1998a). The change in experimental setup was necessary because no flexible PAR-blocking filters were available to wrap around the tubes. Therefore, comparisons were made to three sets of control thalli: 1) those held at the same period of time under a blue filter in these holders (no blue pretreatment), or 2) those held under a UG5 filter (no blue pretreatment), or 3) blue pretreated thalli that then remained in blue light but in the new holders.

Light conditions at Helgoland during these periods were monitored continuously by a PUV-500 radiometer (Biospherical Instruments, Inc., San Diego, CA) mounted permanently on the roof of the research laboratory.

Analysis of MAAs and photosynthetic pigments. Several times during the treatment period, samples were collected for HPLC and spectral analysis of MAAs. Samples were divided in half longitudinally, weighed, and then dried in silica gel. One half of each sample was extracted for 2 h in 25% aqueous methanol (vol/vol) at 45° C, and the extracts were scanned spectrophotometrically (Schimadzu UV-2101PC, Kyoto, Japan). The other half was extracted in an identical manner, and the MAAs were quantified by HPLC (Karsten and Garcia-Pichel 1996, Karsten et al. 1998a). Briefly, MAAs were separated on a Waters HPLC system (Waters, Milford, MA) fitted with a Knauer Spherisorb RP-8 column (Knauer, Berlin, Germany). The mobile phase was 25% aqueous methanol (vol/vol) plus 0.1% acetic acid (vol/vol), run isocratically. Peaks were detected at 335 nm, and absorption spectra from 290 nm to 400 nm were recorded for each peak detected. MAAs were identified by spectra, retention time, and, in some cases, by cochromatography with standards kindly provided by D. Karentz (University of California, San Francisco, CA). Quantification was made using published extinction coefficients (Tsujino et al. 1980, Dunlap et al. 1986, Karsten et al.

1998a). Results of the HPLC analysis are expressed as mg·g⁻¹ dry weight (DW). Additional samples were collected for extraction of chl *a* (Porra et al. 1989) and phycobiliproteins (Beer and Eshel 1985).

UV stress test. Before and at the end of the month-long treatment, samples were tested for UVR sensitivity by means of a standard 3-h UV "stress test" applied in the laboratory. Although the quantity of UV-B in the test was set to an ecologically relevant level, the test was not designed to mimic the solar spectrum in the field. Rather, this method has been previously shown to be effective in assessing the relative degree of acclimation to UVR (Franklin et al. 1999). In this test, we used 320 nm as the division between UV-B and UV-A because of the spectral characteristics of our filters. The test was applied in a temperature-controlled water bath (18° C) to 1.5-cm-long pieces of similar shape cut from the thallus tips. The UV stress treatments were made using UVA 340 fluorescent lamps (Q-Panel Co., Cleveland, OH), in combination with 20 μmol photons·m⁻²·s⁻¹ PAR from daylight fluorescent lamps (Daylight Delux). The emission spectrum of the UVA 340 lamps was similar to the solar spectrum at wavelengths less than 345 nm and contained no radiation below 295 nm. Lamp spectra were measured using the LI-1800UW spectroradiometer, and the distance between the lamps and the samples was adjusted to approximate the unweighted UV-B irradiance at midday on a typical summer day on Helgoland. Irradiances were as follows: UV-B (300–320 nm) 1.4 W·m⁻², UV-A (320–400 nm) 20 W·m⁻², and PAR (400–700 nm) 20 μmol photons·m⁻²·s⁻¹. The effective (weighted) UV-B irradiance was 0.139 W·m⁻², calculated using Caldwell's generalized plant action spectrum (PAS₃₀₀) (Björn and Murphy 1985). Because MAAs have substantial absorption in the UV-A spectrum, the effects of UV-A and UV-B were tested separately. One tip of each thallus was exposed to both UV-A+B in the stress test, whereas another tip was exposed only to UV-A, the UV-B having been removed by a sheet of the 320-nm-long pass foil.

Sensitivity of the algae to the test was indicated by differences in chlorophyll fluorescence characteristics measured three times: before and after the test and after 3 h recovery at 20 μmol photons·m⁻²·s⁻¹ PAR. Fluorescence was measured with a portable Pulse Amplitude Modulation (PAM-2000) fluorometer (H. Walz, Effeltrich, Germany). The optimal quantum yield, F_v/F_m ($F_v = F_m - F_o$), was determined by the following procedure: After 10 min of darkness, a 10-s pulse of dim far red light was applied, the F_o value was recorded, and F_m was determined by a saturating flash (Schreiber et al. 1986, 1995). Proceeding immediately, 3 min of 35 μmol photons·m⁻²·s⁻¹ from the PAM 2000 internal light-emitting diode array (emission maximum 655 nm) was applied to activate photosynthetic dark reactions, and then a light response curve of fluorescence was

recorded from 7 to 350 μmol photons·m⁻²·s⁻¹. At each irradiance, the steady state fluorescence level (F_s) was allowed to stabilize and then a saturating flash was applied (F_m'), followed by 5 s of darkness (F_o'). The effective quantum yield, $\phi_{II} = (F_m' - F_s) / F_m'$ (Genty et al. 1989), and reduction status of the primary PSII acceptor, Q, 1 - qP = 1 - ($F_m' - F_s$) / ($F_m' - F_o'$) (Bilger and Björkman 1990), were calculated. Samples were held in a stirred temperature-controlled (18° C) chamber during the measurements. Irradiance was measured using a cosine-corrected quantum sensor (Li-Cor).

Statistical analyses. Under given conditions, differences in photosynthetic pigments and fluorescence characteristics among the red, green, blue, and white samples were assessed by analysis of variance (Genstat 5 Release 3.1, Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden, Hertfordshire, UK).

RESULTS

Irradiance and general appearance of thalli. The cumulative irradiance for the treatment periods can be found in Table 2. Only those wavelengths relevant to the treatments are presented. Many days in these periods were cloudy or completely overcast. By late September, global irradiance had declined substantially from the summer values, and approximately half the amount of irradiance was received in a given period as during the summer.

The major photosynthetic light-harvesting pigments in *C. crispus* are the phycobiliproteins, which absorb light between 460 nm and 570 nm. Though not measured directly, thalli under the blue filter treatment would have initially received the least amount of photosynthetically usable radiation (PUR) (Morel 1978) on a quantum basis and those in the white light the most. After 40 days, the absolute concentrations of photosynthetic pigment in all treatments changed significantly relative to the initial condition, but these changes affected the ratio of phycobiliproteins to chl *a* only in samples under blue light (Table 3), apparently in response to low PUR. In these samples, the ratio doubled due to increases in both phycoerythrin and phycocyanin, and samples appeared almost purple. The phycoerythrin content of green and red light acclimated samples was similar to white light controls

TABLE 2. Cumulative irradiance values summed over the period when the spectral response of MAA induction was tested (19 July to 27 June 1998) and when the effect of a prior exposure to blue light was tested (3 September to 5 October 1998).

Experiment, dates and days of treatment	305 nm	320 nm	340 nm	380 nm	400–700 nm (mol·m⁻²)
	(kJ·m⁻²·nm⁻¹)				
Spectral response of MAA induction					
19 June to 27 July 1998					
7 d W, B, G, R				99.3	247
14 d W, B, G, R				214.3	531
29 d W, B, G, R				403.5	980
40 d W, B, G, R				568.9	1404
Sequential blue and UV treatment					
3 September to 5 October 1998					
18 d B pretreatment				111.6	263
2 d UV or B	0.18	2.6	5.2	8.0	16
3 d UV or B	0.47	7.1	14.3	22.11	55
7 d UV or B	0.76	14.0	29.1	44.22	106
14 d UV or B	1.20	25.3	53.7	82.28	195

The number of days refers to the total length of time a sample set was under the treatment conditions. Measurements were made with a Biospherical PUV-500 radiometer mounted on the roof of the research building on Helgoland. Actual amounts of radiation received were reduced as indicated by the filter spectra in Figure 1. W, white; B, blue; G, green; R, red.

TABLE 3. Photosynthetic pigments in *Chondrus crispus* before and after 40 days of acclimation to white, blue, green, or red light.

Treatment	Phycoerythrin	Phycocyanin	Chl a	Phycobiliproteins/chl a
	$\mu g \cdot mg^{-1}$ fresh weight			
Initial	1.51 (0.20)[a,b]	0.21 (0.02)[a]	0.41 (0.06)[a,b]	4.26 (0.87)[a]
White	1.30 (0.23)[a,b]	0.19 (0.02)[a]	0.31 (0.04)[c,d]	4.86 (0.73)[a]
Blue	2.81 (0.36)[c]	0.33 (0.04)[c]	0.37 (0.06)[b,c]	8.75 (1.94)[b]
Green	1.68 (0.40)[b]	0.22 (0.03)[a]	0.44 (0.01)[a]	4.33 (0.95)[a]
Red	1.08 (0.31)[a]	0.28 (0.02)[b]	0.28 (0.03)[d]	4.87 (1.34)[a]

Averages (SD), $n = 4$. Statistically different values are marked by different superscripts ($P < 0.05$).

but higher under blue light. The concentration of phycocyanin increased from equal amounts in the white and green treatments to higher in the red and even higher in the blue treatments. The chl a content was highest in green light samples, progressively decreasing in blue, white, and red samples.

Growth rates were estimated from the change in total biomass from one sampling time to the next. Over the course of 40 days, the growth rate of samples in white light was approximately twice that of those in red or green light and seven times higher than in blue light (data not shown). This agrees with the hypothesis of low PUR in the blue treatment and suggests that phycobiliprotein synthesis under this condition did not compensate for the lower PUR in terms of growth. Microscopic observation revealed that red light-acclimated samples developed very thick cell walls as compared with all other treatments (not shown).

Spectral response of MAA induction. Before transfer to the treatment conditions, extracts from initial samples, either cultured or collected fresh from the sublittoral, contained no clear absorbance peaks in the UV-A or UV-B range (Fig. 2). Differential UV absorbance among the red, green, blue, and white treatments was observed within 7 days of the start of the experiment. Representative spectra of extracts obtained

from thalli of equal weight suggested that, relative to the initial condition, there was similar synthesis of UV-absorbing compounds in white and blue treatments, but a loss of compounds under red and green light. Thalli in the white or blue treatments contained substances absorbing strongly between 320 and 370 nm, with broad peaks at 330 nm and 360 nm. Differences between white and blue versus green and red were maintained over the remainder of the 40-day experimental period.

The presence of MAAs was confirmed by HPLC analysis of samples collected throughout the duration of the experiment (Fig. 3). After the first week, thalli in both white and blue treatments contained primarily palythine, though samples in white light contained approximately four times as much on a DW basis. Samples in white light also contained small amounts of shinorine. The palythine content in blue light-treated thalli rose nearly linearly over the course of 40 days to 0.8 mg·g^{-1} DW, and at the end of the experiment there was no significant difference between samples in the blue or white treatment. Induction was light saturated at the levels used in the blue treatment, because the 50% more blue light in the PAR treatment (Table 1) did not result in any greater MAA content. In contrast, there was less than 0.15 mg·g^{-1}
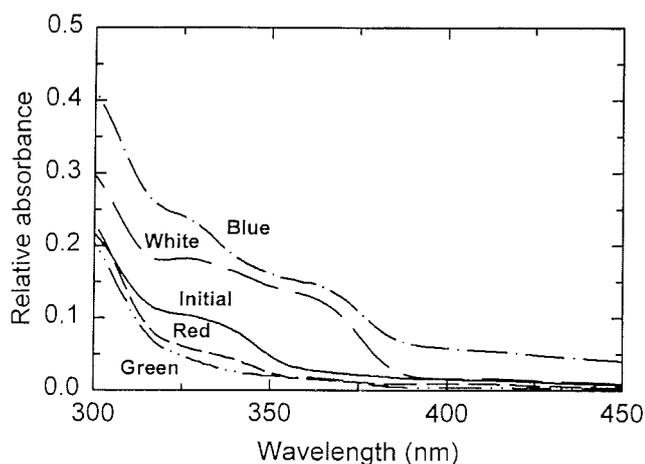

FIG. 2. Extracts of UV-absorbing compounds from *Chondrus crispus* after 1 week in the blue, green, red, or white filter treatments. Samples were of equal fresh weight, extracted in equal volumes of 25% aqueous methanol.

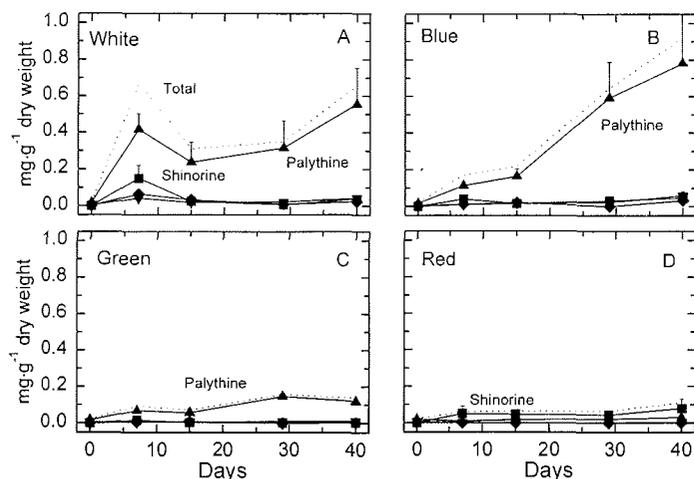262   LINDA A. FRANKLIN ET AL.

FIG. 3. Time course of the accumulation of MAAs in *Chondrus crispus* under (A) PAR, (B) blue, (C) green, or (D) red filter treatments, expressed on a thallus dry weight basis. The major MAAs are identified on the panels; only trace amounts of asterina-330 and palythene were detected. The dotted line is the sum of all MAAs. Means ± SD, $n = 5$.

DW palythine measured in the green treatment and less than 0.1 mg·g^{-1} DW shinorine in the red treatment at any time within the 40 days. Only trace amounts of asterina-330 and palythene were detected in any treatment at any time.

Response to a standard UV stress before synthesis of MAAs. PAM chlorophyll fluorescence characteristics of cultured *C. crispus* are shown in Figure 4, before and after the 3-h UV-A or the UV-A+B stress test and after 3 h recovery in low PAR. Before exposure to any UV stress, F_v/F_m was 0.65 (Fig. 4A, value at 0 irradiance). Upon exposure to increasing irradiance in a light response curve, ϕ_{II} declined from 0.65 to 0.17 as more excitation energy was received by the thallus than was required for electron transport. At the same time, the reduction status of Q increased in response to increasing irradiance, from a fully oxidized state at low irradiance to just under 60% reduction at 380 μmol photons·m^{-2}·s^{-1} (Fig. 4D). After exposure to 3 h UV-A or UV-A+B in low background PAR, both F_v/F_m and ϕ_{II} were reduced to 0.1 or less (Fig. 4B). Although there was no statistically significant difference between stress treatment with UV-A or UV-A+B, thalli treated with UV-A+B had slightly but consistently lower quantum yields. The Q pool was 30%–60% reduced at low irradiance, increasing to nearly 100% at 380 μmol photons·m^{-2}·s^{-1} (Fig. 4E). A greater degree of reduction was seen due to the UV-A+B test compared with the UV-A test, but the response was highly variable. The degree of recovery after the stress differed among the fluorescence parameters. Quantum yield recovered somewhat during the next 3 h in low PAR, with the tendency for lesser recovery when the stress test contained UV-B (Fig. 4C), whereas the reduction status of the Q pool recovered completely in each case, differing only slightly from initial values at low irradiance (Fig. 4F).

Response to a standard UVR stress after acclimation to spectral conditions and MAA synthesis. The response of thalli to the 3-h stress test was measured again at the end of the 40-day acclimation and MAA synthesis period. Results of measurements performed before, after the 3-h treatment, and after the 3-h recovery are presented in Figures 5–7. Complete fluorescence versus irradiance curves were measured in each case, but only the results for F_v/F_m (Fig. 5), ϕ_{II} (Fig. 6), and Q pool reduction status (Fig. 7) at the low and high irradiance endpoints (7 and 380 μmol photons·m^{-2}·s^{-1}) are shown for clarity. In almost every case, there was a significant effect (usually $P < 0.001$) of acclimation filter color on the results of the stress test, as indicated in the figures.

The ratio F_v/F_m of thalli acclimated to white or blue light declined significantly less after the 3-h exposure to UV-A or UV-A+B compared with that of green- or red-acclimated samples (Fig. 5) and less than observed initially (cf. Fig. 4A). Recovery was also significantly greater, especially after UV-A exposure, indicating increased capacity to cope with UVR stress. In contrast, there was no difference in response between red-acclimated samples and initial thalli. Thus, thalli with the higher amount of MAAs had a higher optimal quantum yield under these circumstances. However, samples from the white and blue treatments differed in their response to UV-A, even though they contained similar amounts of MAAs. "White" thalli maintained a significantly higher optimal quantum yield than those from the blue treatment after exposure to the 3-h UV-A, but this difference disappeared when UV-B was included in the test radiation, and the F_v/F_m of all samples was lower. Differences among treatments at the start of the test, although statistically significant, were quite small relative to changes due to the stress and therefore were probably not biologically significant.
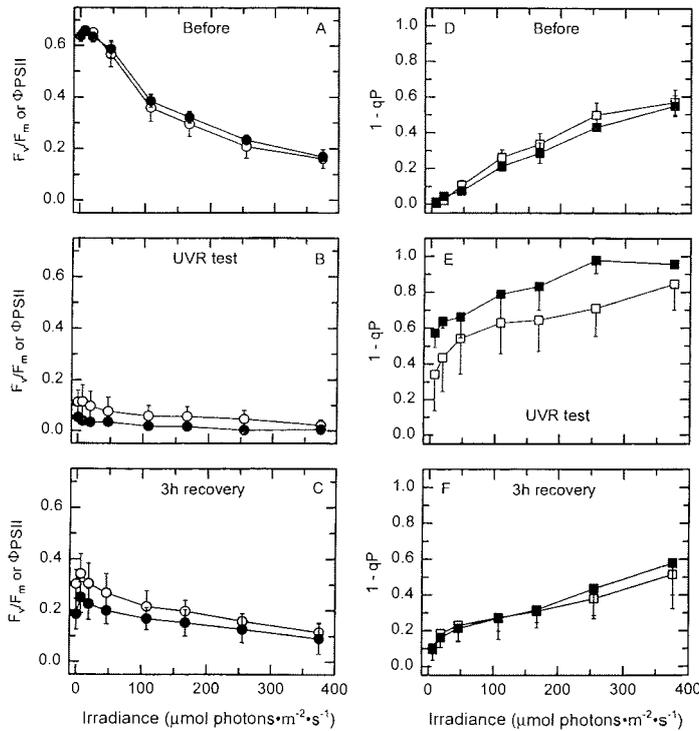
FIG. 4. Fluorescence versus light response curves of *Chondrus crispus* thalli before growth under white, blue, green, or red filters. Measurements were made before and after a 3-h test exposure to UV-A (open symbols) or UV-B (filled symbols) and after 3-h recovery in PAR. Unweighted test irradiance: 1.4 W·m⁻² UV-B (300–320 nm), 20 W·m⁻² UV-A (320–400 nm), and 20 μmol photons·m⁻²·s⁻¹ PAR (400–700 nm). Weighted UV-B (PAS₃₀₀): 0.139 W·m⁻². For the UV-A only treatment, UV-B lamp output was blocked using a Folex filter. Recovery occurred in 20 μmol photons·m⁻²·s⁻¹ PAR. (A–C) Changes in the optimal quantum yield, F_v/F_m, after a 5-min period of darkness and then changes in ϕ_{II} in response to PAR between 7 and 380 μmol photons·m⁻²·s⁻¹. (D–F) Changes in the reduction status of the Q pool. Means ± SD, $n = 4$.

When measured at 7 μmol photons·m⁻²·s⁻¹, ϕ_{II} of samples acclimated to white or blue light was also higher after the UVR test than that of thalli acclimated to green or red light (Fig. 6, A and B). Again, testing with UV-A lowered ϕ_{II} of thalli from the blue treatment more than those from the white treatment, despite equal amounts of MAAs. Again, this difference disappeared when UV-B was present. There was no significant difference between green and red samples in either case. After the 3-h recovery from the UV-A test, ϕ_{II} was close to the starting values in the case of white and blue samples but significantly lower in the case of green and red samples (Fig. 6A). Recovery from the UV-A+B test was greater in white samples than in blue, green, or red samples (Fig. 6B). Despite the fact that red light-acclimated thalli had little accumulation of MAAs and performed worst among the treatments under UVR stress, these samples did show some signs of increased capacity to cope with UV, as recovery was greater than that seen initially (cf. Fig. 4C).

When measured at 380 μmol photons·m⁻²·s⁻¹, the UV-A test (Fig. 6C) did not further reduce ϕ_{II} of white samples or blue samples. Again, samples from the red treatment had significantly lower ϕ_{II}, and green samples

were intermediate. The UV-A+B test (Fig. 6D) reduced ϕ_{II} in all cases, but this time blue, green, and red samples were equally affected despite differing amounts of MAAs. After the recovery period, ϕ_{II} was highest in white and blue (UV-A) or just white thalli (UV-A+B).

Before applying the UVR test, the quinone pool was highly oxidized at 7 μmol photons·m⁻²·s⁻¹ (Fig. 7, A and B) and approximately 50% reduced at 380 μmol photons·m⁻²·s⁻¹ (Fig. 7, C and D). No clear trends or correlations with MAA content were observed. After the test, a slightly higher proportion of Q was reduced at low light in green- and red-acclimated thalli, but after recovery there was no difference among treatments. At high light, the UV-A test had no effect on samples grown in white, blue, or green light, but almost 100% of Q was reduced in red-treated thalli. In contrast, the UV-A+B test led to 100% reduction in blue- and green-acclimated thalli as well. Again, inclusion of UV-B separated the response of white and blue samples, despite equal MAA content. White light-acclimated samples recovered fully after either test. In contrast, thalli from the blue treatment recovered to a similar extent after the UV-A test but less so when the test contained UV-B. The least recovery was found in red samples. In summary, those thalli with more MAAs
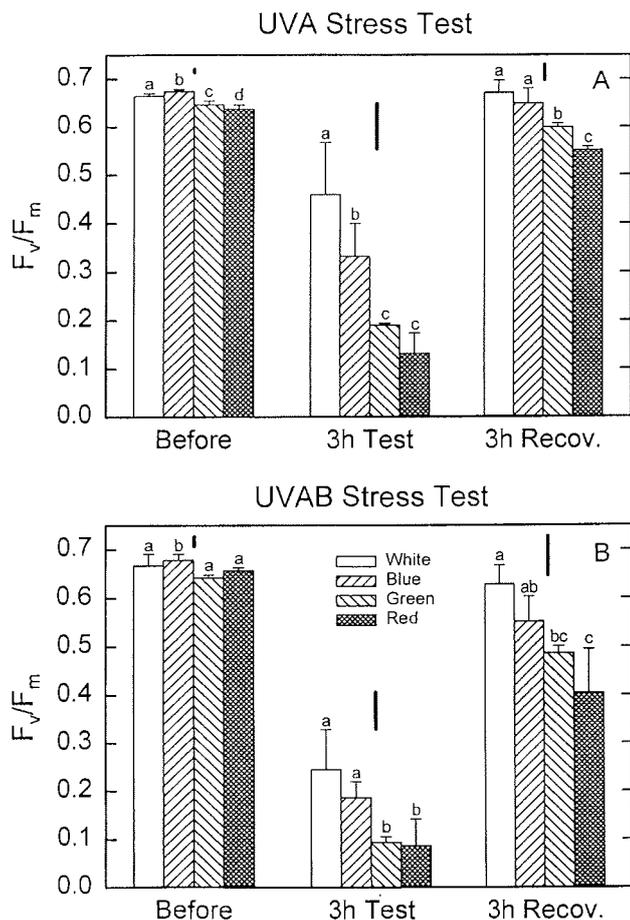
UVA Stress Test

UVAB Stress Test

FIG. 5. Comparison of F_v/F_m of *Chondrus crispus* thalli acclimated 40 days to white, blue, green, or red light. Changes are due to exposure to the laboratory UV-A (A) or UV-A+B (B) stress tests and during subsequent recovery. Test and recovery conditions as in Figure 4. Means ± SD, $n = 4$; heavy lines above each group of bars are the least significant difference.

usually had high photosynthetic performance under UVR stress, but those with equally high MAA content (white and blue) differed with respect to relative protection from UV-A or UV-A+B.

Effect of prior exposure to blue light on UVR-induced MAA synthesis. Neither fresh subtidal *C. crispus* (Fig. 8, A–C, "0" days) nor samples that had already been exposed to the 2-week pretreatment in blue light (Fig. 8, D–F, "0" days) contained any detectable MAAs. This was in contrast to results from the earlier experiments (Fig. 3) but was likely due to the reduced global irradiance at this later date. MAA synthesis in *C. crispus* has been shown previously to be dose dependent (Karsten et al. 1998a). During the next 2 weeks, a small increase in the level of palythine was observed in samples in blue light, occurring faster if there had been previous blue light exposure (Fig. 8, A and D)

but to the same extent at the end of the experiment. Total MAA content was lower than in the first experiment, in agreement with the lower global irradiance at that time of year. In contrast, the amount of shinorine present in both field and blue-pretreated samples rose significantly within 3 days of transfer to UV-A or UV-A+B (Fig. 8, B, C, E, and F). However, the sequential application of blue light and UVR led to a 10-fold greater initial synthesis of shinorine than any treatment alone, a more than additive effect of either blue or UV-A or UV-A+B (note different scales in E and F). The presence of UV-B in the spectrum made little difference in the levels of MAAs measured but might have contributed to a decline in MAA content at the end of 2 weeks. Samples that had not been previously treated in blue light also contained a significant amount of an unidentified UV-absorbing compound (Fig. 8, B
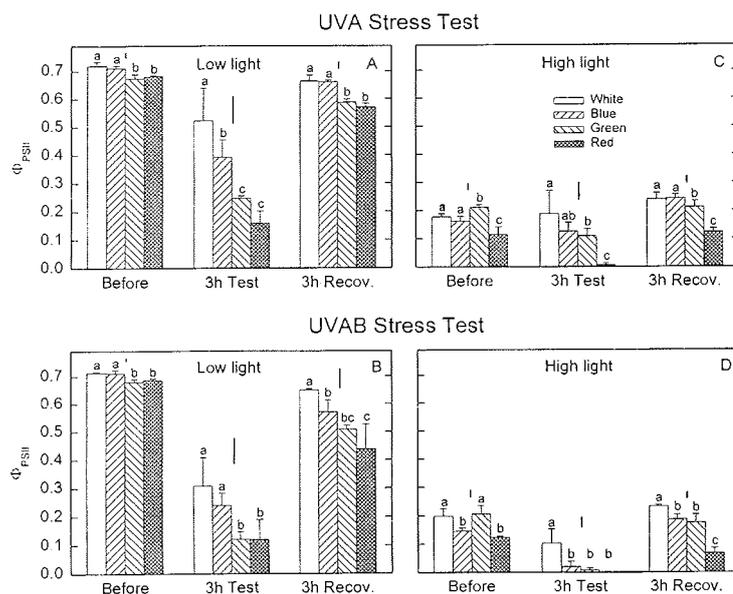
Fig. 6. Comparison of ϕ_{II} of *Chondrus crispus* thalli acclimated 40 days to white, blue, green, or red light. (A and B) Differences among treatments when measured at low light (7 μmol photons·m^{-2}·s^{-1}). (C and D) Differences among treatments when measured at high light (380 μmol photons·m^{-2}·s^{-1}). (A and C) Changes due to exposure to the laboratory UV-A stress test and subsequent recovery. (B and D) Changes due to exposure to the laboratory UV-A+B stress test and subsequent recovery. Test and recovery conditions as in Figure 4. Means ± SD, n = 4. Different letters designate significantly different treatments; heavy lines above each group of bars are the least significant difference.

and C), but total UV-absorbing compounds were still much less than observed in blue-pretreated samples.

DISCUSSION

MAA synthesis and protection of photosynthesis from UV stress. Natural UV-B radiation clearly has the potential to inhibit photosynthesis of many macroalgae *in situ* (e.g. Häder et al. 1996, Sagert et al. 1997) and especially when thalli are moved from low radiation environments at depth to near-surface levels of UV-B (e.g. Wood 1987, 1989, Larkum and Wood 1993, Herrmann et al. 1995, Hanelt et al. 1997). For species occurring over a wide range of depths, the degree of inhibition is usually markedly less in samples from shallow water (Dring et al. 1996, Bischof et al. 1998). There are a number of possible photosynthetic targets for damage by UV-B, including RUBISCO (Nogués and Baker 1995, Lesser 1996) and the donor and/or acceptor side of PSII reaction centers (Post et al. 1996, Vass et al. 1999). The widespread occurrence of MAAs in organisms growing in high irradiance and the broad UV absorption spectrum support the hypothesis that MAAs act as sunscreens in macroalgae. Most evidence relating MAAs to reduced UV sensitivity are correlative, but in the dinoflagellate *Gymnodinium sanguineum*, MAAs specifically reduced the action

spectrum of damage to photosynthesis in UV wavelengths (Neale et al. 1998). In contrast, there are also cases where accumulation of MAAs or unidentified UV absorbing compounds offered only limited protection of photosynthesis, pigment content, or growth rate of macroalgae (Wood 1989, Lesser 1996, Franklin et al. 1999).

Because the photosynthetic efficiency of lab-cultured *C. crispus* was equally reduced by UV-A and UV-A+B and the combination of MAAs in *C. crispus* results in a broad UV absorption spectrum, we were interested in whether there was a differential change in the MAA content of thalli under different light treatments and whether there was differential UVR sensitivity based on the accumulation of MAAs. In agreement with other studies, the photosynthetic efficiency of thalli with higher MAA content was generally more resistant to UVR, either when the comparison was made to thalli sampled before the growth experiment (Fig. 4) or among the treatments after the experiment (Figs. 5 and 6). In those treatments where MAAs were synthesized, the differences in the results of the UV-A tests before and after MAA synthesis were much greater than the differences in the results of the UV-A+B test. This was unexpected because the major compound palythine has an absorption maximum of 320 nm. But
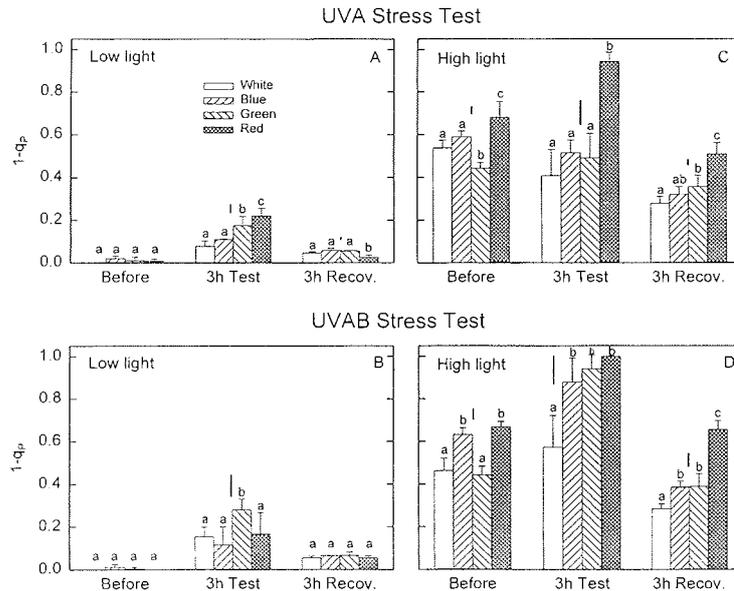
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FIG. 7. Comparison of the reduction status of the Q pool of *Chondrus crispus* thalli acclimated 40 days to white, blue, green, or red light. A–D as in Figure 6. Test and recovery conditions as in Figure 4. Means ± SD, n = 4. Different letters designate significantly different treatments; heavy lines above each group of bars are least significant differences where significant differences were detected.

the transfer to higher irradiance in general appeared to have a positive effect on the response to UV. Even without substantial MAA synthesis, red and green light-treated samples recovered to a greater degree after the UV stress than observed initially.

Additionally, there was often a significant difference between samples with equal MAA content (white and blue) in their change in photosynthetic efficiency after UV-A versus UV-A+B stress. Blue light-grown thalli were usually significantly more sensitive to UV-A than were thalli grown in white light but equally susceptible to the more damaging combination of UV-A+B. This may have reflected a greater capacity of white light-grown samples for damage repair or UV-A screening by means other than MAAs. Or, it may simply be an artifact arising from the fact that white light thalli contained lower amounts of phycoerythrin and phycocyanin relative to chl *a* than blue light samples and therefore had smaller photosynthetic unit targets. On the other hand, UV-B stress continued to have a significant effect on the reduction status of Q that was independent of MAA content and the ratio of phycobiliprotein/chl *a*. After the UV-A+B stress, Q pools in blue-treated samples were as highly reduced at light saturation as in green and red samples. One interpretation of these results is that exposure of blue-, green-, or red-treated thalli to UV-B disrupted electron flow beyond the plastoquinone pool, perhaps through

damage to ATP synthase or RUBISCO, but that growth in white light somehow reduced this effect independently of MAA content. A highly reduced Q pool and high degree of thylakoid membrane energization would have the additional effect of increasing the likelihood of photoinhibition by PAR and damage to PSII (Osmond et al. 1993).

It is quite likely that growth under the three different spectra led to a number of additional metabolic changes besides MAA synthesis. For example, blue and red light are well known to favor synthesis of proteins and carbohydrates, respectively (Kowallik 1987). Therefore, we hesitate to draw any specific conclusions as to exact mechanism by which the presence of MAAs in white versus blue light-grown thalli is correlated with improved photosynthetic performance after UV-A or UV-A+B treatment.

Induction of MAA synthesis in Chondrus crispus. In a number of algae, the synthesis of MAAs has been shown to be induced either by UV-B, UV-A, or PAR, or a combination of these wavelengths (Carreto et al. 1990, Riegger and Robinson 1997, Karsten et al. 1998a). In other species, MAAs are constitutively expressed for generations under laboratory culture conditions (Jeffrey et al. 1999) or are apparently uninducible (Hoyer et al. 2001). This variation of cause and effect makes it difficult to come to a consensus about particular triggering mechanisms. Furthermore, the final
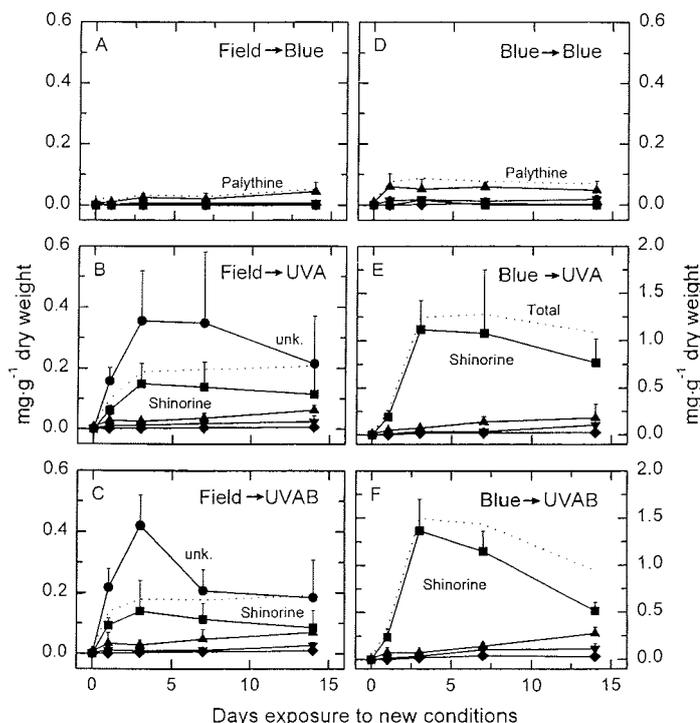
FIG. 8.   A comparison of the time course of the accumulation of MAAs in *Chondrus crispus* collected directly from 6 m (A–C) or preexposed to blue light (D–F). The two types of thalli were treated as follows for the induction of MAAs: (A and D) 2 weeks exposure under blue light, (B and E) 2 weeks exposure under UV-A (no PAR, UG5 + Folex filter), and (C and E) 2 weeks exposure under UV-A+B (no PAR, UG5 + Ultraphan 290 filter). Amounts are expressed on a thallus dry weight basis. Please note the different scale in E and F. The major MAAs are identified on the panels; only trace amounts of asterina-330 and palythene were detected. The dotted line is the sum of all identified MAAs. Means ± SD, *n* = 5.

concentrations of MAAs in an organism, and possibly the particular MAA composition, reflects the quantity of radiation applied (Carreto et al. 1990, Riegger and Robinson 1997, Karsten et al. 1998a, Franklin et al. 1999). Part of the difficulty in interpretation comes from the fact that in a number of cases, an increase in MAA concentration from some previous particularly low amount is reported. Thus, it is difficult to distinguish between signals for synthesis and control of signal transduction pathways that have already been initiated. It is possible that more that one photoreceptor or signal transduction pathway might be involved in the overall process leading to high MAA concentrations, analogous to interactions among various photoreceptors that have been reported in higher plants (Casal 2000).

In the present experiments, evidence has been found for specific blue and UV-A radiation-mediated MAA synthesis from conditions where no MAAs were present and for synergism between the two responses

with respect to synthesis of a single MAA, shinorine. Synthesis of palythine, the principle MAA found in eulittoral populations of *C. crispus*, was clearly induced by blue light. The limited palythine synthesis observed in green light may have been due to the spectral overlap of the filters in the 450- to 550-nm region. Elimination of wavelengths less than 380 nm from the white light treatment dramatically reduced the shinorine synthesis seen previously in PAR (Franklin et al. 1999), and no shinorine was detected under blue light. In contrast, shinorine synthesis was specifically induced by exposure of samples to UV-A radiation, confirming our earlier results with this species (Karsten et al. 1998a). Shinorine is by far the most common of MAAs reported in macroalgae so far, in species collected from tropical to Arctic waters (Banaszak et al. 1998, Karsten et al. 1998b), and was the most rapidly accumulated compound synthesized in *Stylophora* colonies exposed to UVR under controlled laboratory conditions (Shick et al. 1999). Wavelength

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dependence for MAA synthesis in some species of microalgae has been shown previously. In particular, Carreto et al. (1990) found that growth in blue light increased the total UV absorbance of *Alexandrium excavatum* cells, but not to the same degree as white light. A species-specific effect was reported by Riegger and Robinson (1997), with MAA synthesis in Antarctic diatoms responding maximally to wavelengths between 370 nm and 460 nm but not to UV-B and in the prymnesiophyte *Phaeocystis antarctica*, maximally to 340 nm, down to 305 nm.

Shick et al. (1999) used the inhibitor glyphosate to demonstrate that synthesis of 10 MAAs, including shinorine and palythine, proceeds along a portion of the shikimate pathway, as had been inferred from the work of Favre-Bonvin et al. (1987) on fungal mycosporine. With more that 20% of fixed carbon passing along this route (Herrmann 1995), the shikimate pathway is the process by which the aromatic amino acids phenylalanine, tyrosine, and tryptophan and a number of precursors for secondary metabolites are made in microorganisms and plants. In particular, phenylalanine acts as a precursor for the synthesis of UV-absorbing flavinoids. Recent evidence for the complex photoregulation of flavin synthesis in *Arabidopsis* appears relevant to the discussion of regulation of MAA synthesis in *C. crispus* and perhaps in other species. The first step of the flavinoid pathway is catalyzed by chalone synthase (CHS). Christie and Jenkins (1996) and Fuglevand et al. (1996) demonstrated that UV-B and UV-A/blue light induced CHS expression by separate pathways, only the latter of which involved the chryptochrome CRY1 photoreceptor. In addition, UV-A acted synergistically with UV-B to generate a transient signal for stimulating the CHS gene promoter, whereas blue light acted synergistically with UV-B by a stable signal. These data were interpreted as representing separate pathways for signal transduction in the flavinoid pathway.

The details of the pathway responsible for synthesis of specific MAAs and its relationship to specific steps in the shikimate pathway remains to be elucidated. However, support for the hypothesis that two photoreceptors or signal transduction pathways account for MAA synthesis in *C. crispus* comes from the observation that maximal shinorine synthesis occurred when blue light preceded the UV-A treatments, to quantities greater than those predicted from an additive response. The advantage of such a signal transduction system for a marine organism would lie in the preferential attenuation of UVR in the water column by dissolved organic material, or gelbstoff, with a characteristic absorption spectrum that increases exponentially at decreasing UV wavelength. Organisms able to sense a change in the amount of blue light present and respond by directing more carbon skeletons toward the synthesis of UV-absorbing compounds would have an advantage if the change in blue light presaged an increase in UV, as seen on a seasonal basis at high latitudes. In a climate change scenario, the depletion of stratospheric O_3 only leads to an increase in the shortest UV-B wavelengths; thus, at first consideration this triggering system would not be of particular advantage during periods of enhanced UV-B. However, UV-B has been shown to directly affect water column clarity by the photooxidation of gelbstoff (Morris and Hargreaves 1997). In this case, the gradual progression to ever shorter UVR as gelbstoff was oxidized would lead to greater inhibition of photosynthesis (Arrigo and Brown 1996) unless a photoprotective response was triggered before the shortest UV wavelengths were transmitted.

We did not measure a strict dose-response in these experiments, but a rough calculation suggests that in *C. crispus*, the blue light receptor saturates at irradiances that are at most 30% of incident (Table 1). Neither have we identified a specific photoreceptor molecule, though detailed action spectra for MAA synthesis in *C. crispus* is underway (G. Kräbs and U. Karsten, Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven, Germany, unpublished data). It is also interesting to note that based on the difference in pigment concentration between white and blue light-grown samples (Table 3) and the amount of total radiation received (Table 1), the amount of PUR received by the thallus appeared to have no direct effect on MAA synthesis in the short term but affected the rate of growth. It is clear that there are many more parts to the MAA induction puzzle to be clarified, for example, the induction of mycosporine-glycine synthesis by osmotic stress in darkness (Portwich and Garcia-Pichel 1999) and the basis by which macroalgae can be divided into those that do not ever appear to have MAAs, those in which MAAs can be induced, and those that constitutively contain them (Hoyer et al. 2001).

Arrigo, K. W. & Brown C. W. 1996. Impact of chromophoric dissolved organic matter on UV inhibition of primary productivity in the sea. *Mar. Ecol. Prog. Ser.* 140:207–16.
Banaszak, A. T., Lesser, M. P., Kuffner, I. B. & Ondrusek, M. 1998. Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAs) in marine organisms. *Bull. Mar. Sci.* 63:617–28.
Beach, K. S. & Smith, C. M. 1996. Ecophysiology of tropical rhodophytes. 1. Microscale acclimation in pigmentation. *J. Phycol.* 32:701–10.
Beer, S. & Eshel, A. 1985. Determining phycoerythrin and phycocyanin concentrations in aqueous crude extracts of red algae. *Aust. J. Mar. Freshw. Res.* 36:785–92.
Bilger, W. & Björkman, O. 1990. Role of the xanthophyll cycle in

photoprotection elucidated by measurements of light induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. *Photosynth. Res.* 25:173–85.

Bischof, K., Hanelt, D. & Wiencke, C. 1998. UV-radiation can affect depth-zonation of Antarctic macroalgae. *Mar. Biol.* 131:597–605.

Björn, L. O. & Murphy, T. M. 1985. Computer calculation of solar ultraviolet radiation at ground level. *Physiol. Veg.* 23:555–61.

Carreto, J. I., Carignan, M. O., Daleo, G. & De Marco, S. G. 1990. Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? *J. Plankton Res.* 12:909–21.

Casal, J. J. 2000. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. *Photochem. Photobiol.* 71:1–11.

Christie, J. M. & Jenkins, G. I. 1996. Distinct UV-B and UV-A blue light signal transduction pathways induce chalcone synthase gene expression in *Arabidopsis* cells. *Plant Cell* 8:1555–67.

Dring, M. J., Wagner, A., Boeskov, J. & Lüning, K. 1996. Sensitivity of intertidal and subtidal red algae to UVA and UVB radiation, as monitored by chlorophyll fluorescence measurements—influence of collection depth and season, and length of irradiation. *Eur. J. Phycol.* 31:293–302.

Dunlap, W. C., Chalker, B. E. & Oliver, J. K. 1986. Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. 3. UV-B absorbing compounds. *J. Exp. Mar. Biol. Ecol.* 104:239–48.

Dunlap, W. C. & Shick, J. M. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms—a biochemical and environmental perspective. *J. Phycol.* 34:418–30.

Favre-Bonvin, J., Bernillon, J., Salin, N. & Arpin, N. 1987. Biosynthesis of mycosporines: mycosporine-glutaminol in *Trichotecium roseum*. *Phytochemistry* 26:2509–19.

Franklin, L. A., Yakovleva, I., Karsten, U. & Lüning, K. 1999. Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. *J. Phycol.* 35:682–93.

Fuglevand, G., Jackson, J. A. & Jenkins, G. I. 1996. UV-B, UV-A, and blue light signal transduction pathways interact synergistically to regulate chalcone synthase gene expression in *Arabidopsis*. *Plant Cell* 8:2347–57.

Garcia-Pichel, F., Wingard, C. E. & Castenholz, R. W. 1993. Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. *Appl. Environ. Microbiol.* 59:1170–6.

Genty, B., Briantais, J. M. & Baker, N. R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990:87–92.

Häder, D. P., Lebert, M., Mercado, J., Aguilera, J., Salles, S., Flores-Moya, A., Jimenez, C. & Figueroa, F. L. 1996. Photosynthetic oxygen production and PAM fluorescence in the brown alga *Padina pavonica* measured in the field under solar radiation. *Mar. Biol.* 127:61–6.

Hanelt, D., Wiencke, C. & Nultsch, W. 1997. Influence of UV radiation on the photosynthesis of Arctic macroalgae in the field. *J. Photochem. Photobiol. B Biol.* 38:40–7.

Hannach, G. & Sigleo, A. C. 1998. Photoinduction of UV-absorbing compounds in six species of marine phytoplankton. *Mar. Ecol. Prog. Ser.* 174:207–22.

Herrmann, H., Ghetti, F., Scheuerlein, R. & Häder, D. P. 1995. Photosynthetic oxygen and fluorescence measurements in *Ulva laetevirens* affected by solar irradiation. *J. Plant Physiol.* 145:221–7.

Herrmann, K. M. 1995. The shikimate pathway—early steps in the biosynthesis of aromatic compounds. *Plant Cell* 7:907–19.

Hoyer, K., Karsten, U., Sawall, T., & Wiencke, C. 2001. Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages. *Mar. Ecol. Prog. Ser.* 211:117–29.

Jeffrey, S. W., MacTavish, H. S., Dunlap, W. C., Vesk, M. & Groenewoud, K. 1999. Occurrence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae. *Mar. Ecol. Prog. Ser.* 189:35–51.

Karentz, D., McEuen, F. S., Land, M. C. & Dunlap, W. C. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. *Mar. Biol.* 108:157–66.

Karsten, U., Bischof, K., Hanelt, D., Tug, H. & Wiencke, C. 1999. The effect of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). *Physiol. Plant.* 105:58–66.

Karsten, U., Franklin, L. A., Lüning, K. & Wiencke, C. 1998a. Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga *Chondrus crispus* (Rhodophyta). *Planta* 205:257–62.

Karsten, U. & Garcia-Pichel, F. 1996. Carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcoleus* (Cyanobacteria)—a chemosystematic study. *System. Appl. Microbiol.* 19:285–94.

Karsten, U., Sawall, T., Hanelt, D., Bischof, K., Figueroa, F. L., Flores-Moya, A. & Wiencke, C. 1998b. An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. *Bot. Mar.* 41:443–53.

Karsten, U. & Wiencke, C. 1999. Factors controlling the formation of UV-absorbing mycosporine-like amino acids in the marine red alga *Palmaria palmata* from Spitsbergen (Norway). *J. Plant Physiol.* 155:407–15.

Kowallik, W. 1987. Blue light effects on carbohydrate and protein metabolism. *In* Senger, H. [Ed.] *Blue Light Responses: Phenomena and Occurrence in Plants and Microorganisms*. CRC Press, Boca Raton, pp. 7–16.

Larkum, A. W. D. & Wood, W. F. 1993. The effect of UV-B radiation on photosynthesis and respiration of phytoplankton, benthic macroalgae and seagrasses. *Photosynth. Res.* 36:17–23.

Lesser, M. P. 1996. Acclimation of phytoplankton to UV-B radiation—oxidative stress and photoinhibition of photosynthesis are not prevented by UV-absorbing compounds in the dinoflagellate *Prorocentrum micans*. *Mar. Ecol. Prog. Ser.* 132:287–97.

Morel, A. 1978. Available, usable, and stored radiant energy in relation to marine photosynthesis. *Deep Sea Res.* 25:673–88.

Morris, D. P. & Hargreaves, B.R. 1997. The role of photochemical degradation of dissolved organic carbon in regulating the UV transparency of three lakes on the Pocono Plateau. *Limnol. Oceanogr.* 42:239–49.

Nakamura, H., Kobayashi, J. & Hirata, Y. 1982. Separation of mycosporine-like amino acids in marine organisms using reversed-phase high performance liquid chromatography. *J. Chromatogr.* 250:113–8.

Neale, P. J., Banaszak, A. T. & Jarriel, C. R. 1998. Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. *J. Phycol.* 34:928–38.

Nogués, S. & Baker, N. R. 1995. Evaluation of the role of damage to photosystem II in the inhibition of CO_2 assimilation in pea leaves on exposure to UV B radiation. *Plant Cell Environ.* 18:781–7.

Oren, A. 1997. Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. *Geomicrobiol. J.* 14:231–40.

Osmond, C. B., Ramus, J., Levavasseur, G., Franklin, L. A. & Henley, W. J. 1993. Fluorescence quenching during photosynthesis and photoinhibition of *Ulva rotundata* Blid. *Planta* 190:97–106.

Porra, R. J., Thompson, W. A. & Kriedemann, P. E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta* 975:384–94.

Portwich, A. & Garcia-Pichel, F. 1999. Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogloeopsis* PCC 6912. *Arch. Microbiol.* 172:187–92.

Post, A., Lukins, P. B., Walker, P. J. & Larkum, A. W. D. 1996. The effects of ultraviolet irradiation on P680+ reduction in PS II core complexes measured for individual S-states and during repetitive cycling of the oxygen-evolving complex. *Photosynth. Res.* 49:21–7.

Riegger, L. & Robinson, D. 1997. Photoinduction of UV-absorbing

compounds in Antarctic diatoms and *Phaeocystis antarctica*. *Mar. Ecol. Prog. Ser.* 160:13–25.

Sagert, S., Forster, R. M., Feuerpfeil, P. & Schubert, H. 1997. Daily course of photosynthesis and photoinhibition in *Chondrus crispus* (Rhodophyta) from different shore levels. *Eur. J. Phycol.* 32:363–71.

Schreiber, U., Endo, T., Mi, H. & Asada, K. 1995. Quenching analysis of chlorophyll fluorescence by the saturation pulse method: particular aspects relating to the study of eukaryotic algae and cyanobacteria. *Plant Cell Physiol.* 36:873–82.

Schreiber, U., Schliwa, U. & Bilger, W. 1986. Continuous recording of photochemical and nonphotochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. *Photosynth. Res.* 10:51–62.

Shibata, K. 1969. Pigments and a UV absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. *Plant Cell Physiol.* 10:325–35.

Shick, J. M., Romaine-Lioud, S., Ferrier-Pages, C. & Gattuso, J. P. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. *Limnol. Oceanogr.* 44:1667–82.

Sivalingam, P. M., Ikawa, T., Yokohama, Y. & Nisizawa, K. 1974. Distribution of a 334 UV-absorbing substance in algae, with special regard of its possible physiological roles. *Bot. Mar.* 17:23–9.

Starr, R. C. & Zeikus, J. A. 1993. UTEX—the culture collection of algae at the University of Texas at Austin: list of cultures. *J. Phycol.* 29:1–106.

Tsujino, I., Yabe, K. & Sekikawa, I. 1980. Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendoi* Yamada et Mikami. *Bot. Mar.* 23:65–8.

Tsujino, I., Yabe, K., Sekikawa, I. & Hamanaka, N. 1978. Isolation and structure of a mycosporine from the red alga *Chondrus yendoi. Tetrahedr. Lett.* 16:1401–2.

Vass, I., Kirilovsky, D. & Etienne, A. L. 1999. UV-B radiation-induced donor- and acceptor-side modifications of photosystem II in the cyanobacterium *Synechocystis* sp PCC 6803. *Biochemistry* 38: 12786–94.

Wood, W. F. 1987. Effect of solar ultra-violet radiation on the kelp *Ecklonia radiata. Mar. Biol.* 96:143–50.

Wood, W. F. 1989. Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultra-violet radiation. *Aquat. Bot.* 33:41–51.

Photosynthesis, photosynthetic pigments and mycosporine-like amino acids after exposure of the marine red alga *Chondrus crispus* (Gigartinales, Rhodophyta) to different light qualities

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Abstract

Low light adapted laboratory grown thalli of *Chondrus crispus* (Stackhouse) were transplanted into shallow water and exposed to the natural irradiance prevailing in May/June at Helgoland (German Bight). A set of cut-off filters was used to study the wavelength dependent response of photosynthesis as well as pigment and mycosporine-like amino acid (MAA) formation. Due to the higher natural irradiance and in some cases additional UV radiation, a depression of maximal quantum yield coinciding with a decline in maximal electron transport rate could be observed during the first days of the experiment. Faster recovery of maximal quantum yield and maximal electron transport rate might be related to the increase in lutein concentration. In addition, higher rates of electron transport rates might be supported by increased chlorophyll a concentration. In parallel, MAA concentration increased resulting in an effective UV sunscreen. Our data suggest, that lutein is involved in the recovery of photochemical capacity, whereas MAAs provide a sufficient UV sunscreen reducing the light available within the algae to long wavelength UV-A and PAR.

Running title: Exposure of *Chondrus crispus* to different light qualities

Key words: *Chondrus crispus*, mycosporine-like amino acids, photosynthesis, pigments

Introduction

Chondrus crispus (Stackhouse) is an abundant red alga of the North Atlantic inhabiting the intertidal and upper sublittoral zone of rocky shorelines (Lüning 1990). Therefore, specimens have to cope with either extreme periodical variations in solar irradiance, but also with relatively constant low irradiances in deeper or turbid waters, depending on the respective vertical position on the shore, the state of the tide as well as seasonal and diurnal changes in solar elevation.

The occurrence of this species in a wide variety of different habitats and the fact that pronounced differences in UV sensitivity have been observed between intertidal and subtidal macrophytes species and individuals (Bischof et al 1998 a, b, Karsten et al. 2001, van de Poll et al. 2001, 2002), have spurred the interest to investigate acclimation responses of *C. crispus* to various ecological parameters as well as UV effects (Kübler & Davison 1993, 1995, Sagert et al. 1997, Collén & Davison 1999, Franklin et al. 1999, Bischof et al. 2000, Yakovleva & Titlyanov 2001).

One mechanism in the acclimation to changing light especially UV conditions is the accumulation of UV absorbing mycosporine-like amino acids (MAAs; for overview see Bandaranayake 1998, Shick et al. 2000, Shick & Dunlap 2002), although the degree of protection varies (Garcia-Pichel 1993, Lesser et al 1996, Franklin at al. 1999). The synthesis of MAAs is both dependent on the quality and dose of radiation applied (Carreto et al. 1990, Riegger & Robinson 1997, Karsten et al. 1998).

Previously, we have studied the wavelength dependence of MAA synthesis of laboratory grown algae transferred to shallow water under natural solar radiation (Kräbs et al. 2002), but one result remained unclear. Apart from the main response maximum for MAA formation in the UV-A band, a pronounced negative response to UV-B wavelengths has been detected. Differences in maximal quantum yield of photosynthesis (Fv/Fm) between algae exposed under different cut-off filters were small. On the other hand, Franklin et al. (1999) found a significant UV-B induced growth inhibition of sublittoral *C. crispus* transplanted into shallow water. Consequently, it is unclear whether the negative effect of UV-B radiation on MAA accumulation is due to an impaired metabolism or to some unknown wavelengths dependent characteristics of the mechanism triggering MAA formation.

Therefore, in the present study we measured photosynthesis versus irradiance curves using a similar set-up and took samples for pigment analysis, additionally to samples for

MAA analysis in order to get more information on the general effects of different light qualities on the metabolism of *C. crispus* during acclimation.

Material and Methods

Algal material and experimental conditions

Thalli of *C. crispus*, originally isolated on Helgoland (German Bight, North Sea; 54°11'N, 7°53'E) were cultivated in glass beakers in the laboratory (15°C, light-dark-cycle 16:8, 30 μmol m^{-2} s^{-1} PAR). In May 2001, approximately 2 cm long tips were transplanted into boxes (18 × 18 × 5 cm) within an open flow through basin (150 × 50 × 10 cm) mounted on the roof of the laboratory (BAH, Helgoland) and exposed to natural solar radiation. The spectral response to photosynthesis as well as pigment and MAA induction was studied by placing samples into boxes covered with different cut-off filters (WG305, WG320, WG335, WG360, GG400, GG420 and GG495; Schott, Mainz, Germany), thus generating different spectral radiation conditions. Additionally, one box was left uncovered ("unfiltered") to study the effect of the whole light spectra.

Throughout the experiment, the whole set up was additionally covered with two layers of black gauze (Haver & Boecker, Oelde, Germany; transmission = 40%) to reduce light intensity neutrally. The boxes were perforated at opposite sides to allow water-exchange. The basin was continuously flushed with filtered North Sea water. The water column above the samples was 5 cm. Algae were inserted into the experimental set-up in the evening. Samples for photosynthesis measurements and pigment analysis were taken at 6:00 h. Replica for MAA analyses were sampled at 6:00 h and 18:00 h.

Light measurements

Radiation conditions at Helgoland during the experiment were continuously monitored by a PUV-500 radiometer (Biospherical Instruments, San Diego, USA) mounted on the roof of the institute. Additionally, a spectrum of solar radiation was recorded at noon on a sunny day (Tab. 1) by a fast scanning double monochromator spectroradiometer (UV320D, Instruments Systems, Munich, Germany) equipped with a cosine sensor.

Table 1: Spectral irradiance measured on a sunny day at noon (unfiltered) and theoretical spectral irradiance under the used cut-off filter calculated by the measured spectrum against the transmittance spectrum of the respective filter

	UV-B	UV-A $W\ m^{-2}\ s^{-1}$	PAR
unfiltered	1,04	51,54	438,49
WG305	0,65	44,91	393,18
WG320	0,33	45,41	398,61
WG335	-	37,88	388,66
WG360	-	27,60	388,40
GG400	-	1,44	374,53
GG420	-	-	358,42
GG495	-	-	257,04

Table 2: Statistical significance of difference between two sequential cut-off filters. Statistical significant differences ($p<0,05$) are marked with x

	Shinorine	Palythine	Asterina	Palythene	Total
Unfiltered/WG305	x	x	x	x	x
WG305/WG320	x				x
WG320/WG335					
WG335/WG360		x	x		
WG360/GG400	x	x	x	x	x
GG400/GG420					
GG420/GG495		x	x	x	x

Biological variables

During exposure, maximal quantum yield of photosystem II electron transport of dark adapted algae (4 min) was determined by the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) with a pulse amplitude modulated chlorophyll fluorometer (PAM 2000, Walz, Effeltrich, Germany), following the protocol described in detail by Hanelt (1998). Photosynthesis versus irradiance curves were recorded with the same fluorometer as described by Bischof et al. (1999). Measurements of Fv/Fm and photosynthesis versus irradiance curves were conducted in triplicates from randomly collected samples.

Changes in the pigment (chlorophyll a, lutein, α- + β-carotene, zeaxanthin) composition and content were analysed as described by Bischof et al. (2002). Pigment data were obtained from independent triplicate samples.

Changes in MAA composition and content were analysed as described by Kräbs et al. (2002) with the modification of the mobile phase to 5 % aqueous methanol (v/v) plus 0,1 % acetic acid (v/v) in water. MAA data were obtained from five independent samples.

Polychromatic response spectra

To facilitate intercomparison of results obtained from the different filter treatments, a theoretical spectral irradiance from every single filter was calculated from each wavelength according to the following equation:

$$I_{th} = I_{m}*T/100$$

with the measured irradiance I_m at a given wavelength, the corresponding transmission T of the filter and the theoretical spectral irradiance I_{th} below the filter (Tab. 1).

Subsequently, polychromatic response spectra were calculated from the respective MAA concentration accumulated in *C. crispus* grown under different filter conditions. First, a polynom second order (shinorine) and each a linear equation (palythine, asterina-330, palythene), respectively was fitted through single measured data points for the respective MAA concentration accumulated in algae exposed under each filter. Using the obtained equation a "theoretical" concentration of the respective MAA was calculated for the sixth day of the experiment. A mean value was calculated and used for a group of filter treatments with no statistically difference in MAA concentration, while the calculated value was used directly if the pattern in MAA accumulation under the filter in question was significantly different from neighbour filter treatments (Tab. 2). To calculate the polychromatic response spectrum for the total MAA concentration, the mean value for the steady state was calculated. Statistically significant differences between different filter treatments were taken into consideration as described above.

Response spectra were calculated using this statistically adjusted data set: The difference in MAA concentration in algae grown under two sequentially numbered cut-off filters was divided by the difference in total irradiance between the two light fields under the filters (Tab. 3; Rundel 1983). All calculation of polychromatic response spectra are based on the median wavelength of the difference in total irradiance obtained by subtraction of two sequential light fields as described by Riegger and Robinson (1997).

Table 3: Irradiance of the difference spectra calculated from the theoretical filter spectra of Tab.1 and respective Median Wavelength

Difference spectra	W m^{-2} s^{-1}	nm
WG305-WG320	0,5543	313
WG320 – WG335	7,5297	329
WG335 – WG360	10,3010	345
WG360 – GG400	37,4390	386
GG400 – GG420	18,2770	411
GG420 – GG495	107,0200	466
GG495	257,0400	495

Data treatment

Statistical significance between Fv/Fm values and *PI*-curves was calculated using the Mann & Whitney Test (p<0,05; Lozán & Kausch 1998).

Mean values and standard deviation were calculated from the respective replicates. Statistical significance (p<0,05) of difference in MAA and pigment content accumulated under each cut-off filter and for different times as well as their combined effect was tested by two way analysis of variance (ANOVA) followed by Least Significant Difference-Test (LSD). Calculations were done using the program Statistica Kernel-Version 5.5A (StatSoft, Inc., Tulsa, OK, USA).

Results

Environmental conditions

Solar irradiation varied markedly in the course of the experiment due to prevailing weather conditions. Sunny and cloudy conditions often changed rapidly, sometimes interrupted by rainfall, as indicated by the course of PAR (Fig.1). The respective daily dose of solar irradiance at 305, 320, 340, 380 and 400-700 nm is presented in Tab.4.

Figure 1: Changes in the course of PAR irradiance at the study site in the course of the experiment
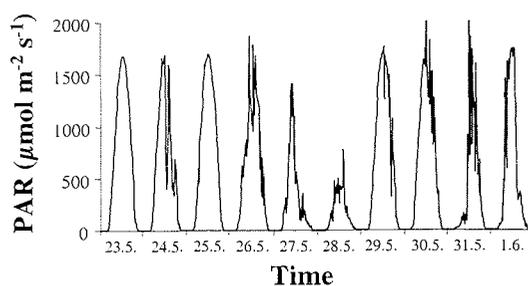
Table 4: Daily dose of solar radiation during the course of experiment

Date	Day	305 nm	320 nm	340 nm	380 nm	400-700 nm
			kJ m^{-2} nm^{-1}			mol m^{-2}
23 May 2001	0	0,576	7,118	12,974	22,141	55,695
24 May 2001	1	0,408	5,748	10,722	18,126	43,556
25 May 2001	2	0,597	7,296	13,296	22,980	56,435
26 May 2001	3	0,423	5,786	10,533	17,529	42,106
27 May 2001	4	0,207	3,172	5,885	9,651	22,572
28 May 2001	5	0,181	2,134	3,687	5,797	12,214
29 May 2001	6	0,604	7,340	13,334	22,643	55,618
30 May 2001	7	0,550	7,175	13,133	22,192	54,415
31 May 2001	8	0,285	4,321	7,959	13,339	30,481
01 June 2001	9	0,431	5,728	10,333	17,177	39,970

Effects on photosynthesis

During the first days of the experiment a pronounced inhibition of maximal quantum yield of photosynthesis (Fv/Fm) could be observed in the morning (Tab. 5). Even so there was no clear trend, thalli exposed under cut-off filters to UV radiation of shorter wavelengths (WG305-WG360) were more inhibited than thalli exposed to PAR, while thalli exposed unfiltered to the full solar radiation exhibited relatively high Fv/Fm values almost throughout the experiment. In the morning of the fifth and ninth day of the experiment Fv/Fm values recovered to approximately 76 % of the start value, with no significant variations between the different filter treatments.

PI-curves recorded in the morning, showed a decline in maximal electron transport rate during the time of low Fv/Fm values (Fig. 2). Parallel to the recovery of Fv/Fm the maximal electron transport rate increased over the values of the start measurements.

Table 5: Maximal quantum yield of photosynthesis (Fv/Fm) of thalli exposed under different cut-off filters. MW ± SD

	Initial (0 d)	1st day	2nd day	3rd day	5th day	9th day
Unfiltered	0,694 ± 0,001	0,430 ± 0,064	0,618 ± 0,079	0,542 ± 0,109	0,577 ± 0,071	0,547 ± 0,007
WG305	0,679 ± 0,031	0,367 ± 0,155	0,170 ± 0,098	0,324 ± 0,063	0,467 ± 0,062	0,446 ± 0,070
WG320	0,691 ± 0,027	0,230 ± 0,114	0,233 ± 0,223	0,315 ± 0,158	0,393 ± 0,018	0,468 ± 0,083
WG335	0,692 ± 0,010	0,457 ± 0,151	0,286 ± 0,033	0,214 ± 0,094	0,497 ± 0,052	0,558 ± 0,004
WG360	0,673 ± 0,025	0,419 ± 0,167	0,264 ± 0,067	0,391 ± 0,081	0,513 ± 0,064	0,587 ± 0,056
GG400	0,693 ± 0,009	0,382 ± 0,050	0,455 ± 0,046	0,540 ± 0,058	0,557 ± 0,039	0,538 ± 0,026
GG420	0,688 ± 0,007	0,472 ± 0,042	0,346 ± 0,111	0,461 ± 0,083	0,550 ± 0,023	0,580 ± 0,023
GG495	0,677 ± 0,015	0,633 ± 0,045	0,514 ± 0,097	0,539 ± 0,023	0,543 ± 0,029	0,566 ± 0,010
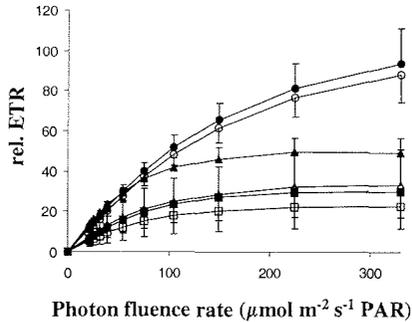
Figure 2: *PI*-curves showing relative electron transport rate (rel. ETR) of mean values calculated from all measured thalli irrespective of the filter treatment; ▲ = day 0, △ = day 1, □ = day 2, ■ = day 3, ○ = day 5, ● = day 9; MW ± SD
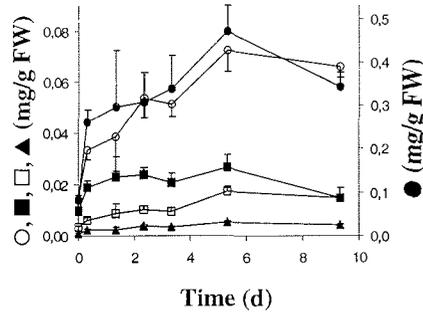
Figure 3: Changes in the content of chlorophyll a (●), lutein (○), α- (■) + β-carotene (□) and zeaxanthin (▲) during the experiment; statistical significant differences between the different filter treatments could not be observed; MW ± SD

Changes in the pigment content

The internal concentration of chlorophyll a, lutein, α- and β-carotene increased significantly during the experiment, while the concentration of zeaxanthin remained constant (see GG495; Fig. 3). Significant variations in the pigment composition and content between the different filter treatments could not be observed.

MAA content and induction

Samples taken before the start of the exposure contained only traces of the MAA palythine. After thalli were transferred to the experimental treatments and a short lag phase, a substantial induction of MAAs could be observed (Fig. 4).

Shinorine was accumulated more rapidly than the other MAAs. Depending on the filter treatment, the maximal shinorine content was reached between the third and fifth day of the experiment, followed by a significant decline of shinorine concentration. All other MAAs were accumulated more steadily throughout the experiment.

In general, a significantly lower MAA concentration was accumulated in algae exposed to the full solar irradiance ("unfiltered"; Tab. 1). The total MAA concentration exhibited in algae exposed under the filter WG305 was slightly higher than in algae exposed under the filters WG320, WG335 and WG360. The MAA content of this filter
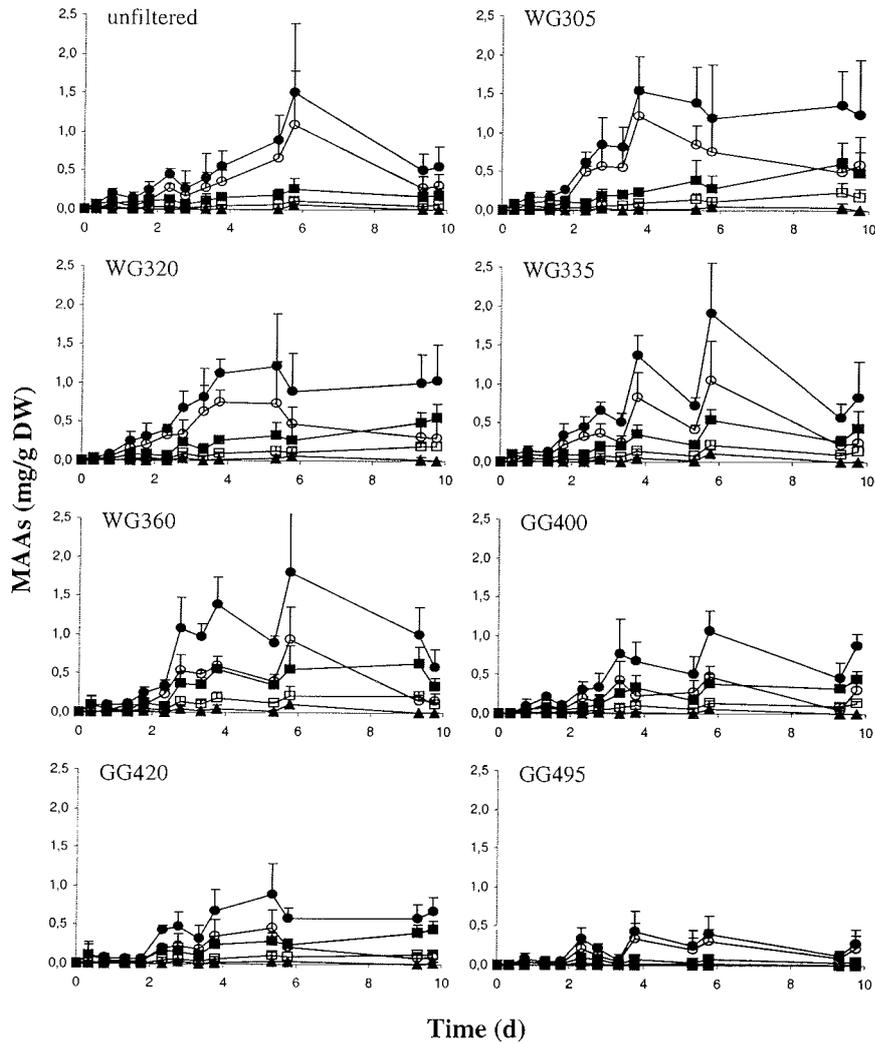
Figure 4: Time course of accumulation of shinorine (O), palythine (■), asterina-330 (□) and palythene (▲) as well as the total concentration of mycosporine-like amino acids (●) in C. crispus exposed to natural solar radiation under different cu-off filters; MW ± SD

treatment group was significantly higher than the synthesised MAA concentration of algae exposed under the filters GG400 and GG420. In algae exposed to PAR excluding the major part of blue light (GG495) the lowest MAA concentration could be detected (see also Fig. 5).

Polychromatic response spectra calculated from shinorine and the total MAA concentration indicated, that UV-B has the highest quantum efficiency for the accumulation of MAAs (Fig. 6). However, the response spectra calculated for palythine, asterina-330 and palythene revealed a difference in the wavelength dependent response between the steadily accumulated MAAs and shinorine. Thus, the response spectra for palythine, asterina-330 and palythene exhibit the highest quantum efficiency in the long waveband UV-A (λ_{max} = 386 nm) and a second response peak in the blue light region of the spectra (λ_{max} = 466 nm).
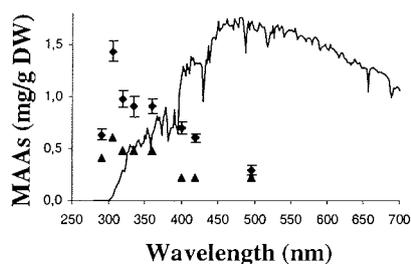
Figure 5: Final MAA concentration (◆; MW ± SE; n = 20-25) and calculated shinorine concentration (▲) put against the number of the respective cut-off filter (wavelength of 50 % emission) on top of the measured irradiance spectrum (Tab. 1, unfiltered)
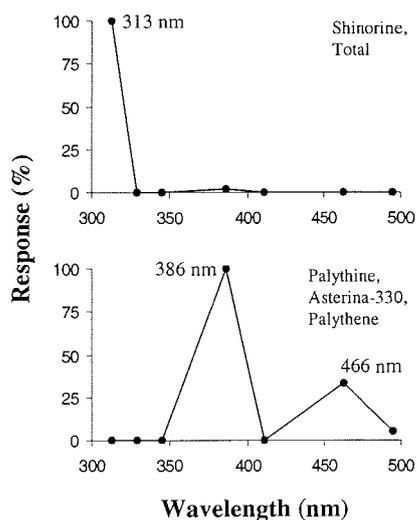
Figure 6: Polychromatic response spectra of the wavelength dependent induction of mycosporine-like amino acids exposed under natural solar radiation (see Material and Methods for the calculation)

Discussion

Our results show that low light adapted laboratory grown thalli of *C. crispus* transferred to high natural sun light can acclimate to the severely changed irradiance conditions (see also Franklin et al. 1999).

Due to higher irradiance and additional UV wavelengths a depression of maximal quantum yield could be observed within the first days. The relatively constant and high Fv/Fm values of algae exposed unfiltered to full solar radiation might be a temperature artefact. Although the whole experimental set-up (including cut-off filters) was submerged in cooled seawater (flow-through), the water underneath the cut-off filters may have been warmer than the surrounding water adding an additional stress factor. In parallel to the depressed Fv/Fm values, maximal electron transport rate declined. Similar results were found in several Arctic macrophytes after prolonged exposure to UV radiation (Bischof et al. 2000). Even though photosynthetic capacity was impaired, a rapid induction of chlorophyll a and lutein formation could be observed, indicating that photosynthetic activity was sufficient to provide the necessary energy equivalents for pigment synthesis.

An increase in lutein concentration may be an important part of the acclimation to higher irradiance, because lutein contributes to the nonphotochemical quenching of excess light energy (Niyogi et al. 1997, Pogson et al. 1998). By dissipating excessively absorbed light energy lutein, similar to the de-epoxidation of violaxanthin, prevents an increased formation of reactive oxygen species during photosynthetic activity and thus the photo-oxidation of the photosynthetic apparatus (Asada and Takahasi 1987). This function may be essential, because *C. crispus* lacks the xanthophyll cycle, which protects plants and macroalgae from photodamage induced through high PAR (Versihinin and Kamnev 1996, Hanelt et al. 1997, Schofield et al. 1998, Harker et al. 1999, Bischof et al. 2002 a, b). Low and constant concentrations of zeaxanthin, which also contributes to the nonphotochemical quenching (Niyogi et al. 1997, Pogson et al. 1998, Wilson et al. 2003) further emphasize the importance of lutein in *C. crispus*. Therefore, increased lutein concentration may be responsible for the recovery of maximal quantum yield and thus also for the recovery of maximal electron transport rate. Together with the recovery of optimal quantum yield, the initial shape of the *PI*-curves typical for shade-adapted thalli (start of the experiment) was altered to a course

characteristic for thalli exposed to high irradiance. This enhancement of maximal electron transport rate might be related to the change in chlorophyll a concentration.

Parallel to the pigment synthesis a steady accumulation of MAAs was observed, with a shift from shinorine to palythine as major MAA during the course of MAA induction as previously described (Franklin et al. 1999, Kräbs et al. 2002). While pigment content increases irrespective of the filter treatment a wavelength dependent difference in MAA accumulation could be observed. Algae exposed to PAR without the major part of blue light exhibit the lowest MAA concentration, followed by specimens exposed to PAR. Additional UV radiation increases the concentration of MAAs markedly, while short-wavelength UV-B leads to pronounced lower MAA concentration ("unfiltered").

This results in two polychromatic response spectra (calculated for the cut-off filters WG280 to GG495), one for shinorine accumulation and another on for palythine, asterina-330 and palythene accumulation. The polychromatic response spectrum for the total concentration of MAAs coincides with the one of shinorine. As discussed earlier (Kräbs et al. 2002), this might be explained by the following slightly improved scenarios:

1) according to Caldwell and Flint (1997) the ratio of UV:PAR affects biological response and might therefore be responsible for the differences in qualitative and quantitative MAA composition, although the same photoreceptor is involved

2) the synthesis of palythine, asterina-330 and palythene may be stimulated by a different photoreceptor than shinorine, or

3) an interconversion within the MAAs occurs as postulated by Franklin et al. (1999); on this background the polychromatic response spectra coincides merely with the wavelength dependency of enzyme induction/inhibition necessary for the interconversion.

According to Coohill (1992, 1994) polychromatic response spectra tend to obscure the photoreceptor molecule and can not conclusively show what the respective absorption spectrum would look like. This is the case for the present data. Because MAA accumulation occurs already in specimens exposed under PAR without the major part of blue light (GG495), the polychromatic response spectrum calculated for shinorine as well as the total MAA concentration can not reflect the absorption spectrum of the photoreceptor triggering MAA formation. On the contrary, the broader the light spectrum under which algae were grown the more MAAs were accumulated (see Fig. 5, exception short-wavelength UV-B ("unfiltered"). Hence, a combination of both

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calculated polychromatic response spectra might be a better fit for the absorption characteristics of the photoreceptor responsible for MAA induction. This unknown photoreceptor must therefore be able to absorb blue light, UV-A and UV-B, indicating that one or two chromophores or even photoreceptors interact to trigger MAA synthesis (see also Franklin et al. 2001).

So far, two molecules have been suggested as photoreceptor chromphore. Portwich and Garcia-Pichel (2000) suggested a reduced pterin (tetrahydroform) as photoreceptor molecule, because of the similarity between the conducted action spectrum and the absorption spectrum of pterins as well as the inhibitory effect on MAA synthesis due to an inhibitor of the biosynthetic pathway of pterins and an antagonist of pterin excite states. As the action spectrum is only based on a wavelengths between 290 and 340 nm and because the authors failed to check the effect of the used inhibitors on the target molecule, this proposal should be considered carefully.

An other candidate as the responsible photoreceptor might be a flavin-based cryptochrome, because the induction of shinorine is boosted if blue light and UV radiation interact (Franklin et al. 2001), similar to the photoregulation of flavin synthesis (Christie and Jenkins 1996, Fugelevand 1996).

Nevertheless, a carefully constructed action spectrum (monochromatic irradiance) will be necessary to identify the absorption characteristics of the target chromophore (Coohill 1991).

Based on our results, long wavelength UV-B has by far the highest quantum efficiency for MAA accumulation, followed by UV-A and blue light. The assumed interconversion of MAAs with shinorine as precursor (also proposed by Franklin et al. 1999, Shick et al. 2000) may be light regulated, with UV-A and blue light inducing and UV-B inhibiting presupposed enzymes catalysing the interconversion.

Until now, we kept the MAA concentration accumulated in specimens exposed unfiltered to the full solar radiation out of the consideration. These specimens accumulated significant lower MAA concentrations than those exposed under the filter WG305, which would result in a pronounced negative effect of short wavelength UV-B. This is in line with our previous results. Specimens exposed in a similar experimental set up under the filter WG305 exhibited a significantly lower MAA concentration than those under the filter WG320 (Kräbs et al. 2002). Therefore, we can rule out that differences in MAA concentration are caused by different temperatures, as assumed for Fv/Fm values. On the contrary, they seemed to be a response to UV-B radiation.

Because we could show that pigment induction is similar in all algae irrespective of filter treatment, we know that this is not due to an UV-B induced inhibition of algal metabolism. Therefore, the negative effect of short-wavelength UV-B on MAA formation may indicate an inhibitory effect of UV-B radiation on the biosynthetic pathway of MAA formation.

Differences of these results and previous data (Kräbs et al. 2002) are mainly due to the lower irradiance during this experiment (1-3 d ~ 75 %, 4-9 d ~40 % of the previous data). The depression of optimal quantum yield is due to higher irradiance during the first day (0 d) and the lack of a stepwise acclimation as we have done during the previous experiment. Optimal quantum yield and maximal electron transport rate recover when the lutein concentration has reached the steady state, indicating that lutein plays an important role during acclimation to high irradiance (PAR). Higher electron transport rates may be due to increased chlorophyll a concentrations.

The generally lower MAA concentrations in the present data set reflect the lower irradiance during the experiment. Even though UV-B radiation had an effect on maximal quantum yield, we could demonstrate that this is not an unequivocal sign of an impaired metabolism. Thus, pigment synthesis was unaffected by UV-B radiation, suggesting that a decline in MAA concentration is neither due to missing energy equivalents nor to an impaired metabolism, but to an UV-B induced inhibition of MAA formation. Nevertheless, in all treatments internal MAA concentration was sufficient to protect specimens against UV radiation.

References

ASADA K. & TAKAHASHI M. 1987. Production and scavenging of active oxygen species in photosynthesis. In: *Photoinhibition. Topics in photosynthesis* (Ed. by D.J. Kyle, C.B. Osmond & C.D. Arntzen), pp. 89-109. Elsvier Science Publisher, Amsterdam.

BANDARANAYAKE W.M. 1998. Mycosporines: are they nature's sunscreens? *Natural Product Report* 15: 159-172.

BISCHOF K., HANELT D. & WIENCKE C. 1998a. UV-radiation can affect depth-zonation of Antarctic macroalgae. *Marine Biology* 131: 597-605.

BISCHOF K., HANELT D., TÜG H., KARSTEN U., BROUWER P. E. M. & WIENCKE C. 1998b. Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitzbergen, Norway). *Polar Biology* 20: 388-395.

BISCHOF K., HANELT D. & WIENCKE C. 1999. Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biology* 1: 435-444.

BISCHOF K., HANELT D. & WIENCKE C. 2000a. Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. *Planta* 211: 555-562.

BISCHOF K., KRÄBS G., HANELT D. & WIENCKE C. 2000b. Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline? *Helgoland Marine Research* 54: 47-52.

BISCHOF K., KRÄBS G., WIENCKE C. & HANELT D. 2002a. Solar ultraviolet radiation affects the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L.. *Planta* 215: 502-509.

BISCHOF K., PERALTA G., KRÄBS G., POLL W.H. VAN DE, PÉREZ-LLORÉNS J. & BREEMAN A.M. 2002b. Effects of solar UV-B radiation on canopy structure of *Ulva* communities from southern Spain. *Journal of Experimental Botany* 53: 2411-2421.

CALDWELL M.M. & FLINT S.D. 1997. Uses of biological spectral weighting functions and the need of scaling for the ozone reduction problem. *Plant Ecology* 128: 66-76.

CARRETO J.I., LUTZ V.A., MARCO S.G. DE & CARIGNAN M.O. 1990. Fluence and wavelength dependance of mycosporine-like amino acids synthesis in the dinoflagellate *Alexandrium excavatum*. In: *Toxic Marine Phytoplankton*. (Ed. by E. Garneli, B. Sunström, L. Edler & D.M. Anderson), pp. 275-279. Elsevier, Amsterdam.

CHRISTIE J.M. & JENKINS G.J. 1996. Distinct UV-B and UV-A/blue light transduction pathway induce chalcone synthase gene expression in *Arabidopsis* cells. *Plant Cell* 8: 1555-1567.

COLLÉN J. & DAVISON I.R. 1999. Stress tolerance and reactive oxygen metabolism in the intertidal red seaweeds *Mastocarpus stellatus* and *Chondrus crispus*. *Plant Cell Environment* 22: 1143-1151.

COOHILL T.P. 1991. Action spectra again? *Photochemistry and Photobiology* 54: 859-870.

COOHILL T.P. 1992. Action spectra revisited. *Journal of Photochemistry and Photobiology B: Biology* 13: 95-100.

COOHILL T.P. 1994. Exposure response curves action spectra and amplification factors. In *Stratospheric Ozone Depletion/UV-B Radiation in the Biosphere* (Ed. by R.H. Biggs & M.E.B. Joyner), vol. 108, pp. 57-62. NATO ASI Series, Springer Berlin.

FRANKLIN L. A, YAKOVLEVA I.; KARSTEN U. & LÜNING K. 1999. Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae). *Journal of Phycology* 35: 682-693.

FRANKLIN L. A., KRÄBS G. & KUHLENKAMP R. 2001. Blue light controls the synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae). *Journal of Phycology* 37: 257-270.

FUGLEVAND G., JACKSON J. A. & JENKINS G. I. 1996. UV-B, UV-A, and blue light signal transduction pathway interact synergistically to regulate chalcone synthase gene expression in *Arabidopsis*. *Plant Cell* 8: 2347-2357.

GARCIA-PICHEL F., WINGARD C. E. & CASTENHOLZ R. W. 1993. Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacterium *Gloeocapsa* sp. *Applied Environmental Microbiology* 59: 170-176.

HANELT D. 1998. Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Marine Biology* 131: 361-369.

HANELT D., WIENCKE C., KARSTEN U. & NULTSCH W. 1997. Photoinhibition and recovery after high light stress in different development stages of *Laminaria saccharina* (Phaeophyta). *Journal of Phycology* 33: 387-395.

HARKER M., BERKALOFF C., LEMOINE Y., BRITTON G., YOUNG A.J., DUVAL J.C., RMIKI N.E. & ROUSSEAU B. 1999. Effects of high light and desiccation on the operation of the xanthophylls cycle in two marine brown algae. *European Journal of Phycology* 34: 35-42.

KARSTEN U., FRANKLIN L.A., LÜNING K. & WIENCKE C. 1998. Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga *Chondrus crispus*. *Planta* 205: 257-262.

KARSTEN U., BISCHOF K. & WIENCKE C. 2001. Photosynthetic performance of Arctic macroalgae after transplantation from deep to shallow waters. *Oecologia* 127: 11-20.

KRÄBS G., BISCHOF K., HANELT D., KARSTEN U. & WIENCKE C. 2002. Wavelength-dependent induction of UV-absorbing mycosporine-like amino acids in the red alga *Chondrus crispus* under natural solar radiation. *Journal of Experimental Marine Biology and Ecology* 268: 69-82.

KÜBLER J.E. & DAVISON I.R. 1993. High temperature tolerance of photosynthesis in the red alga *Chondrus crispus*. *Marine Biology* 117: 327-335.

KÜBLER J.E. & DAVISON I.R. 1995. Thermal acclimation of light-use characteristics of *Chondrus crispus* (Rhodophyta). *European Journal of Phycology* 30: 189-195.

LESSER M.P. 1996. Acclimation of phytoplankton to UV-B radiation: oxidative stress and photoinhibition of photosynthesis are not prevented by UV-absorbing compounds in the dinoflagellate *Prorodentrum micans*. *Marine Ecology Progress Series* 132: 287-297.

LÜNING K. 1990. *Seaweeds*. Wiley, New York. 527pp.

LOZÁN J.L. & KAUSCH H. 1998. *Angewandte Statistik für Naturwissenschaftler*. Parey, Berlin. 287 pp.

NIYOGI K.K., BJÖRKMAN O. & GROSSMAN A.R. 1997. The roles of specific xanthophylls in photoprotection. *Proceedings of the National Academy of Sciences. USA* 94: 14162-14167.

POGSON B.J., NIYOGI K.K., BJÖRKMAN O. & DELLAPENNA D. 1998. Altered xanthophyll composition adversely affect chlorophyll accumulation and nonphotochemical quenching in *Arabidopsis* mutants. *Proceedings of the National Academy of Sciences. USA* 95: 13324-13329.

POLL W.H. VAN DE, EGGERT A., BUMA A.G.I. & BREEMAN A.M. 2001. Effects of UV-B-induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-releted differences in UV-B tolerance. *Journal of Phycology* 37: 30-37.

POLL W.H. VAN DE, EGGERT A., BUMA A.G.I & BREEMAN A.M. 2002. Temperature-dependence of UV radiation effects in Arctic and temperate isolates of three red macrophytes. *European Journal of Phycology* 37: 59-68.

PORTWICH A. & GARCIA-PICHEL F. 2000. A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912. *Photochemistry and Photobiology* 71: 493-498.

RIEGGER L. & ROBINSON D. 1997. Photoinduction of UV-absorbing compounds in Antarctic diatoms and *Phaeocystis antarctica*. *Marine Ecology Progress Series* 160: 13-25.

RUNDEL RD. 1983. Action spectra and estimation of biologically effective UV radiation. *Physiologia Plantarum* 58: 360-366.

SAGERT S., FORSTER R.M., FEUERPFEIL P. & SCHUBERT H. 1997. Daily course of photosynthesis and photoinhibition in *Chondrus crispus* (Rhodophyta) from different shore levels. *European Journal of Phycology* 32: 363-371.

SCHOFIELD O., EVENS T.J. & MILLIE D.F. 1998. Photosystem II quantum yields and xanthophylls-cycle pigments of the macroalga *Saragassum natans* (Phaeophyceae): responses under natural sunlight. *Journal of Phycology* 34: 104-112.

SHICK J.M. & DUNLAP W.C. 2002. Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annual Reviews of Physiology* 64: 223-262.

SHICK J.M., DUNLAP W.C. & BUETTNER G.R. 2000. Ultraviolet (UV) protection in marine organisms II. Biosynthesis accumulation, and sunscreening function of mycosporine-like amino acids. In *Free Radicals in Chemistry, Biology and Medicine* (Ed. by T. Yoshikawa, S. Tonokuni, Y. Yamamoto & Y. Naito), pp. 215-228. OICA International, London.

VERSHININ A. O & KAMNEV A.N. 1996. Xanthophyll cycle in marine macroalgae. *Botanica Marina* 39: 421-425.

WILSON K.E, KRÓL M. & HUNER N.P. 2003. Temperature-induced greening of *Chlorella vulgaris*. The role of the cellular energy balance and zeaxanthin-dependent nonphotochemical quenching. *Planta* 217: 616-627.

YAKOVLEVA I.M & TITLYANOV E.A. 2001. Effects of high visible and UV irradiance on subtidal *Chondrus crispus*: stress, photoinhibition and protective mechanisms. *Aquatic Botany* 71: 47-61.

Polychromatic response spectra for the accumulation of UV-absorbing mycosporine-like amino acids in the red alga *Chondrus crispus* Stackh.

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Abstract

Under laboratory conditions the red alga *Chondrus crispus* synthesises up to three different mycosporine-like amino acids (MAAs): shinorine, palythine and asterina-330. By exposing specimens under a set of various cut-off filters to three different irradiances emitted by a Philips MSR 400 HR lamp emitting a solar-like spectrum, we calculated polychromatic response spectra for MAA accumulation under the full light spectrum and UV radiation. We were able to show that reciprocity for shinorine, palythine and asterina-330 accumulation holds during the first days, but fails as soon as a steady state is reached, indicating that there is a specific maximal internal MAA concentration for given radiation conditions. Furthermore, we found a strong indication of a precursor relationship between shinorine and palythine, whereby the shinorine concentration decreased at the same rate as the concentration of palythine increased in algae transferred from UV radiation to the full light spectrum. The highest quantum efficiency for MAA formation was observed for UV-A radiation in all calculated polychromatic response spectra. Our results support the proposal that the MAAs of the studied species are induced through a UV-A photoreceptor, with an as yet unknown photoreceptor molecule. The absorption maximum of this molecule is also unknown, because both absorption characteristics of the photoreceptor molecule and harmful/inhibitory effects of UV-B radiation influence the shape of the calculated response spectra. Beside the UV-A response peak, palythine response spectra also have a maximum in the blue light region, pointing to a second photoreceptor and the wavelengths responsible for shinorine interconversion to palythine. The results

demonstrate the capacity of the alga to acclimate the internal MAA concentration to spectrally different radiation conditions.

Introduction

Mycosporine-like amino acids (MAAs), a class of approximately 19 compounds, are chemical derivates of a cyclohexenone or cyclohexenine chromophore conjugated with one or two amino acids and/or amino alcohols (for overview see Bandaranayake 1998, Shick et al. 2000, Shick and Dunlap 2002 and references therein). The biosynthetic pathway is not conclusively known, but a study of MAA synthesis in coral, and another in cyanobacteria indicate that synthesis proceeds via early steps of the shikimate pathway (Shick et al. 1999, Portwich and Garcia-Pichel 2003).

Because of their absorption maxima between 310 and 360 nm, it has been proposed that MAAs protect organisms against UV radiation induced damage (e.g. Dunlap et al. 1986, Dunlap and Shick 1998, Conde et al. 2000), although the degree of protection varies (Garcia-Pichel et al. 1993, Lesser 1996, Franklin et al. 1999). Recent results of Misonou and co-workers (2003) indicate that this protection acts not only on photosynthesis, but that MAAs also protect DNA molecules by quenching the excited thymine residue.

MAAs occur in a number of taxonomically diverse organisms, particularly corals (Dunlap et al. 1986, Dunlap and Shick 1998), microalgae (Jeffrey et al. 1999) and rhodophyte algae (Sivalingam et al. 1974, Karentz et al. 1991, Karsten et al. 1998b, Hoyer et al 2001). The synthesis of MAAs is dependent both on the quality and quantity/duration of radiation applied (Carreto et al. 1990, Riegger and Robinson 1997, Karsten et al. 1998, Franklin et al. 2001, Gröninger and Häder 2002, Kräbs et al. 2002).

The photoreceptor responsible for the signal transduction initialising MAA formation is as yet, unknown. A carefully measured action spectrum for monochromatic light is necessary to identify the absorption characteristics of the target chromophore responsible for MAA synthesis (Schäfer and Fukshansky 1984, Coohill 1990). However, this approach is highly artificial.

An alternative is the calculation of a polychromatic response spectrum, found by exposing specimens to polychromatic light using a set of different cut-off filters.

Polychromatic response spectra are closer to natural conditions and combine in the measured response natural repair and mitigating mechanisms (Coohill 1994), but they tend to obscure individual chromophores (Coohill 1992) and therefore can not be applied to answer conclusively the question of the absorption spectrum of the photoreceptor involved (Coohill 1994).

Thus action spectra and polychromatic response spectra have their advantages and limitations, which have to be considered when discussing data.

In recent years many authors have investigated the wavelength dependent induction of MAA biosynthesis. With one exception (Portwich and Garcia-Pichel 2000), authors used polychromatic irradiance for their experiments (Carreto et al. 1990, Riegger and Robinson 1997, Gröniger and Häder 2002, Kräbs et al. 2002, Sinha et al. 2002), revealing a diverse response of MAA formation due to different wavebands. Using the same polychromatic experimental set up, Riegger and Robinson (1997) demonstrated that wavelengths between 370 and 460 nm exhibit the highest quantum efficiency for MAA formation in two Antarctic diatoms, while the prymnesiophyte *Phaeocystis antarctica* showed a response maximum at around 345 nm. UV-B radiation was most effective for MAA accumulation in two cyanobacteria and the green alga *Prasiola stipitata* (Portwich and Garcia-Pichel 2000, Gröniger and Häder 2002, Sinha et al. 2002) In addition to UV radiation, blue light induces MAA accumulation in the dinoflagellate *Alexandria excavatum* (Carreto et al. 1990) and the red alga *Chondrus crispus* (Franklin et al. 2001). It has been proposed that a photoreceptor molecule is responsible for triggering MAA formation (Shick et al. 2000, Portwich and Garcia-Pichel 2000, Franklin et al. 2001). However, information on both the nature of the regulating mechanism of MAA formation and the putative photoreceptor is limited and raises the question of the general distribution of these characteristics within the algal classes.

This study aimed to obtain more information about the wavelength dependence of MAA formation. Thalli of *C. crispus* were exposed to three different irradiances covered with different cut-off filters. Polychromatic response spectra were calculated according to Rundel (1983), weighting the difference in MAA formation with the difference in radiation applied. Because MAA induction in *Chondrus crispus* is triggered by UV-B, UV-A and blue light (Karsten et al. 1998a, Franklin et al. 2001, Kräbs et al. 2002), we were able to use one organism to test all known wavebands effective for MAA synthesis.

Material and Methods

Algal material and experimental conditions

Thalli of *C. crispus*, originally isolated from Helgoland (German Bight, North Sea; 54°11'N, 7°53'E) were cultivated in glass beakers in temperature-controlled rooms (15 ± 1°C, light-dark-cycle 16:8, 30 μmol m^{-2} s^{-1} PAR) until they reached 2,5 to 3 cm in size. Provasoli enriched North Sea water was used as the culture medium (McLachlan 1973).

Experimental conditions

In the first experiment, thalli were grown under continuous light of "high", "medium" and "low" irradiance (Tab. 1) for seven days, covered with nine different cut-off filters (WG280, WG305, WG320, WG335, WG345, WG375, GG400, GG420 and GG495, with 50 % transparency at the given wavelength; Schott, Mainz, Germany; Fig. 1A). Samples for MAA analysis and Fv/Fm measurements were taken at the beginning and after 1, 2, 4 and 7 days.

In the second experiment, thalli were exposed to a light-dark cycle of 16:8 h of "high" irradiance for seven days. The same filter set up which was used under continuous light was applied and the same sampling times were used. Additionally, samples for pigment analysis were taken after 7 days.

The experimental set up for the third experiment was the same as in the second experiment. In addition to the filters WG280 to GG400, algae were covered with the band path filter UG5 (transparent for UV radiation and wavelengths above 650 nm; Schott, Mainz, Germany). Samples for MAA analysis and Fv/Fm measurements were taken at the beginning and after 2, 4 and 7 days.

In the fourth experiment, algae were loaded with MAAs by exposing them under a combination of both a UG5 and a WG320 filter to "high" continuous irradiance for three days. The initial filter combination was then removed and sub samples of thalli were covered with the filters WG280 to GG420, respectively. Samples for MAA analysis and Fv/Fm measurements were taken 0 and 1, 2, 3 and 4 days after the removal of the initial filters.

The light source for all experiments was a 400 W metalogen lamp (Philips MSR 400 HR).
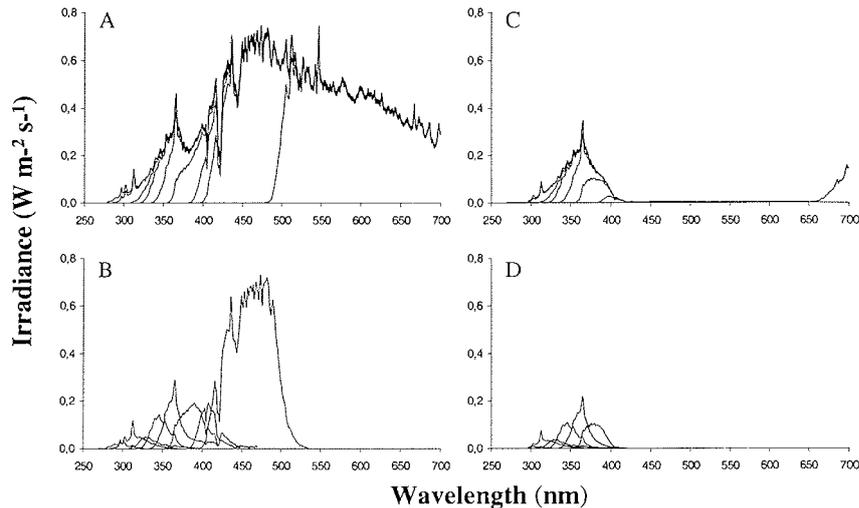
Figure 1: Spectral irradiances under the used cut-off filters during the experiment with "high" UV+PAR (A) and "high" UV (C) irradiance (see also Tab. 1); the spectra from left to right are WG280, WG305, WG320, WG335, WG345, WG375, GG400, GG420 and GG495; for the UV treatment (C) a UG5 was additionally placed over the filters WG280 to GG400; (B, D) Difference spectra generated by subtracting irradiance under two sequential cut-off filters; for median wavelength see Tab.3.

Light measurements

The light spectra of "high", "medium" and "low" irradiance (Tab. 1, Fig. 1A, C) were recorded by a fast scanning double monochromator spectroradiometer (UV320D, Instruments Systems, Munich, Germany) equipped with a cosine sensor.

Biological variables

During exposure, maximal quantum yield of photosystem II electron transport of dark adapted algae (4 min) was determined by the ratio of variable to maximal chlorophyll fluorescence (Fv/Fm) with a pulse amplitude modulated chlorophyll fluorometer (PAM 2000, Walz, Effeltrich, Germany), following the protocol described in detail by Hanelt (1998). Measurements of Fv/Fm were conducted in triplicates from randomly collected samples.

Changes in the pigment (chlorophyll a, lutein, α- + β-carotene, zeaxanthin) composition and content were analysed as described by Bischof et al. (2002a). Pigment data were obtained from independent triplicate samples.

Changes in MAA composition and content were analysed as described by Kräbs et al. (2002) with the modification of the mobile phase to 5 % aqueous methanol (v/v) plus 0,1 % acetic acid (v/v) in water. MAA data were obtained from 4-5 independent samples.

Table 1: Spectral irradiance under the four experimental conditions and the used cu-off filters

		UV B $W\ (m^{-2}\ s)$	UV A $W\ (m^{-2}\ s)$	PAR $W\ (m^{-2}\ s)$
High	WG280	1,49	18,42	142,5
	WG305	0,95	18,79	145,8
	WG320	0,03	17,65	145,8
	WG335		16,09	145,1
	WG345		12,32	145,6
	WG375		6,41	142,2
	GG400		0,92	141,7
	GG420		0,01	136,7
	GG495			89,30
Medium	WG280	0,57	7,03	54,64
	WG305	0,35	7,02	54,83
	WG320	0,01	6,57	54,66
	WG335		5,94	54,31
	WG345		4,52	54,31
	WG375		2,43	54,33
	GG400		0,34	52,41
	GG420			52,32
	GG495			33,43
Low	WG280	0,23	3,03	24,15
	WG305	0,14	3,04	24,31
	WG320		2,80	23,86
	WG335		2,63	24,34
	WG345		1,98	24,07
	WG375		1,08	24,26
	GG400		0,16	23,71
	GG420			23,21
	GG495			15,23
UV – High	WG280	0,57	11,92	3,6
	WG305	0,42	12,15	3,7
	WG320	0,02	11,32	3,7
	WG335		10,20	3,6
	WG345		7,34	3,7
	WG375		3,16	3,6
	GG400		0,25	3,5

Table 2: Equations fitted to data points to model the pattern of shinorine, palythine and asterina-330 accumulation (x = time (d)), as well as MW ± SD of the steady state in total MAA concentration

		Shinorine	Palythine	Asterina-330	Total mg g^{-1} DW
Low continuous light	WG280	$-0,0024\ x^2 + 0,0175\ x$	$0,0216\ x$	$0,0028\ x$	$0,113 \pm 0,118$
	WG305	$-0,0065\ x^2 + 0,00457\ x$	$0,0167\ x$	$0,0034\ x$	$0,150 \pm 0,088$
	WG320	$-0,0046\ x^2 + 0,0367\ x$	$0,0193\ x$	$0,0062\ x$	$0,205 \pm 0,114$
	WG335	$-0,006\ x^2 + 0,0429\ x$	$0,0337\ x$	$0,0086\ x$	$0,199 \pm 0,132$
	WG345	$-0,002\ x^2 + 0,0165\ x$	$0,008\ x$	$0,0025\ x$	$0,084 \pm 0,109$
	WG375	$-0,0023\ x^2 + 0,0168\ x$	$0,0029\ x$	$0,0006\ x$	$0,039 \pm 0,043$
	GG400	$-0,0008\ x^2 + 0,0059\ x$	$0,0043\ x$	$0,0011\ x$	$0,012 \pm 0,005$
	GG420	$-0,0001\ x^2 + 0,0072\ x$	$0,0091\ x$	$0,0025\ x$	$0,111 \pm 0,118$
	GG495	$-0,0009\ x^2 + 0,0065\ x$	$0,0087\ x$	$0,0023$	$0,017 \pm 0,015$
Medium continuous light	WG280	$-0,018\ x^2 + 0,0734\ x$	$0,0485\ x$	$0,0157\ x$	$0,249 \pm 0,073$
	WG305	$-0,0202\ x^2 + 0,1475\ x$	$0,0661\ x$	$0,0127\ x$	$0,636 \pm 0,481$
	WG320	$-0,0163\ x^2 + 0,1207\ x$	$0,0605\ x$	$0,0129\ x$	$0,544 \pm 0,182$
	WG335	$-0,0199\ x^2 + 0,1494\ x$	$0,0648\ x$	$0,0206\ x$	$0,612 \pm 0,275$
	WG345	$-0,0259\ x^2 + 0,1121\ x$	$0,0242\ x$	$0,008\ x$	$0,126 \pm 0,075$
	WG375	$-0,0099\ x^2 + 0,0746\ x$	$0,0384\ x$	$0,0102\ x$	$0,345 \pm 0,114$
	GG400	$-0,001\ x^2 + 0,0088\ x$	$0,0162\ x$	$0,0038\ x$	$0,166 \pm 0,087$
	GG420	$0,0249\ x^2 + 0,1038\ x$	$0,0312\ x$	$0,0104\ x$	$0,404 \pm 0,241$
	GG495	$0,0003\ x^2 + 0,0045\ x$	$0,0117\ x$	$0,002\ x$	$0,134 \pm 0,073$
High continuous light	WG280	$-0,0404\ x^2 + 0,1644\ x$	$0,0582\ x$	$0,0069\ x$	$0,274 \pm 0,128$
	WG305	$-0,0198\ x^2 + 0,1413\ x$	$0,1551\ x$	$0,0227\ x$	$1,082 \pm 0,448$
	WG320	$-0,0475\ x^2 + 0,3509\ x$	$0,1949\ x$	$0,0527\ x$	$1,852 \pm 0,286$
	WG335	$-0,0371\ x^2 + 0,2785\ x$	$0,1777\ x$	$0,0372\ x$	$1,637 \pm 0,420$
	WG345	$-0,0406\ x^2 + 0,02959\ x$	$0,1236\ x$	$0,0335\ x$	$1,254 \pm 0,618$
	WG375	$-0,0265\ x^2 + 0,1971\ x$	$0,1451\ x$	$0,0265\ x$	$1,197 \pm 0,413$
	GG400	$-0,0194\ x^2 + 0,1377\ x$	$0,1168\ x$	$0,0229\ x$	$0,551 \pm 0,310$
	GG420	$-0,0271\ x^2 + 0,1961\ x$	$0,0975\ x$	$0,0226\ x$	$0,762 \pm 0,305$
	GG495	$-0,0101\ x^2 + 0,111\ x$	$0,0182\ x$	$0,0038\ x$	$0,369 \pm 0,155$
High light/dark cycle	WG280	$-0,0329\ x^2 + 0,2273\ x$	$0,1223\ x$	$0,0143\ x$	$0,975 \pm 0,255$
	WG305	$-0,04606\ x^2 + 0,2883\ x$	$0,1597\ x$	$0,013\ x$	$1,330 \pm 0,377$
	WG320	$-0,0345\ x^2 + 0,2529\ x$	$0,1131\ x$	$0,016\ x$	$1,065 \pm 0,402$
	WG335	$-0,0358\ x^2 + 0,2505\ x$	$0,1467\ x$	$0,0182\ x$	$1,125 \pm 0,214$
	WG345	$-0,0072\ x^2 + 0,0583\ x$	$0,0992\ x$	$0,0207\ x$	$0,898 \pm 0,365$
	WG360	$-0,0207\ x^2 + 0,15\ x$	$0,1128\ x$	$0,0098\ x$	$0,986 \pm 0,328$
	GG400	$-0,0138\ x^2 + 0,1082\ x$	$0,0921\ x$	$0,0155\ x$	$0,854 \pm 0,118$
	GG420	$-0,0328\ x^2 + 0,2347\ x$	$0,1238\ x$	$0,0149\ x$	$1,051 \pm 0,244$
	GG495	$0,0034\ x^2 + 0,2032\ x$	$0,0391\ x$	$0,002\ x$	$1,921 \pm 1,036$

Polychromatic response spectra

Polychromatic response spectra were calculated from the respective MAA concentrations accumulated in *C. crispus* grown under different filter conditions. First, second order polynomial (shinorine) and linear (palythine, asterina-330) equations were fitted through the measured data points for the respective MAA concentration accumulated in algae exposed under each filter. Using the equations obtained (Tab. 2) a "theoretical" concentration of the respective MAA was calculated for the second (shinorine) and fourth day (palythine, asterina-330) of the experiment. A mean value was calculated and used for a group of filter treatments with no statistically significant difference in MAA concentration, while the individually calculated value was used directly if the pattern in MAA accumulation under the filter in question was significantly different from neighbouring filter treatments (Tab. 3). To calculate the polychromatic response spectrum for the total MAA concentration, the mean value for the steady state was calculated. Statistically significant differences between different filter treatments were taken into consideration as described above.

Response spectra were calculated with this statistically adjusted data set: the difference in MAA concentration in algae grown under two sequentially numbered cut-off filters was divided by the difference in total irradiance between the two light fields beneath the filters (Tab. 4) according to Rundel (1983). All calculations of polychromatic response spectra are based on the median wavelength of the difference in total irradiance obtained by subtraction of two sequential light fields as described by Riegger and Robinson (1997).

Data treatment

Mean values and standard deviations were calculated from the respective replicates. Statistical significance ($p < 0.05$) of difference in MAA and pigment content accumulated under each cut-off filter and for different times as well as their combined effect was tested by two way analysis of variance (ANOVA) followed by Least Significant Difference-Test (LSD). Calculations were done using the program Statistica Kernel-Version 5.5A (StatSoft, Inc., Tulsa, OK, USA).

Table 3: Statistical significance of differences between two sequential cut-off filters; statistically significant difference (p<0,05) is marked with x; O = no statistical difference, but pronounced step between the response of algae of two filter groups, which is considered in the calculation dividing these filters into two filter groups

| | Continuous light | | | | | | | | | | | | Light/dark cycle | | | | | | | |
| | Low | | | | Medium | | | | High | | | | High | | | | UV – High | | | |
	Shinorine	Palythine	Asterina-330	Total	Shinorine	Palythine	Asterina-330	Total	Shinorine	Palythine	Asterina-330	Total	Shinorine	Palythine	Asterina-330	Total	Shinorine	Palythine	Asterina-330	Total
WG280/WG305	x				x	x		x	x	x	x	x					x			x
WG305/WG320									x	x	x	x		x						
WG320/WG335											x			x			x	x		x
WG335/WG345	x		x							x		x	x	x		x	x	x		x
WG345/WG375					x	x	x	x	O											
WG375/GG400								x		x		x					x			x
GG400/GG420			x																	
GG420/GG495			x				x		x	x		x	x	x		x				

Table 4: Irradiances and median wavelengths for the difference spectra generated by subtracting irradiance under two sequential cut-off filters

	High	Medium	Low	UV-High	Median-wavelength
		W m^{-2} s^{-1}			nm
WG280 – WG305	0,587	0,246	0,096	0,147	298
WG305 – WG320	2,076	0,794	0,353	1,264	316
WG320 – WG335	1,461	0,594	0,184	1,139	335
WG335 – WG345	3,775	1,425	0,647	2,859	347
WG345 – WG360	6,431	2,216	0,941	4,210	365
WG360 – GG400	7,481	3,220	1,347	3,024	389
GG400 – GG420	5,073	1,597	0,748	3,75	409
GG420 – GG495	48,747	18,725	8,187		462
GG495	89,300	33,430	15,230		495

Results

MAA formation under continuous light

Samples taken before the start of the exposure contained only traces of the MAA palythine. After thalli were transferred to the experimental conditions a substantial induction of MAAs was observed (Tab. 2, Fig. 2).

Shinorine was accumulated rapidly until the second or fourth day, depending on filter treatment. After that, the shinorine concentration declined, returning finally to the initial low levels. In contrast, palythine and asterina-330 were steadily accumulated throughout the experiment. Under most filter treatments a steady state was reached after the fourth day.

For each filter treatment a linear relationship exists between applied irradiance and the concentration of shinorine, palythine, and asterina-330 as well as with the total MAA concentration. Statistically significant differences became more pronounced with higher irradiances (Tab. 3).
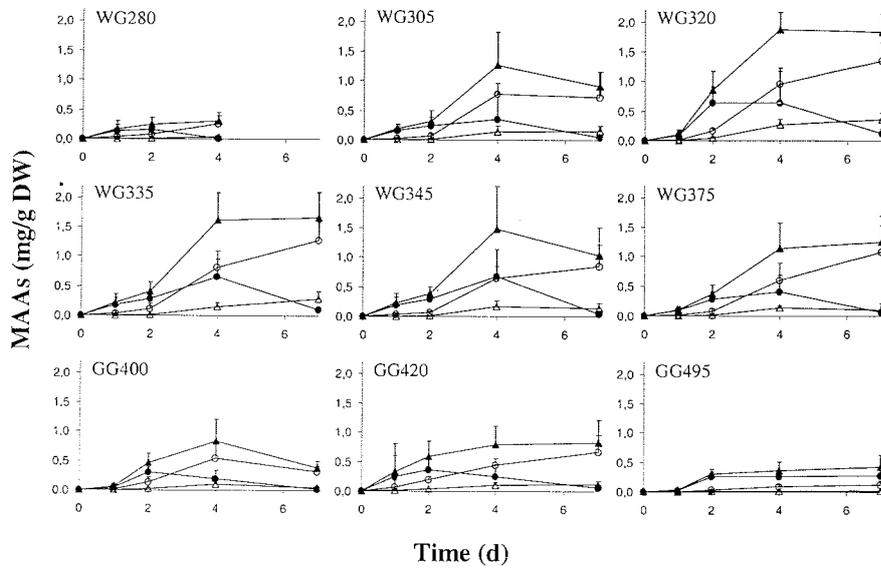

Time (d)

Figure 2: Time course of the variation of the concentration of shinorine (●), palythine (○) and asterina-330 (△) as well as of the total concentration of mycosporine like amino acids (▲) in *Chondrus crispus* exposed to continuous "high" radiation conditions; MW ± SD
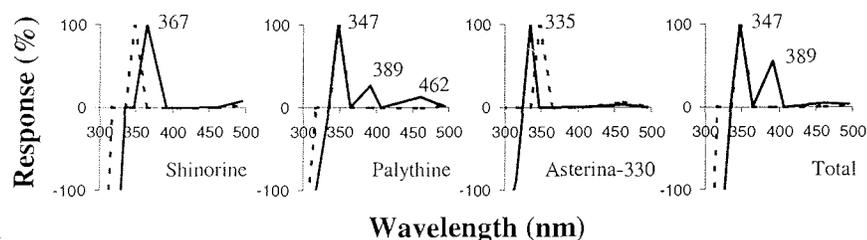
Figure 3: Polychromatic response spectra for the wavelength-dependent induction of mycosporine-like amino acids calculated for *Chondrus crispus* exposed under continuous "medium" (dotted line) and "high" (solid line) radiation conditions; numbers mark the maximum wavelength of the respective response peak of the response spectra calculated for MAA induction of algae exposed to "high" irradiance

Polychromatic response spectra revealed that UV-A radiation elicited the highest quantum efficiency for the induction of all MAAs, with maxima between 334 and 367 nm (Fig.3). The response spectra of palythine synthesis and of total MAA concentration have a second maximum at 389 nm.

Maximal quantum yield of photosynthesis (Fv/Fm) declined after the first day of exposure to higher light intensities and UV radiation and remained depressed throughout the experiment (Tab.5).

For algae exposed under the filter WG280 to "high" irradiances a visible bleaching of thalli could be observed. They were totally bleached after the fourth day. All other algae appeared to be normally pigmented throughout the experiment.

Table 5: Maximal quantum yield of photosynthesis (Fv/Fm) of thalli exposed for seven days under different cut-off filters and continuous light

	high	medium	low
Control	$0,503 \pm 0,054$	$0,539 \pm 0,039$	$0,534 \pm 0,016$
WG305	$0,297 \pm 0,042$	$0,291 \pm 0,109$	$0,456 \pm 0,034$
WG320	$0,329 \pm 0,072$	$0,423 \pm 0,070$	$0,538 \pm 0,052$
GG400	$0,325 \pm 0.048$	$0,504 \pm 0,057$	$0,513 \pm 0,052$
GG495	$0,318 \pm 0,118$	$0,523 \pm 0,032$	$0,481 \pm 0,011$

Figure 4: Time course of the variation of the concentration of shinorine (●), palythine (O) and asterina-330 (△) as well as of the total concentration of mycosporine like amino acids (▲) in *Chondrus crispus* exposed to a light-dark cycle of 16:8 h of "high" irradiance (UV+PAR); MW ± SD
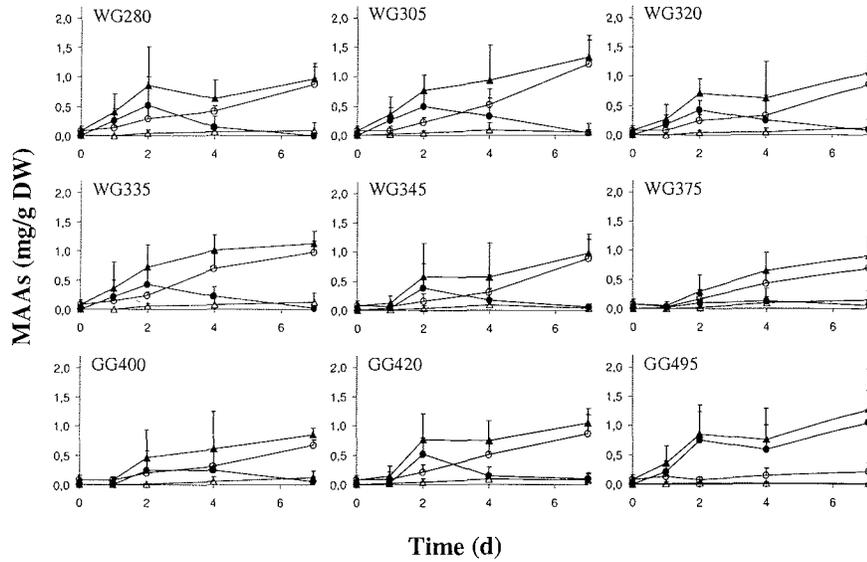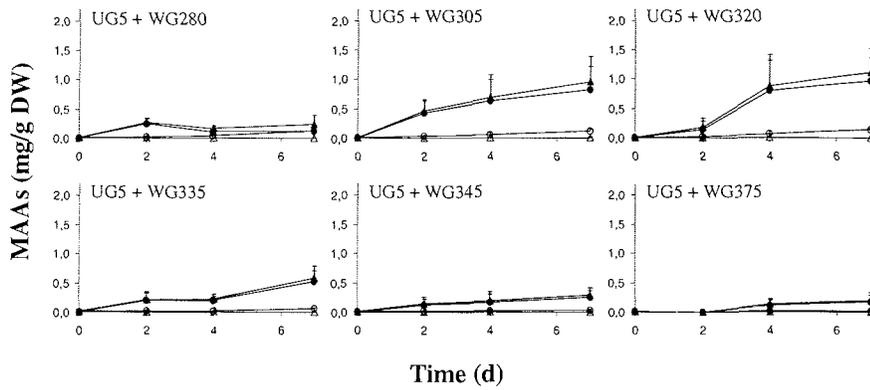

Figure 5: Time course of the variation of the concentration of shinorine (●), palythine (O) and asterina-330 (△) as well as the of total concentration of mycosporine like amino acids (▲) in *Chondrus crispus* exposed to a light-dark cycle of 16:8 h of "high" irradiance (UV); MW ± SD

MAA formation under a light-dark cycle

In specimens exposed to a light-dark cycle of "high" irradiance a similar accumulation pattern was found as in thalli exposed to continuous light (Fig. 4), while thalli exposed only to the UV band of the same light field, showed a different pattern (Fig. 5). Under UV radiation only, shinorine was also accumulated steadily throughout the experiment. It was the major MAA accumulated under these light conditions, while only low concentrations of palythine were synthesised. Asterina-330 was not induced throughout the experiment.

UV-A radiation elicited the highest quantum efficiency for shinorine and palythine accumulation as well as the total concentration of MAAs (Fig. 6), irrespective of the light conditions. Under the full light spectrum the accumulation of palythine had a second, slightly higher response peak with a maximum at 316 nm.
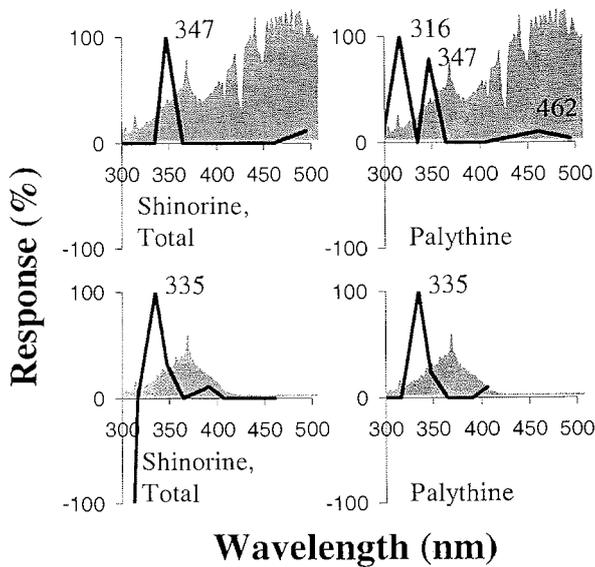

Figure 6: Polychromatic response spectra for the wavelength-dependent induction of mycosporine-like amino acids calculated for *Chondrus crispus* exposed under a light-dark cycle of 16:8 h of "high" light conditions; shaded areas represent the light field under which algae were exposed when covered with the filter WG280; numbers mark maximum wavelength of respective response peak

Maximal quantum yield of photosynthesis was inhibited by exposure to shorter wavelengths in algae exposed to the full light spectrum (Tab. 6). For all algae exposed under the full light spectrum Fv/Fm values were significantly lower than for specimens exposed to the UV band of the same spectrum.

The pigment composition and content of algae exposed for seven days to the full light spectrum was similar to the initial pigment concentration, with no significant differences between the different filter treatments (Tab. 7).

Table 6: Maximal quantum yield of photosynthesis (Fv/Fm) of thalli exposed for seven days under different cut-off filters and light-dark cycle of 16:8 h

	UV+PAR	UV
Control	0,561 ±0,029	0,545 ± 0,027
WG280	0,226 ± 0,092	0,556 ± 0,020
WG305	0,175 ± 0,062	0,570 ± 0,016
WG320	0,292 ± 0,027	0,576 ± 0,033
WG335	0,293 ± 0,073	0,578 ± 0,025
WG345	0,265 ± 0,056	0,624 ±0,033
WG375	0,255 ± 0,062	0,622 ± 0,016
GG400	0,276 ± 0,109	0,600 ± 0,037
GG420	0,393 ± 0,091	
GG495	0,378 ± 0,090	

Table 7: Content of chlorophyll a, lutein, zeaxanthin, α- and β-carotene, with respect to the filter treatment under which algae were exposed to a light-dark cycle of 16:8 h, MW ± SD

	Chlorophyll a	Lutein	Zeaxanthin mg g^{-1} FW	a-Carotene	b-Carotene
control	0,083 ± 0,051	0,020 ± 0,013	0,001 ± 0,001	0,002 ± 0,001	0,005 ± 0,003
WG280	0,129 ± 0,053	0,034 ± 0,013	0,003 ± 0,001	0,004 ± 0,002	0,008 ± 0,003
WG305	0,161 ± 0,077	0,037 ± 0,014	0,003 ± 0,001	0,005 ± 0,003	0,009 ± 0,004
WG320	0,106 ± 0,007	0,028 ± 0,001	0,002 ± 0,000	0,003 ± 0,000	0,006 ± 0,000
WG335	0,097 ± 0,048	0,025 ± 0,012	0,001 ± 0,001	0,002 ± 0,001	0,005 ± 0,003
WG345	0,116 ± 0,011	0,028 ± 0,000	0,002 ± 0,000	0,003 ± 0,000	0,006 ± 0,000
WG375	0,116 ± 0,047	0,025 ± 0,011	0,002 ± 0,001	0,003 ± 0,001	0,005 ± 0,002
GG400	0,103 ± 0,022	0,024 ± 0,007	0,002 ± 0,000	0,003 ± 0,001	0,005 ± 0,001
GG420	0,091 ± 0,014	0,018 ± 0,003	0,001 ± 0,000	0,003 ± 0,000	0,004 ± 0,001
GG495	0,082 ± 0,029	0,016 ± 0,005	0,001 ± 0,001	0,003 ± 0,001	0,003 ± 0,001

Interconversion of MAAs

For specimens loaded with MAAs, an interconversion of shinorine to palythine was observed, with a similar pattern for all filter treatment. For example, figure 7 shows the time course of interconversion for algae exposed under the filter GG400. Approximately two days after thalli were covered with different cut-off filters, the shinorine concentration declined steadily, while the concentration of palythine increased by a similar amount. Nevertheless, the total concentration of MAAs changed for some filter treatments, leading to significantly lower MAA concentration in algae exposed under the filter WG280 (Fig. 8).

Throughout the experiment Fv/Fm values were between 0.48 and 0.5.

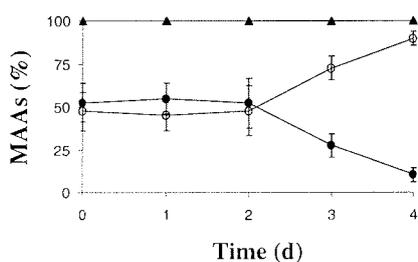Figure 7: Interconversion of UV induced shinorine (●) to palythine (○), while thalli of *Chondrus crispus* were exposed to PAR (GG400); ▲ = total MAA concentration
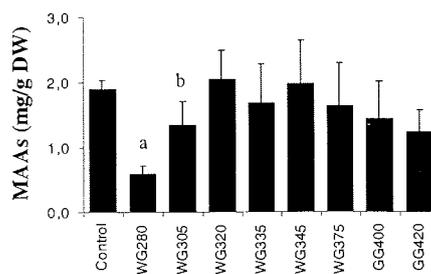
Figure 8: Final MAA concentration in *Chondrus crispus* exposed under different cut-off filters, after MAA induction under UV light; a = significant difference from the control and all other filters; b = significant difference from WG320

Discussion

Our results show that reciprocity between irradiance and specific as well as total MAA concentration in algae exposed under any specific cut-off filter, holds during the first days, but fails as soon as the steady state is reached. These results indicate that not only the light intensity, but also the duration of exposure determines the maximal internal MAA concentration. Beside this common response, the wavelength dependent accumulation varies for specific MAAs, revealing different wavebands to be most effective for the accumulation of a specific MAA. The polychromatic response spectra calculated for the total MAA concentrations is either a mixture of the calculated response spectra for shinorine, palythine and asterina-330 (Fig. 3; continuous light) or compares to that of shinorine (Fig. 6; light-dark cycle).

The common feature of polychromatic response spectra calculated for MAA accumulation in algae exposed to continuous irradiance are the main response maxima in the UV-A band and a pronounced negative effect of UV-B radiation. Under continuous light the negative response to UV-B radiation can mostly be explained by the harmful effects of these wavelengths, which induce damage to DNA, RNA and proteins (Vincent and Neale 2000, van de Poll et al. 2001, 2002) and inhibits photosynthesis, growth and reproduction (Wiencke et al. 2000, Kuhlenkamp et al. 2001, Bischof et al. 2002a, b). A reduction in growth rate due to UV-B radiation was shown in *C. crispus* (Franklin et al. 1999, van de Poll et al. 2003). Additionally, high UV-B irradiance lead to an accumulation of cyclobutane-pyrimidine dimers in *C. crispus*, although the authors regard these results cautiously due to the low PAR levels during the experiment (van de Poll et al. 2003). Under "high" irradiance algae exposed under the filter WG280 bleached completely, revealing severe damage to algal metabolism. Algae exposed under the filter WG305 may also have suffered a depressed metabolism, but appeared to be normally pigmented. As polychromatic response spectra calculated for MAA accumulation in algae exposed to "medium" irradiance had a similar shape as those calculated for algae exposed to "high" irradiance, there may also be some other responses responsible for the negative effect of UV-B radiation. Beside the indirect effect based on insufficient energy equivalents a direct inhibitory effect on MAA biosynthesis may be involved.

The main maximum in the UV-A band present in all response spectra indicates the wavelengths with the highest quantum efficiency for MAA formation. The shift in the

response maximum may be caused by the experimental set up. In addition to the main maximum, response spectra for palythine and total MAA concentration reveal other wavebands with high quantum efficiency MAA formation.

According to Coohill (1992, 1994), polychromatic response spectra tend to obscure the photoreceptor molecule and can not conclusively reveal what the respective absorption spectrum would look like. This is the case with the present data. Because shinorine accumulation is already present in algae exposed to PAR without the major part of blue light (GG495), the respective polychromatic response spectra can not reflect the absorption spectrum of the photoreceptor triggering MAA synthesis. Therefore, a combination of the calculated response spectra or the one calculated for the total MAA concentration might be a better fit for the absorption spectrum of the photoreceptor molecule. The individual polychromatic response spectra may represent wavelengths with a regulatory effect on enzymes catalysing the synthesis of specific MAAs.

The comparison of polychromatic response spectra calculated for MAA accumulation in algae exposed to continuous irradiance with those exposed to a light-dark cycle reveals marked differences. Under the light-dark cycle (full light spectrum) the pronounced negative effect of UV-B radiation is missing. Equally pigmented algae in all filter treatments indicate that UV-B radiation has no harmful effect on algal metabolism. Therefore, the difference in the shinorine response maximum may be due to harmful effects of shorter wavelengths under continuous irradiance.

The differences in the response spectra for palythine accumulation are difficult to explain. Maxima present in both response spectra may indicate wavelengths with high quantum efficiency for MAA accumulation, while differences may indicate wavebands involved in the photoregulation of enzymes catalysing MAA biosynthesis. This also indicates that light and dark phases have different effects on MAA formation.

Until now, we have only discussed polychromatic response spectra for algae exposed under PAR with UV additions. Including polychromatic response spectra for algae exposed under UV radiation, MAA formation becomes more complicated and yet easier to explain.

Under UV radiation, shinorine is steadily accumulated and it is the major MAA (see also Karsten et al. 1998a). This is in contrast to algae exposed under the full light spectrum, where shinorine concentrations decline after a few days and palythine becomes the major MAA, indicating an interconversion within the MAAs. Shinorine is the most common MAA reported in macroalgae (Banaszak et al. 1998, Karsten et al.

1998b) and many authors assume a precursor relationship between shinorine and the other MAAs (e.g. Franklin et al. 1999, Shick et al. 2000, Kräbs et al. 2002). With this background polychromatic response spectra calculated for algae exposed under UV radiation reveal that UV-A elicits the highest quantum efficiency for MAA formation and that PAR is important to trigger the interconversion within MAAs. Thus, polychromatic response spectra, especially those calculated for MAA formation in algae exposed to PAR with UV additions, may give a hint as to which wavelengths trigger MAA formation, but – more importantly – they indicate wavebands regulating enzymes involved in MAA synthesis. In the case of those calculated for MAA formation in algae exposed to UV radiation, polychromatic response spectra may show the absorption characteristics of the photoreceptor chromophore within the UV band.

So far we avoided mention of photoinduction of MAAs. Based on a great number of induction experiments it is a standing assumption that MAAs are photoinduceable. However, UV radiation increases the production of reactive oxygen species during photosynthesis (Fridovich 1986). Therefore, MAA formation may be stimulated by reactive oxygen species as discussed by Shick and co-workers (2000). Using the bandpath filter UG5 in addition to different cut-off filters we found a good indicator that MAAs are photoinduced.

Because the filter UG5 is also transparent for wavelengths above 650 nm, algae show a constant though low photosynthetic activity throughout the experiment. High Fv/Fm values indicate that the photosynthetic efficiency was not affected. Since production of reactive oxygen species is dependent on photosynthetic electron transport during photosynthesis (see Rijstenbil et al. 2000), we can assume that reactive oxygen species were low during the experiment. This is supported by results of Bischof and co-workers (2003) who found unchanged malondialdehyde concentration, which indicate production of reactive oxygen species, when *Ulva rotundata* was exposed to UV radiation. Because similar MAA concentrations were accumulated in algae exposed to either the full light spectrum or UV radiation, we can conclude that MAA formation is photoinduced.

Furthermore, the negative effect of UV-B radiation, also present in polychromatic response spectra calculated for shinorine and total MAA concentration in algae exposed to UV radiation, may be a result of different UV-B:UV-A:PAR ratios. This is supported by the high Fv/Fm values during the experiments and the results of Bischof and co-workers (2002b) who found that chlorophyll and total protein content, Rubisco

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concentration and photosynthesis was not affected when *Ulva rotundata* was exposed to UV radiation.

To test the hypothesis of MAA interconversion, we loaded specimens with shinorine by exposing them to UV radiation. Thereafter, sub samples were covered with different cut-off filters and exposed to the full light spectrum. After approximately two days shinorine was converted into palythine in the ration 1:1. Because no significant differences between the filter treatments could be found, PAR radiation in general seems to trigger the interconversion.

An interesting, but unclear result of this experiment was the significant reduction of the MAA concentration in algae exposed under the filter WG280. Because MAAs are photostable (Adams and Shick 1996, 2001, Conde et al. 2000), this can not be explained through a UV-B induced destruction of MAAs. Adding the high cost for the biosynthesis of MAAs (see Shick and Dunlap 2002) an active reduction of a useful UV screen becomes even more puzzling.

In summary, MAA formation is photoinduced and the final MAA concentration and composition is controlled by light quality, irradiance and duration of exposure. Polychromatic response spectra indicate that the photoreceptor responsible for MAA induction must be able to absorb UV-A radiation and blue light. Nevertheless, an action spectrum (monochromatic irradiance) will be necessary to reveal the absorption characteristics of the photoreceptor. Furthermore, MAAs are interconverted and polychromatic response spectra may show the wavebands regulating the enzymes involved.

The experiment demonstrates, once more, the ability of *C. crispus* to acclimate the internal MAA concentration flexibly to the spectral distribution of irradiance and its duration.

References

Adams, N. L. & Shick, J. M. 1996. Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin, *Strongylocentrotus droebachiensis*. *Photochem. Photobiol.* 64:149-158.

Adams, N. L. & Shick, J. M. 2001. Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin *Strongylocentrotus droebachiensis*. *Mar. Biol.* 138:267-280.

Banaszak, A. T., Lesser, M. P., Kuffner, I. B. & Ondrusek, M. 1998. Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAs) in marine organisms. *Bull. Mar. Sci.* 63:617-628.

Bandaranayake, W. M. 1998. Mycosporines: are they nature's sunscreens? *Natural Product Reports.* 15:159-172.

Bischof, K., Kräbs G., Wiencke, C. & Hanelt, D. 2002a. Solar ultraviolet radiation affects the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L. *Planta* 215:502-509.

Bischof, K. Peralta, G., Kräbs, G., van de Poll, W. H., Pérez-Lloréns, J. L. & Breeman A. M. 2002b. Effects of solar UV-B radiation on canopy structure of Ulva comunities from southern Spain. *J. Exp. Bot.* 53:2411-2421.

Carreto, J. I., Lutz, V. A., de Marco, S. G. & Cardigan, M. O. 1990. Fluence and wavelength dependance of mycosporine-like amino acid synthesis in the dinoflagellate *Alexandrium excavatum*. *In* Garneli E., Sundström, B., Edler & L. Anderson, D. M. (Eds) *Toxic Marine Phytoplankton*. Elsevier, Amsterdam, pp. 275-279.

Coohill, T. P. 1990. Action spectra again? *Photochem. Photobiol.* 54:859-870.

Coohill, T. P. 1992. Action spectra revisited. *J. Photochem. Photobiol. B: Biol.* 13:95-100.

Coohill, T. P. 1994. Exposure response curves action spectra and amplification factors. *In* Biggs R. H. & Joyner M. E. B. (Eds) *Stratospheric Ozone Depletion/UV-B Radiation in the Biosphere*, NATO ASI Series, Springer, Berlin, vol. 108, pp.57-62.

Conde, F. R., Churio, M. S. & Previtali, C. M. 2000. The photoprotector mechanism of mycosporine-like amino acids: Excited-state properties and photostability of porphyra-334 in aqueous solution. *J. Photochem. Photobiol. B: Biol.* 56:139-144.

Dunlap, W. C., Chalker, B. E. & Oliver, J. K. 1986. Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Austalia. III. UV-B absorbing compounds. *J. Exp. Mar. Biol. Ecol.* 104:239-248.

Dunlap, W. C. & Shick, J. M. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. *J. Phycol.* 34:418-430.

Franklin, L. A., Yakovleva, I., Karsten, U. & Lüning, K. 1999. Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. *J. Phycol.* 35:682-693.

Franklin, L. A., Kräbs, G. & Kuhlenkamp, R. 2001. Blue light controls the synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae). *J. Phycol.* 37:257-270.

Fridovich, I. 1996. Biological effects of the superoxide radical. *Arch. Biochem. Biophys.* 247:1-11.

Garcia-Pichel, F., Wingard, C. E. & Castenholz, R. W. 1993. Evidence regarding the UV sunscreen role of mycosporine-like compounds in the cyanobacterium *Gloeocapsa* sp. *Appl. Environ. Microbiol.* 59:170-176.

Gröniger, A. & Häder, D.-P. 2002. Induction of the synthesis of an UV-absorbing substance in the green alga *Prasiola stipitata*. *J. Photochem. Photobiol. B: Biol.* 66:54-59.

Hanelt, D. 1998. Capability of dynamic photoinhibition in Arctic macroalgaes is related to their depth distribution. *Mar. Biol.* 131:361-369.

Hoyer, K., Karsten, U., Sawall, T. & Wiencke, C. 2001. Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and development stages. *Mar. Ecol. Prog. Ser.* 211:117-129.

Jeffrey, S. W., MacTavish,, H. S., Dunlap, W. C. Vesk, M. & Groenewoud, K. 1999. Occurrence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae. *Mar. Ecol. Prog. Ser.* 189:35-51.

Karentz, D., McEuen, F. S. & Dunlap, W. C. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. *Mar. Biol.* 108:157-166.

Karsten, U., Franklin, L. A., Lüning, K. & Wiencke, C. 1998a. Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalgae *Chondrus crispus. Planta* 205:257-262.

Karsten, U., Sawall, T., Hanelt, D., Bischof, K., Figueroa, F. L., Flores-Moya, A. & Wiencke, C. 1998b. An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm-temperate regions. *Bot. Mar.* 41:443-453.

Kräbs, G., Bischof, K., Hanelt, D., Karsten, U. & Wiencke C. 2002. Wavelength-dependent induction of UV-absorbing mycosporine-like amino acids in the red alga *Chondrus crispus* under natural solar radiation. *J. Exp. Mar. Biol. Ecol.* 268:69-82.

Kuhlenkamp, R., Franklin, L. A. & Lüning, K. 2001. Effects of solar and ultraviolet radiation on growth in the marine macroalgae *Dictyota dichotoma* (Phaeophyceae) at Helgoland and its ecological consequences. *Helgol. Mar. Res.* 55:77-86.

McLachlan J. 1973. Growth media –marine. *In* Stein JR (Ed) *Handbook of Phycological Methods.* Cambridge University press, Cambridge, pp. 25-51.

Misonou, T., Saitoh, J.,Oshiba, S., Tokitomo, Y., Maegawa, M., Inoue, Y., Hori, H. & Sakurai, T. 2003. UV-absorbing substance in the red alga *Porphyra yezoensis* (Bangiales, Rhodophyta) block thymine photodimer production. *Mar. Biotechnol.* 5:194-200.

Lesser, M. P. 1996. Acclimation of phytoplankton to UV-B radiation: oxidative stress and photoinhibition of photosynthesis are not prevented by UV-absorbing compounds in the dinoflagellate *Prorodentrum micans. Mar. Ecol. Prog. Ser.* 132:287-297.

Portwich, A. & Garcia-Pichel, F. 2000. A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912. *Photochem. Photobiol.* 71:493-498.

Portwich, A. & Garcia-Pichel F. 2003. Biosynthetic pathway of mycosporines (mycosporine-like amino acids) in the cyanobacterium *Chlorogoeopsis* sp. strain PCC 6912. *Phycologia* 42:384-392.

Riegger, L. & Robinson, D. 1997. Photoinduction of UV-absorbing compounds in Antarctic diatoms and *Phaeocystis antartica. Mar. Ecol. Prog. Ser.* 160:13-25.

Rijstenbil, J. W., Coelho, S. M. & Eijsackers, M. 2000. A method for the assessment of light-induced oxidative stress in embryos of fucoid algae via confocal laserscan microscopy. *Mar. Biol.* 137:763-774.

Rundel, R. D. 1983. Action spectra and estimation of biologically effective UV radiation. Physiol. *Plantarum* 58:360-366.

Schäfer E & Fukshansky L. 1984. Action spectroscopy. *In* Smith H. & Holmes M. G. (Eds) *Techniques in photomorphogenesis*, Academic Press, London, pp. 109-129.

Shick, J. M., Romaine-Lioud S., Ferrier-Pagès & C. Gattusom, J. P. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral *Stylophora pistillata* despite decreases in its population of symbiotic dinoflagellates. *Limnol. Oceanogr.* 44:1667-1682.

Shick, J. M., Dunlap, W. C. & Buettner, G. R. 2000. Ultraviolet (UV) protection in marine organisms II. Biosynthesis, accumulation, and sunscreening function of mycosporine-like amino acids. *In* Yoshikawa T., Tonokuni S., Yamamoto Y. & Naito Y. (Eds)*Free radicals in chemistry, biology and mededine*, OICA International, London, pp. 215-228.

Shick, J. M. & Dunlap, W. C. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu. Rev. Physiol.* 64:223-262.

Sinha, R. P., Sinha, J. P., Gröniger, A. & Häder, D. P. 2002. Polychromatic action spectrum for the induction of mycosporine-like amino acid in a rice-field cyanobacterium, *Anabaena* sp. *J. Photochem. Photobiol. B* 66:47-53.

Sivaligam, P. M., Ikawa, T., Yokohama, Y. & Nisizawa, K. 1974. Distribution of a 334 UV-absorbing substance in algae, with special regard of its possible physiological roles. *Bot. Mar.* 17:23-29.

van de Poll, W. H., Eggert, A., Buma, A. G. J. & Breeman, A. M. 2001. Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related differences in UV-B tolerance. *J. Phycol.* 37:30-37.

van de Poll, W. H., Hanelt, D., Hoyer, K., Buma, A. G. J. & Breeman, A. M. 2002. Ultraviolet-B induced cyclobutane-pyrimidine dimer formation and repair in Arctic marine macrophytes. *Photochem. Photobiol.* 76:493-501.

van de Poll, W. H., Bischof K., Buma, A. G. J. & Breeman, A. M. 2003. Acclimation of the temperate marine red macrophyte *Chondrus crispus* to Ultraviolet-B radiation. *In* van de Poll, W: H. (Ph. D. thesis) *Patterns in ultraviolet radiation sensitivity of tropical, temperate and Arctic marine macroalgae*, Stichting Drukkerij C. Regenboog, Haren; p. 73-83

Vincent, W. F. & Neale, P. J. 2002. Mechanisms of UV damage to aquatic organisms. *In* de Mora S., Demers S., Verent M. (Eds) *The effects of UV radiation in the marine environment*. Cambridge University, Cambridge, pp. 149-176.

Wiencke, C., Gómez, I., Pakker, H., Flores-Moya, A., Altermirano, M., Hanelt, D., Bischof, K. & Figueroa, F. L. 2000. Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implication for depth zonation. *Mar. Ecol. Prog. Ser.* 197:217-229.

A monochromatic action spectrum for the photo induction of the UV-absorbing mycosporine-like amino acid shinorine in the red alga *Chondrus crispus* Stackh.

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ABSTRACT

To determine the action spectrum for photo induction of the UV absorbing mycosporine-like amino acid shinorine, specimens of the marine red alga *Chondrus crispus* Stackh. were irradiated with monochromatic light of various wavelengths using the Okazaki Large Spectrograph at the National Institute for Basic Biology in Okazaki, Japan. Fluence response curves were determined for the wavelengths between 280 and 750 nm, by irradiating the algae with monochromatic light for 10 hours, followed by 4 hours of 25 μmol m^{-2} s^{-1} photosynthetic active radiation and 10 hours' darkness. Samples were taken after the second exposure interval. A linear correlation between fluence rate and accumulated shinorine concentration was detected for wavelengths between 350 and 490 nm in the fluence-rate range of 20-30 μmol m^{-2} s^{-1}, whereas there was no induction above 490 nm. Below 350 nm a decline in shinorine concentration could be observed at fluence rates above 30 μmol m^{-2} s^{-1}, probably due to an inhibition of photosynthetic activity and a subsequent impairment of shinorine biosynthesis. The constructed action spectrum indicated that the photoreceptors mediating shinorine photo induction might be an unidentified UV-A type photoreceptor with absorption peaks at 320, 340 and 400 nm.

Keywords: action spectra, *Chondrus crispus*, UV-absorbing substances

INTRODUCTION

The marine red alga *Chondrus crispus* Stackh. is an abundant species along the coasts of the North Atlantic and inhabits the intertidal and upper sublittoral zone of rocky shorelines (1). Therefore, representatives of this species must cope with extreme periodical variations in solar radiation, and also with relatively constant low irradiances in deeper or turbid water, depending on the vertical position of specimens on the shore.

Because of the high-energy quanta of shorter wavelengths, ultraviolet (UV) radiation has a disproportionally large biological effect. Thus, upon absorption, UV radiation can degrade or transform DNA, RNA and proteins photo chemically (2-4) and inhibit photosynthesis, growth and reproduction (5-7)

To counteract the harmful effects of UV radiation, algae have developed several physiological and biochemical defence mechanisms, one of which is the synthesis of UV-absorbing compounds such as mycosporine-like amino acids (MAAs) (e.g. 3, 8-12). MAAs are composed of a group of cyclohexenone or cyclohexenine rings conjugated with one or two amino acids and/or amino alcohols, and occur in a number of marine organisms (for overview see 13-15). Due to their absorption maxima between 310 and 360 nm they are considered to act as natural sunscreens (16-17).

The quality and quantity of MAA formation can be flexibly adjusted to the *in situ* light climate, vertical distribution and seasonal changes (18-19), according to the degree of exposure to solar radiation. Hitherto, only scant information on the mechanisms triggering MAA synthesis as well as their wavelength dependence is available, but photoreceptors may be involved in this process.

Previous studies on *C. crispus* revealed UV-B, UV-A and blue light dependent MAA formation (20-22). Therefore, we have chosen this species to obtain a more detailed view of the mechanism for qualitative and quantitative regulation of MAAs by determining a monochromatic action spectrum for this process. The determined action spectrum should match the absorption characteristics of the putative photoreceptor (23-25).

MATERIAL AND METHODS

Culture conditions. Thalli of *C. crispus* Stackh., originally isolated from Helgoland (German Bight, North Sea; 54°11'N, 7°53'E) were cultivated in glass beakers in the laboratory (15°C, light-dark-cycle 16:8 h, 25 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR)) until they reached 2-2.5 cm long. One week before monochromatic irradiation, the light dark cycle was changed to 14:10 h, thus corresponding to the light cycle during the experiment.

Preliminary experiment for proper irradiation and sampling program. To find the proper experimental conditions regarding sampling time and range of fluence rate to be tested, specimens of *C. crispus* were exposed to 10 h of each of 5, 10, 15 and 20 μmol m^{-2} s^{-1} UV-A radiation (FL20S·BL-B, National, Osaka, Japan) followed by 4 h 25 μmol m^{-2} s^{-1} PAR (FL20SS D18 daylight, Toshiba, Tokyo) to ensure photosynthetic activity and 10 h darkness. The experiments started at 8:00 h and samples were taken daily shortly before the light was switched on. Additionally, samples were taken at 22:00 h on the second day of the experiment.

Monochromatic Response and Action Spectrum. To determine the response and action spectra for MAA induction, specimens were exposed under the Okazaki Large Spectrograph (OLS) (26) at the National Institute for Basic Biology (NIBB), Okazaki, Japan. Monochromatic light was provided by a large spectrograph equipped with a 30 kW xenon arc lamp (Ushio Electric Co., Tokyo, Japan). The light beam was reflected first by a plane mirror and than by a condensing mirror. After reflection by a diffraction grating, it passed through an intercepting plate window of different optical filters into the irradiation room. In each glass dish placed at various wavelengths between 280 and 750 nm, specimens were placed beneath a mirror reflecting the monochromatic light beam directly onto the samples. Short-cut-off filters (Hoya Co., Tokyo, Japan) were placed above the glass dishes. (see Tab. 1). Fluence rates examined at specific wavelengths (see Tab. 1) were adjusted with neutral density filters (Fujitok Co., Tokyo, Japan) and were measured with a photon flux density meter (RMS101, Rayon Ind. Co. Ltd., Tokyo, Japan) equipped with a calibrated silicon photodiode. Specimens were treated with a light dark cycle of 10 h under monochromatic light of different fluence

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rates, followed by 4 h of 25 μmol m^{-2} s^{-1} PAR and 10 h in darkness. After the second exposure interval, samples were taken and the photosynthetic activity was measured as described below to evaluate possible damage of the sample by the UV irradiation. The short period of dim PAR after exposure to the different monochromatic wavelengths was applied to ensure photosynthesis, giving energy equivalents for MAA biosynthesis. The chosen PAR intensity itself is not inductive for MAA synthesis.

Photosynthetic quantum efficiency. After irradiation with the OLS the quantum efficiency of photosystem II electron transport (Yield) was measured at 25 μmol m^{-2} s^{-1} PAR with a pulse amplitude modulated chlorophyll fluorometer (PAM 2000, Walz, Effeltrich, Germany). The definition of yield is the ratio of maximal minus variable chlorophyll fluorescence to maximal chlorophyll fluorescence of a light adapted plant (Yield). According to Genty et al. (27), this parameter is directly proportional to the efficiency of excitation capture by open photosystem II reaction centers (Fv/Fm) of dark adapted plants. A decline in this value is symptomatic of the effect of photo inhibitory stress (28).

Table 1: Used cut-off filters (Hoya Co.) and fluence rates per wavelength for calculating the action spectra of shinorine formation in *Chondrus crispus* thalli exposed to the light field of the Okazaki Large Spectrograph. Tested conditions are marked with x.

nm	Cut-off filter	Fluence rate (μmol m^{-2} s^{-1})						
		5	10	15	20	30	50	85
280	-	x	x	x	x			
290	-	x	x	x	x			
300	UV28	x	x	x	x			
310	UV28		x		x	x	x	
320	UV30		x		x	x	x	
330	UV30		x		x	x	x	
340	UV32		x		x	x	x	
350	UV34		x		x	x	x	
360	UV34		x		x	x	x	
370	L37		x		x	x	x	
380	L37		x		x	x	x	
390	L38		x		x	x	x	
400	L38		x		x	x	x	
410	L38				x	x	x	x
420	L39				x	x	x	x
430	L40				x	x	x	x
440	L40				x	x	x	x
450	L42				x	x	x	x
460	L42				x	x	x	x
470	Y44				x	x	x	x
480	Y44				x	x	x	x
490	Y46				x	x	x	x
500	Y46				x	x	x	x
525	Y48				x	x	x	x
550	Y52				x	x	x	x
575	O54				x	x	x	x
600	O56				x	x	x	x
620	O58				x	x	x	x
640	R60				x	x	x	x
660	R62				x	x	x	x
680	R64				x	x	x	x
700	R64				x	x	x	x
720	R66				x	x	x	x
730	R68				x	x	x	x
750	R70				x	x	x	x

Analysis and identification of MAAs. Samples were oven-dried and MAAs were subsequently extracted for 2 h in 70 % aqueous methanol (v/v) at 60°C. Extracts were separated on a Waters HPLC system fitted with a Spherecolne C8 column (250 × 4 mm, 5 μ; Phenomenex, Aschaffenburg, Germany). The mobile phase was 5 % aqueous methanol (v/v) plus 0.1 % acetic acid (v/v), run isocratically with a flow rate of 0.7 ml min^{-1}. Peaks were detected at 330 nm and absorption spectra were recorded from 290 to 400 nm. The MAAs were identified by absorption spectra, retention time, and in case of shinorine, by co-chromatography with extracts of the red alga *Mastocarpus stellatus*. Quantification was carried out by using published extinction coefficients (18, 29-32). Results were expressed as mg g^{-1} dry weight (DW).

Calculation of the action spectrum. The reciprocal of the fluence rate required for formation of a certain amount of shinorine (0.3 mg g^{-1} DW) was calculated from the linear part of the fluence response curves and plotted against wavelength (for overview see 23, 25, 33).

Data treatment. For yield measurements, mean values and standard deviations were calculated from four independent replicates. For MAA accumulation, mean values and standard errors were calculated from eight independent replicates. Statistical significance ($p < 0.05$) of differences in MAA content generated by the exposure to different wavelengths at a fixed fluence rate and under different fluence rates at a specific wavelength as well as their combined effect was tested by two way analysis of variance (ANOVA) followed by a Least Significant Difference-Test (LSD). Calculations were performed using the program Statistica Kernel-Version 5.5 A (StatSoft, Inc., Tulsa, OK, USA).

RESULTS

Preliminary experiment for proper irradiation and sampling program.

In the preliminary experiment, the time course of MAA accumulation was examined. Whereas 5, 10 and 15 μmol m^{-2} s^{-1} UV-A were not inductive, a distinct induction pattern could be found in algae irradiated with 20 μmol m^{-2} s^{-1} UV-A (Fig. 1). After a lag-phase of one day, a linear accumulation was observed, which continued during darkness. At the third day of exposure a steady state was achieved. Based on these data, we decided to sample after the second exposure interval in order to obtain the maximal MAA concentrations.

Figure 1: Shinorine accumulation induced in *Chondrus crispus* exposed to a light-dark cycle of 10 h UV-A (20 μmol m^{-2} s^{-1}), 4 h PAR (25 μmol m^{-2} s^{-1}) and 10 h darkness; mean value ± standard error; n = 4; shaded areas represent the dark phase of the light dark cycle.
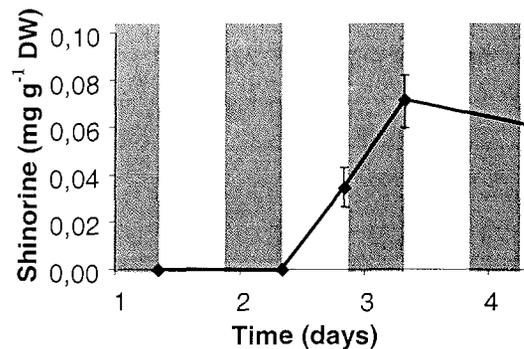
Photosynthetic quantum efficiency.

In the wavelength range between 360 and 750 nm, quantum yield of photosynthesis was unaffected in algae exposed to these wavelengths (Yield ~ 0.57 (± 5%); Fig. 2), whereas in algae exposed to 280 to 350 nm, values declined with shorter wavelengths. This effect became more pronounced at higher fluence rates, showing the adverse effects of short wavelength UV radiation (Standard deviation ± 5-15%). Above 10 μmol m^{-2} s^{-1}, algae exposed to 280, 290 and 300 nm were visibly bleached.
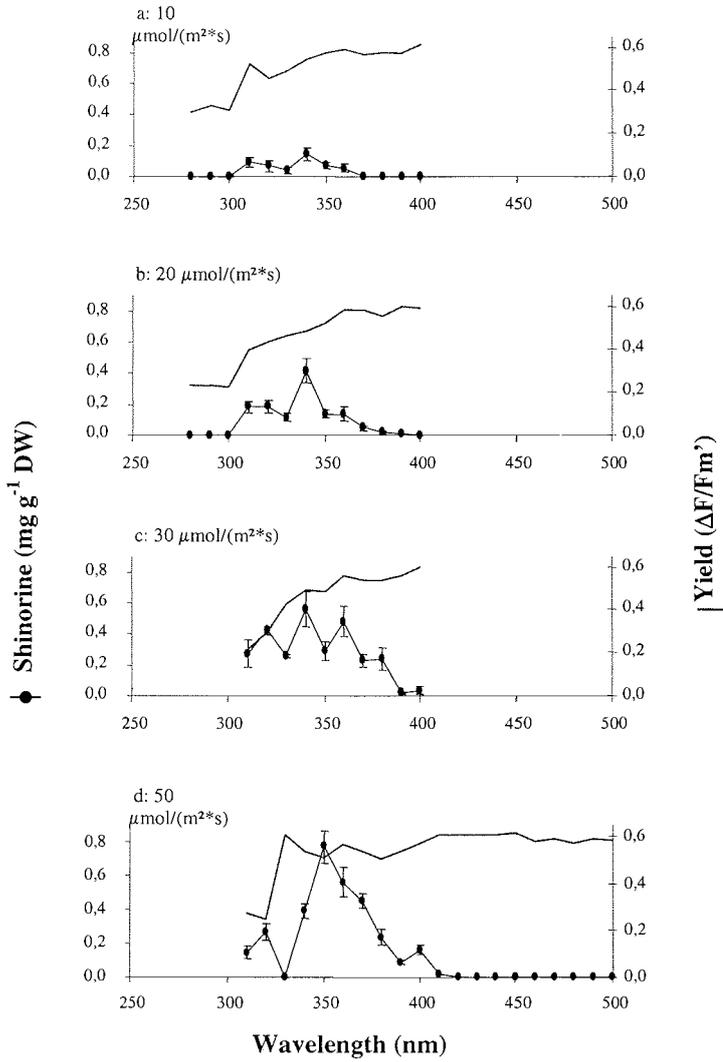
Figure 2: Equal quantum response spectra for photosynthetic quantum yield and shinorine formation in *Chondrus crispus* exposed to a: 10 μmol m^{-2} s^{-1}, b: 20 μmol m^{-2} s^{-1}, c: 30 μmol m^{-2} s^{-1} and d: 50 μmol m^{-2} s^{-1} monochromatic light for two exposure intervals of 10 h monochromatic light, 4 h 25 μmol m^{-2} s^{-1} PAR and 10 h darkness. Yield = black line (mean value, n = 4), shinorine concentration = • (mean value ± standard error; n = 8).

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Equal Quantum Response Spectrum.

With more than 95 % of the total MAA content shinorine was the main MAA accumulated under the given radiation conditions. Beside shinorine only traces of palythine and asterina-330 could be detected, but with no apparent trends.

The equal quantum response spectra at 10, 20, 30 and 50 μmol m^{-2} s^{-1} reveal a broad response peak from 300 nm to 370/390/410 nm (Fig. 2 a-d). It is characterized by a main maximum at 340 nm (except at 50 μmol m^{-2} s^{-1}), a minimum at 330 nm and a second maximum at 320 nm. At higher fluence rates these characteristics became more pronounced. At 50 μmol m^{-2} s^{-1}, a shift in the main maximum to 350 nm was observed. Furthermore, the shape of the response peak changed at higher fluence rates. The rising slope of the peak became steeper between 300 to 340 nm (350 nm), while the tail of the peak extended to longer wavelengths (see also Tab. 2).

Table 2: Shinorine concentration in mg g^{-1} DW (mean value ± standard error; n = 8) in thalli irradiated by the given fluence rates for 10 h monochromatic light of different fluence rates, 4 h 25 μmol m^{-2} s^{-1} PAR and 10 h darkness using the Okazaki Large Spectrograph.

nm	Fluence rate (μmol m^{-2} s^{-1})		
	50	85	110
410	0.180 ± 0.050	0.145 ± 0.038	
420	-	0.075 ± 0.033	
430	-	0.013 ± 0.009	
440	-	-	
450	-	0.043 ± 0.017	-
470	-	-	0.072 ± 0.070
490	-	-	0.024 ± 0.024

Dose response curves.

There was no shinorine induction at 280, 290 and between 490 to 750 nm (data not shown). For wavelengths below 350 nm a decline in shinorine content could be detected for fluence rates above 30 μmol m^{-2} s^{-1} (Fig. 3). For wavelengths between 350 and 390 nm (except 380 nm) a linear correlation between fluence rates and shinorine content was observed.
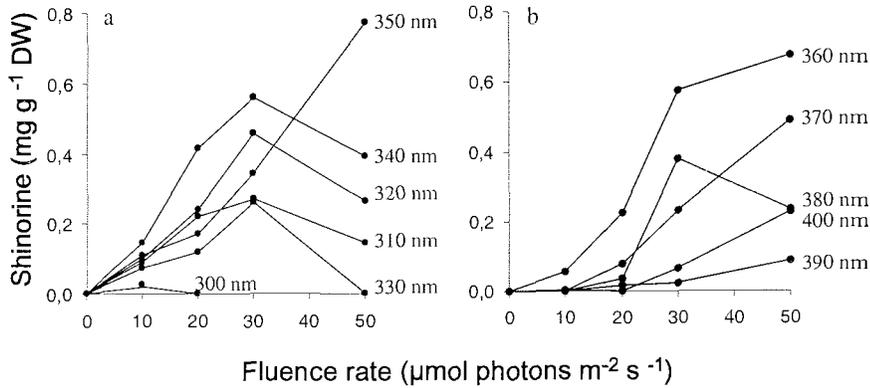
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Figure 3: Fluence response curves for shinorine formation in *Chondrus crispus* thalli exposed to 10 h of monochromatic light of different fluence rates, 4 h 25 μmol m^{-2} s^{-1} PAR and 10 h darkness; mean values; n = 8; a: 310 to 350 nm; b: 360 to 400 nm.

Action spectrum.

The action spectrum (Fig. 4) was determined for the steeply increasing phase of the dose response curves (Fig. 3 and Tab. 2) by plotting the reciprocals of the fluence rate required for the formation of a certain amount of shinorine (shinorine = 0.3 mg g^{-1} DW). The action spectrum has its main maximum at 340 nm, which is divided by a minimum (330 nm) from the second maximum at 320 nm. Beside this double peak, the action spectrum has a smaller third maximum at 400 nm.

Figure 4: Action spectrum for shinorine formation in *Chondrus crispus*. The relative effectiveness i.e. the reciprocal of the fluence rate required for formation of a certain amount of shinorine (0.3 mg g^{-1} DW) was calculated (from the data in Fig. 3 and Tab. 2) and plotted against the wavelength.
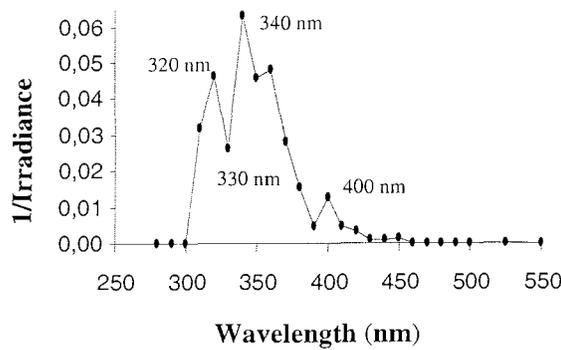
Statistics

Both the difference in shinorine content accumulated in algae exposed to different wavelengths by a certain fluence rate and to different fluence rates at a specific wavelength is statistically significant. The same is true for the combined effect between different wavelengths and fluence rates.

DISCUSSION

Over the last years many authors investigated the wavelength dependent induction of MAA biosynthesis. However the information on both the nature of the regulating mechanisms and the putative photoreceptor is very limited.

With one exception (34), previous studies (e.g. 22, 35-38) have investigated the wavelength dependence of MAA formation under polychromatic light. Because the resulting polychromatic response spectra tend to obscure individual chromophores (39), they cannot be applied to conclusively answer questions about the absorption spectrum of the photoreceptor involved (40). Nevertheless, they are close to a natural setting and combine in the measured response natural repair and mitigating reactions (40), which is useful for ecological predictions.

To identify the target chromophore responsible for MAA induction, an action spectrum carefully constructed under monochromatic light is necessary (24). Nevertheless, this approach is highly artificial.

Polychromatic response spectra and action spectra each have their advantages and limitations. It is therefore necessary to provide detailed information on the experimental set up so that the reader understands the limitations of presented data (39).

The following paragraph will give a short overview on relevant papers concerning MAA photo induction and the respective light conditions of the experiment: The highest quantum efficiency for MAA production in two Antarctic diatoms was found for wavelengths between 370 and 460 nm, while the prymnesiophyte *Phaeocystis antarctica*, examined under the same polychromatic experimental set up, showed a response maximum at around 345 nm (36). In the cyanobacterium *Chlorogloeopsis* PCC 6912, 310 nm was most effective for MAA accumulation (action spectrum from

290 to 340 nm; 34). In addition to UV radiation, polychromatic blue light induces MAA synthesis in the dinoflagellate *Alexandrium excavatum* (35) and the red alga *Chondrus crispus* (21). In the latter species, a wavelength-dependent difference for the accumulation of five different MAAs has been described (polychromatic response spectra; 22). These data reveal apparent species-dependent differences among the photo-sensory processes controlling MAA formation.

Under a daily cycle of polychromatic UV-A radiation followed by dim PAR mainly shinorine (>95 %) was detected in irradiated specimens (Fig. 1). This agrees with the results of Karsten et al. (20), who found that UV radiation stimulates shinorine formation.

The course of shinorine accumulation reveals a lag-phase between the start of exposure and shinorine production. In contrast, high solar radiation induces MAA formation within one day in low light-adapted specimens transplanted to shallow water (17, 22). Based on data of Neale et al. (16), Shick et al. (15) discuss an "up regulation of the biosynthetic pathway's enzymes in response to PAR as part of a suite of differences in allocation of nutrients and patterns of gene expression between high-PAR and low-PAR cells". As this might be an explanation for the delayed MAA production, it is also possible that the photoreceptor chromophores are shielded (i.e. by the cell wall). Thus, a higher fluence of monochromatic light may be necessary to penetrate to the chromophore in question.

Although MAA formation can also be induced by salt stress (36) and possibly by increased levels of reactive oxygen species (15), the persisting MAA production during darkness indicates that a photoreceptor is responsible for the examined process.

In those specimens exposed to the light field of the OLS, again, mainly shinorine could be detected. As blue light induces palythine formation (21), this might indicate that shinorine is synthesized as precursor of other MAAs (as discussed in 17, 41). Therefore the polychromatic response spectra for MAA formation under natural sunlight (22) might only show the wavelength dependence of MAA interconversion and not their photo-induction.

While equal quantum response spectra reflect the absorption characteristics of the photoreceptor involved, carefully determined (classical) action spectra should match the absorption spectra of the photoreceptor in question (23-25). The obtained action spectrum for shinorine formation in *C. crispus* indicates that the absorption spectrum of

this photoreceptor has a main maximum at 340 nm, a second maximum at 320 nm and a smaller third maximum at 400 nm.

Even though these characteristic points of the action spectrum are statistically different, two facts have to be considered:

1.) The minimum at 330 nm might be overestimated because the induction of shinorine, which has an absorption maximum at 334 nm, may decrease the penetration of light quanta of these wavelengths (33, 25). Nevertheless, as this minimum already occurs in the equal quantum action spectrum for 10 μmol m^{-2} s^{-1} (Fig. 2a) with a very low shinorine concentration (maximal reported shinorine concentration $\sim$ 1.8 mg g^{-1} DW), we believe that this would only diminish the relationship between maxima and minimum, and not alter the general form of the action spectrum.

2.) Because algae exposed under monochromatic blue light are able to photosynthesise during the experiment, the maximum at 400 nm might also be overestimated. Thus, the higher availability of energy equivalents resulting from photosynthetic activity might lead to a stimulation of MAA biosynthesis in algae irradiated by these wavelengths (15). Nevertheless, as fluence rates of 110 μmol m^{-2} s^{-1} induce shinorine formation up to 490 nm, we believe that there is a true maximum at 400 nm. This would also be supported by the proposed blue light photoreceptors in *A. excavatum* and *C. crispus* (21, 35).

Because the action spectrum for MAA induction obtained for *Chlorogloeopsis* PCC 6912 matches the absorption spectrum of a reduced pterin, Portwich and Garcia-Pichel (34) suggest a reduced pterin (tetrahydroform) as a possible photoreceptor chromophore. This is additionally supported by a depression in photo sensory efficiency for MAA synthesis by either applying an inhibitor of the biosynthetic pathway for pterins or an antagonist of pterin excite states (34). As the action spectrum is based on a limited number of tested wavelengths and the authors failed to test the effect of the inhibitors used on the target molecule, this should be considered with caution.

Conversely flavins might be the photoreceptor chromophore, as discussed by Franklin et al. (17). They could demonstrate that blue light and UV radiation interact to boost the synthesis of shinorine, which is in line with the photo regulation of flavin synthesis (42-43).

However, neither the absorption spectra of pterins nor those of flavins are similar to the action spectrum determined here. Additionally, the present action spectrum does not compare with the absorption spectrum of the UV-A/blue light type photoreceptor (44),

for which several flavoproteins (cryptochromes, phototropins, photo activated adanylyl cyclase, Appt and WC-1) have been identified (45 and references therein). We propose that one (or two) as yet unidentified UV-A photoreceptor(s) is/are responsible for triggering shinorine formation. Each of the action spectral peaks might well be attributable to the absorption spectral peaks of different pterin chromophores in these UV-A photoreceptor proteins (46).

References

1. Lüning, K. (1990) *Seaweeds.* Wiley, New York

2. Vincent, W. F. and Neale, P. J. (2000) Mechanisms of UV damage to aquatic organisms. In *The effects of UV radiation in the marine environment* (Edited by S. de Mora, S. Demers and M. Verent), pp. 149-176. Cambridge University, Cambridge.

3. van de Poll, W. H., Eggert, A., Buma, A. G. J. and Breeman, A. M. (2001) Effects of UV-B induced DNA damage and photoinhibition on growth of temperate marine red macrophytes: habitat-related difference in UV-B tolerance. *J. Phycol.* 37, 30-37.

4. van de Poll, W. H., Hanelt, D. Hoyer, K., Buma, A. G. J. and Breeman, A. M. (2002) Ultraviolet-B induced cyclobutane-pyrimidine dimmer formation and repair in Artic marine macrophytes. *Photochem. Photobiol.* 76, 493-501.

5. Wiencke, C., Gómez, I., Pakker, H., Flores-Moya, A., Altamirano, M., Hanelt, D., Bischof, K. and Figueroa, F. L. (2000) Impact of UV-radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implication for depth zonation. *Mar. Ecol. Prog. Ser.* 197, 217-229.

6. Kuhlenkamp, R., Franklin, L. A. and Lüning, K. (2001) Effect of solar and ultraviolet radiation on growth in the marine macroalgae *Dictyota dichotoma* (Phaeophyceae) at Helgoland and its ecological consequences. *Helgol. Mar. Res.* 55, 77-86.

7. Bischof, K., Hanelt, D., Aguilera, J., Karsten, U., Vögele, B., Sawall, T. and Wiencke, C. (2002) Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord: Sensitivity of photosynthesis to ultraviolet radiation. *Mar. Biol.* 140, 1097-1106

8. Mitchell, D. and Karentz, D. (1993) The induction and repair of DNA photodamage in the environment. In *Environmental UV photobiology* (Edited by A. Young, L. Björn, J. Moan and W. Nultsch) pp. 345-377. Plenum, New York.

9. Roy, S. (2000) Strategies for minimization of UV-induced damage. In *The effects of UV radiation in the marine environment* (Edited by S. de Mora, S. Demers and M. Vernet) pp. 177-205. Cambridge University, Cambridge.

10. Aguilera, J., Dummermuth, A., Karsten, U. Schriek, R. and Wiencke, C. (2002) Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Artic marine macroalgae. *Polar Biol.* 25, 432-441.

11. Bischof, K., Kräbs, G., Hanelt, D. and Wiencke, C. (2000) Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over *Chondrus crispus* at the Helgoland shoreline? *Helgol. Mar. Res.* 54:47-52

12. Misonou, T., Saitoh, J., Oshiba, S., Tokitomo, Y., Maegawa, M., Inoue, Y., Hori, H. and Sakurai, T. (2003) UV-absorbing substance in the red alga *Porphyra yezoensis* (Bangiales, Rhodophyta) Block Thymine Photodimer Production. *Mar. Biotechnol.* 5, 194-200.

13. Karentz, D., McEuen, F. S., Land, M. C. and Dunlap, W. C. (1991) Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. *Mar Biol.* 108, 157-166.

14. Bandaranayake, W. M. (1998) Mycosporines: are they nature's sunscreens? *Nat. Prod. Rep.* 15, 159-172.

15. Shick, J. M., Dunlap, W. C. and Buettner, G. R. (2000) Ultraviolet (UV) protection in marine organisms II. Biosynthesis, accumulation, and sunscreening function of mycosporine-like amino acids. In *Free Radicals in Chemistry, Biology and Medecine* (Edited by T. Yoshikawa, S. Tonokuni, Y. Yamamoto and Y. Naito), pp.215-228. OICA International, London.

16. Neale, P. J., Banazak, A. T. and Jarriel, C. R. (1998) Ultraviolet sunscreens in *Gymnodium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. *J. Phycol.* 34, 928-938.

17. Franklin, L. A., Yakovleva, I., Karsten, U. and Lüning, K. (1999) Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. *J. Phycol.* 35, 682-693.

18. Dunlap, W. C., Chalker, B. E. and Oliver, J. K. (1986) Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia: III. UV-B absorbing compounds. *J. Exp. Mar. Biol. Ecol.* 104, 239-248.

19. Karsten, U., Bischof, K., Hanelt, D., Tüg, H. and Wiencke, C. (1999) The effect of ultraviolet radiation on photosyntheis and ultraviolet-absorbing substances in the endemic Arctic macroalga *Devaleraea ramentacea* (Rhodophyta). *Physiol. Plant.* 105, 58-66.

20. Karsten, U. Franklin, L. A., Lüning, K. and Wiencke, C. (1998) Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine-like amino acids in the marine macroalga *Chondrus crispus* (Rhodophyta). *Planta* 205, 257-262.

21. Franklin, L. A., Kräbs, G. and Kuhlenkamp. R. (2001) Blue light controls the synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae). *J. Phycol.* 37, 257-270.

22. Kräbs, G., Bischof, K. Hanelt, D., Karsten, U. and Wiencke, C. (2002) Wavelength-dependent induction of UV-absorbing mycosporine-like amino acids in the red alga *Chondrus crispus* under natural solar radiation. *J. Exp. Mar. Biol.* 268, 69-82.

23. Schäfer, E. and Fukshansky, L. (1984) Action spectroscopy. In *Techniques in photomorphogenesis* (Edited by H. Smith and M. G. Holmes) pp. 109-129. Academic Press, London.

24. Coohill, T. P. (1990) Action spectra again? Photochem. Photobiol. 54 (5), 859-870.

25. Holmes, M. G. (1997) Action spectra for UV-B effects on plants: monochromatic and polychromatic approaches for analyzing plant response. In *Plants and UV-B: Response to Environmental Changes* (Edited by P.S. Lumsden) pp. 31-50. Cambridge Univ. Press, Cambridge.

26. Watanabe, M., Furuya, M., Miyoshi, Y., Inoue, Y, Iwahashi, I. and Matsumoto, K. (1982) Design and performance of the Okazaki Large Spectrograph for photobiological reseach. *Photochem. Photobiol.* 36, 491-498.

27. Genty, B., Briantais, J.-M. and Baker, N. R. (1989) The relationship between the quantum yield of photosynthesic electron transport and quenching of chlorophyll fluorescence. Biochim. *Biophys. Acta* 990, 87-92.

28. Baker, N. R. and Horton, P. (1987) Chlorophyll fluorescence quenching during photoinhibition. In "Photoinhibition" (Edited by D. J. Kyle, C. B. Osmond and C. J. Arntzen), pp. 145-168. Elsevier, Amsterdam.

29. Takano, S., Uemura, D. and Hirata, Y. (1978) Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa. Tetrahedron Lett.* 26, 2299-2300.

30. Takano, S., Uemura, D. and Hirata, Y. (1978) Isolation and structure of two new amino acids, palythinol and palythene, from the zoanthid *Palythoa tuberculosa. Tetrahedron Lett.* 49, 4909-4912.

31. Tsujino, I., Yabe, K. and Sekikawa, I. (1980) Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendoi* Yamada et Mikami. *Bot. Mar.* 23, 65-68.

32. Gleason, D. F. (1993) Differential effects of ultraviolet radiation on green and brown morphs of the Caribbean coral *Porites astroides. Limnol. Oceanogr.* 38, 1452-1463.

33. Shropshire, W. (1972) Action spectroscopy. In *Phytochrome* (Edited by K. Mitrakos and W. Shropshire) pp. 161-181. Academic Press, London.

34. Portwich, A. and Garcia-Pichel, F. (2000) A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912. *Photochem. Photobiol.* 71, 493-498

35. Carreto, J. I., Lutz, V.A., de Marco, S. G. and Carignan, M. O. (1990) Fluence and wavelength dependance of mycosporine-like amino acids in the dinoflagellate *Alexandrium excavatum.* In *Toxic Marine Phytoplankton* (Edited by E. Garneli, B. Sundström, L. Elder and D. M. Anderson), pp.275-279. Elviser, Amsterdam.

36. Riegger, L. and Robinson, D. (1997) Photoinduction of UV-absorbing compounds in Antarctic diatoms and *Phaeocystis antarctica. Mar. Ecol. Prog. Ser.* 160, 13-25.

37. Gröniger, A. and Häder, D.-P. (2002) Induction of the synthesis of an UV-absorbing substance in the green alga *Prasiola stipitata. J. Photochem. Photobiol.*, B 66, 54-59

38. Sinha, R. P., Sinha, J. P., Gröniger, A. and Häder, D.-P. (2002) Polychromatic action spectrum for the induction of mycosporine-like amino acids in a rice-field cyanobacterium, *Anabaena* sp. *J. Photochem. Photobiol. B* 66, 47-53.

39. Coohill, T. P. (1992) Action spectra revisited. *J. Photochem. Photobiol. B* 13, 95-100

40. Coohill, T. P. (1994) Exposure response curves action spectra and amplification factors. In *Stratospheric Ozone Depletion/UV-B Radiation in the Biosphere* Vol. 108 (Edited by R. H. Biggs and M. E. B. Joyner), pp. 57-62. NATO ASI Series, Springer, Berlin.

41. Banaszak, A. T., LaJeunesse, T. C. and Trench, R. K. (2000) The synthesis of mycosporine-like amino acids (MAAs) by cultured, symbiotic dinoflagellates. *J. Exp. Mar. Biol. Ecol.* 249, 219-233.

42. Christie, J. M. and Jenkins, G. J. (1996) Distinct UV-B and UV-A/blue light transduction pathways induce chalcone synthase gene expression in *Arabidopsis* cells. *Plant Cell* 8, 1555-1567.

43. Fuglevand, G., Jackson, J.A. and Jenkins, G. I. (1996) UV-B, UV-A, and blue light signal transduction pathways interact synergistically to regulate chalcone synthase gene espression in Arabidopsis. *Plant Cell* 8 ,2347-2357.

44. Watanabe, M. (1995) Action spectroscopy: Photomovement and Photomorphogenesis Spectra. In *Handbook of organic photochemistry and photobiology* (Edited by W. Horspool and P. S. Song) pp. 1276-1288. CRC Press, Boca Raton.

45. Watanabe, M. (2004) Action Spectroscopy for Photosensory Processes. In *Handbook of Organic Photochemsitry and Photobiology*, 2nd ed. (Edited by W. Horspool and P. S. Song) pp. 115.1-115.16. CRC Press, Boca Raton.

46. Galland, P. and Senger, H. (1988) The role of pterins in the photoreception and metabolism of plants. *Photochem. Photobiol.* 48, 811-820.

4. Summary of results and general discussion

In this chapter the main results of the various studies compiled in this thesis will be discussed, referring to the role of MAAs as UV sunscreens, antioxidants or precursor of antioxidants, to a light regulated interconversion of MAAs, to a possible signal transduction in the initiation of MAA biosynthesis and to putative photoreceptors triggering MAA synthesis. A model for MAA biosynthesis will be proposed, summarizing the presented results.

4.1 Role of MAAs as UV sunscreen

Observations that MAA concentrations in algae and corals are correlated with the vertical distribution from which the specimens were collected (Dunlap et al. 1986, Karsten et al. 1998) combined with their absorption spectra between 309 and 360 nm lead to the assumption that MAAs act as photo protective sunscreens against UV radiation (Bandaranayake 1998).

The proposed photo protective function of MAAs was strongly supported by the finding that embryos of green sea urchins developing in eggs with high MAA concentrations were less affected by UV exposure than those in eggs with low MAA concentrations (Adams and Shick 1996, 2001).

The exact photo protective value of MAAs in organisms actively synthesizing these compounds is difficult to evaluate, as MAA concentration usually reflects irradiance conditions of the habitat of the organism. Thus, comparing specimens of one species but with different MAA concentrations always means comparing specimens with a different irradiation history. Specimens with high MAA concentrations are either already acclimated to UV radiation or high PAR intensities, while those with low MAA concentration may neither have been exposed to additional UV radiation nor to high PAR intensities. This results in a different state of acclimation prior to the test irradiance and to difficulties in interpreting the data received.

Thus, specimens of *C. crispus* grown under blue light accumulated similar MAA concentrations to those grown under PAR, yet photosynthesis of blue light-grown thalli were significantly more sensitive to UV-A than those of thalli grown under PAR.

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Because of their different irradiation history, PAR-grown thalli may have a greater capacity for repair and mitigating mechanisms (e.g. more antioxidants and antioxidant enzymes) or they may have smaller photosynthetic unit targets, reflected in lower phycoerythrin and phycocyanin concentrations relative to the concentration of chlorophyll a (Franklin et al. 2001; Pub. III).

Comparing closely related species that exhibit different MAA concentrations when grown under the same irradiance conditions does not help to prove the photo protective value of MAAs, because every species has specific acclimation properties with different repair and mitigating mechanisms. For example, photosynthesis in *C. crispus* was more strongly affected by UV-B radiation than in *Mastocarpus stellatus* (Bischof et al. 2000; Pub. II). This may be due to the 6-fold higher MAA concentration in *M. stellatus*: whilst shinorine, the major MAA in *M. stellatus*, has its absorption maximum at 334 nm, additional extinction coefficients calculated for the wavelengths 320 and 310 nm, were still 87 % of that of palythine (λ_{max} = 320 nm) and 60 % of that of mycosporine-glycine (λ_{max} = 310 nm), indicating that shinorine is also an effective UV-B screen (Adams and Shick 2001). On the other hand, the relative robustness of *M. stellatus* may simply be part of its generally higher stress tolerance (Dudgeon et al 1989, 1990, 1995). Thus, a different genetic adaptation of *M. stellatus* may be responsible for protecting photosynthetic activity and growth during desiccation and freezing while these functions were impaired in *C. crispus* under the same stress conditions.

Nevertheless, both experiments indicate that MAAs protect photosynthesis against UV-A stress. Photosynthetic activity of neither *C. crispus* nor *M. stellatus* was affected by UV-A radiation (Bischof et al. 2000; Pub. II). And although *C. crispus* grown under blue light was more sensitive to UV-A than individuals grown under PAR, they were more resistant than specimens grown under green and red light, in which only traces of MAAs were detected (Franklin et al. 2001; Pub. III). These results are in line with biological weighting functions calculated for the inhibition of photosynthesis by UV radiation in dinoflagellates, showing that photosynthetic sensitivity to UV radiation in specimens with high MAA concentrations is lower at wavelengths strongly absorbed by MAAs (Neale et al. 1998 a, Lichtman et al. 2002).

The sunscreen function of MAAs is one out of many acclimation mechanisms and information about the other acclimation processes is required to estimate how "important" MAAs may be. Among other factors, acclimation to different light conditions involves the pigmental restructuring of PS I and PS II to modulate the light

harvesting efficiency (Lüder et al. 2002), as well as dynamic photo inhibition (Hanelt et al. 1997, Hanelt 1998) and the activation of antioxidative enzymes (Aguilera et al. 2002 b).

Exposing laboratory-grown thalli of *C. crispus* in shallow water to natural irradiance indicated that lutein plays an important role in acclimation to higher irradiance by non-photochemical quenching of excess light energy (Niyogi et al. 1997, Pogson et al. 1998), while MAAs block UV radiation. For thalli grown under the cut-off filters WG320 to GG495, a linear correlation was found between final MAA concentration and "theoretical" irradiance underneath the filters (Pub. IV). Therefore, MAA concentrations can be flexibly adjusted to the radiation conditions, providing exactly the necessary sunscreen effect.

The accumulation of MAAs may, therefore, be estimated as an additional but important acclimation mechanism, which has its advantage over long time scales. In *C. crispus* this additional protection can be established parallel to the acclimation of photosynthetic activity (Pub. IV), while the diatom *Thalassiosira weissflogii* first accomplishes photo acclimation by an increase in xanthophyll cycle pigments and begins MAA synthesis only after the recovery of photosynthesis is complete (Zudaire and Roy 2001). As soon as the maximal MAA concentration is accumulated the concentration of photo protective xanthophyll cycle pigments declines in *T. weissflogii*, which emphasizes the importance of MAAs as photo protective compounds. Yet, these two examples clearly demonstrate the importance of investigating different acclimation mechanisms, because every species may regulate energy and carbon flow to different metabolic mechanisms and within different timescales. This may result in a different sequence of photo-protective compounds.

4.2 Role of MAAs as antioxidants or precursors of antioxidants

Desiccation, which can disrupt respiration and photosynthesis, can lead to an increased formation of ROS (Bowler et al. 1992, Smirnoff 1993, Alscher et al. 1997, Yordanov et al. 2000) and so too can high irradiance and UV radiation (Fridovich 1996, Mahalingan and Fedoroff 2003). ROS are normally subject to rapid enzymatic conversion to harmless molecules or are scavenged by antioxidants (Foyer et al. 1997, Potterat 1997). Nevertheless, they can also function as intercellular messengers during stress, by

regulating both transcriptional and post-transcriptional processes (Sauer et al. 2001, Droge 2002, Ermak and Davies 2002).

It has been suggested that MAAs act as biological antioxidants (Carreto et al. 1990 b, Dunlap and Yamamoto 1995). Imino MAAs are oxidatively stable, which is in line with their primary function as stable biological sunscreens, while mycosporine-glycine has an antioxidant activity which inhibits lipid per oxidation (Dunlap and Yamamoto 1995). *Vibrio* bacteria associated with holothurians convert shinorine and porphyra-334 from their algal diet into mycosporine-glycine and further into 4-deoxigadusol, providing a strong antioxidant (Dunlap and Shick 1998).

Field and laboratory experiments carried out in this study showed for the first time that the MAA concentration and composition in the red algal genus *Porphyra* is dependent on both irradiance and internal water content (Pub. I). In June, the MAA concentration of *Porphyra* spp. was four times higher than in May and September (laboratory experiment), revealing seasonal variations in the MAA concentration. During the experiments the imino MAA concentration was higher in submerged than in desiccated thalli. The decrease/increase in imino MAAs was dependent on the rate of desiccation of algae, which is influenced by irradiance and temperature. In parallel with the decrease/increase in imino MAAs the concentration of mycosporine-glycine changed. In May and during laboratory experiments highest mycosporine-glycine concentrations were found in desiccated thalli, while in June the highest mycosporine-glycine concentrations were found in submerged specimens. The ratio of mycosporine-glycine and imino MAAs was strongly influenced by irradiance. A linear correlation was found under PAR, while an exponential correlation was evident under high PAR and UV radiation.

This observed decrease of imino MAAs combined with a parallel increase of mycosporine-glycine may be interpreted as an antioxidant system to protect algae against ROS. During desiccation the internal ROS concentration in algae may increase. Increased ROS concentrations may lead to increased oxidation of specific senso-scavenging antioxidant compounds within signal transduction pathways, leading to an up regulation of ROS detoxification capacity (Foyer and Noctor 2003). Thus, ROS may activate the putative enzymes bio-converting shinorine and porphyra-334 (oxidatively stable) into mycosporine-glycine (antioxidant), in a way similar to the bioconversion of these MAAs through *Vibrio* bacteria in holoturians (Dunlap and Shick 1998).

The lower increase in mycosporine-glycine compared to the decrease in shinorine and porphyra-334 is explained through the decomposition of oxidized mycosporine-glycine (Dunlap and Yamamoto 1995). In summer, higher PAR and UV intensities may require that a pool of mycosporine-glycine exists prior to emersion, explaining the different kinetics of mycosporine-glycine concentrations. With rehydration the proposed enzymes may be inactivated and the pool of shinorine and porphyra-334 is refilled.

However, data about the actual ROS concentration during desiccation of thalli of *Porphyra* spp. were not measured during the experiment. Furthermore, enzymes catalysing the bioconversion are only proposed and need to be identified in further investigations.

Assuming that MAAs act in an antioxidant system, the different correlations between mycosporine-glycine and imino MAAs (Pub. I) may reflect the ROS production during desiccation and thus the efficiency of the antioxidant system. During desiccation and under moderate PAR fewer ROSs may be generated during photosynthesis and, in consequence, fewer mycosporine-glycine molecules will be oxidized. This may result in a linear correlation between mycosporine-glycine and imino MAAs as found under laboratory conditions. In contrast, during desiccation high PAR and UV intensities increase the production of ROS during photosynthesis, which may result in an immediate oxidation of all available mycosporine-glycine molecules and, thus, in an exponential correlation between mycosporine-glycine and imino MAAs as found in the field study conducted in May. In spring and autumn, when no mycosporine-glycine pool existed prior to emersion, the correlation between mycosporine-glycine and imino MAAs may be an indicator of ROS production during desiccation. Again, the link between ROS production, ROS concentration and MAA bioconversion as a possible antioxidant system must be established before the use of mycosporine-glycine and imino MAA correlations as indicators for ROS production can be established. Even if these factors are interrelated, differences in initial mycosporine-glycine concentrations during the year may make it impossible to define a profile for this process.

The seasonal differences also indicate that, beside the function of MAAs as UV sunscreens (e.g. Dunlap et al. 1986, Conde et al. 2000), a major role of MAAs in *Porphyra* spp. may be their function as antioxidant and as precursor of antioxidants. This is supported by the almost complete loss of MAAs during the tidal cycle in May (Pub. I). The high cost of de novo MAA synthesis further emphasizes the importance of the proposed antioxidant system. Presumably 60 ATP-equivalents or 600 moles of

photons captured in photosynthesis are necessary to synthesize one mole of MAAs (Shick and Dunlap 2002 and references therein). Neglecting an unknown, but possible, turnover of MAAs during some parts of the desiccation process, specimens of *Porphyra* spp. still have to synthesize a very large concentration of MAAs almost once a day (depending on the time of low tide and weather conditions), which reduces energy, carbon and nitrogen available for growth and cell division as well as other metabolic processes. The "daily" de novo synthesis of approximately 15 mg g^{-1} DW under sunny weather conditions exceeds the final MAA concentration reported for most red algae (Karsten et al. 1998 b, c, 2000).

This leads to the question, why is the function of MAAs as antioxidants or precursors of antioxidants more important than their function as UV sunscreens? By blocking UV radiation, MAAs prevent the production of ROS during photosynthesis, at least to some extent. This may lead to the assumption that ROS production triggered by desiccation and high PAR intensities outnumbers the additional ROS production due to UV radiation. Thus, scavenging of ROS in general may protect algal metabolism better than preventing ROS formation due to UV radiation.

In addition, the genus *Porphyra* belongs to the order Bangiales. Because of their simple vegetative and reproductive organization Bangiales are considered to be an ancestral taxa (Kraft and Wölkerling 1990). In the geological past, specimens had to cope with high UV radiation typical for Palaeozoic times, which may indicate that MAAs acting as sunscreens may have enhanced their chances of survival. On the other hand, their evolution took place during a period of high UV radiation, from ancestors adapted to these severe environmental conditions. Therefore, the need for an (additional) effective antioxidant system may have been greater than that for protection from UV radiation.

Nevertheless, MAAs acting in an antioxidant system is only prioritised during spring and autumn. In summer, the MAA concentrations are so high that MAAs can fulfil both antioxidant and sunscreen functions.

Because desiccation tolerance requires both mechanisms to prevent oxidative damage and mechanisms to maintain the native structure of membranes and macromolecules (Hoekstra et al. 2001), this proposed antioxidant system might contribute to the desiccation tolerance of the genus *Porphyra*. Enzymes involved in this process and their regulation needs to be characterized and investigated. Whether it is a common protection mechanism existing in all intertidal algae exhibiting MAAs must also be ascertained.

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4.3 Biosynthesis of MAAs in *Chondrus crispus*

4.3.1 Light regulated interconversion of MAAs

When *C. crispus* is grown under PAR supplemented with UV radiation, shinorine is accumulated rapidly, but concentrations decline after a few days and palythine becomes the major MAA (Pub. IV and V). These different kinetics indicate an interconversion within the MAAs. Shinorine is the most common MAA reported in macroalgae (Banaszak et al. 1998, Karsten et al. 1998) and based on similar kinetics, many authors assume a precursor relationship between shinorine and other MAAs (e.g. Franklin et al. 1999, Shick et al. 2000). To test this hypothesis, specimens were loaded with shinorine by exposing them to UV radiation. Thereafter, sub samples were covered with different cut-off filters and exposed to the full light spectrum. After approximately two days shinorine was converted into palythine in the ratio 1:1, with no significant differences between the different filter treatments. Because the cut-off filter GG495 was not included in this experiment it is not clear whether PAR in general triggers the interconversion or if blue light alone is responsible for the regulation of enzymes involved. Furthermore, asterina-330 was not detected throughout the experiment, yet the interconversion may proceed from shinorine via asterina-330 to palythine (Franklin et al. 1999, Kräbs et al. 2002).

Thalli grown under blue light alone accumulated palythine as the major MAA and only traces of shinorine, asterina-330 and palythene could be detected. A similar MAA composition was found for thalli grown under PAR (Franklin et al. 2001; Pub. III). However, in further experiments shinorine was also found in PAR exposed thalli (Pub. IV and V). Therefore, it may be concluded that palythine is synthesized directly from shinorine, although shinorine may not always be detectable.

In the case of an interconversion of shinorine via asterina-330 to palythine two lyases are necessary: first a decarboxylase catalysing the step from shinorine to asterina-330 and second a lyase separating the ethoxy group of asterina-330, which results in palythine. A similar step-wise interconversion of porphyra-334 via palythenic acid to usujirene or palythene has been postulated (Carreto et al. 1990 b). For this interconversion a hydrolyase and a decarboxylase would be necessary.

On the other hand, Portwich and Garcia-Pichel (2003) postulated a shinorine synthase catalysing the condensation of serine to mycosporine-glycine and further proposed that

all imino MAAs are synthesized directly from mycosporine-glycine by the condensation of the respective amino compound to the C_1 atom of ring structure.

However, the biosynthetic pathway of MAAs is not conclusively known and nor are the identities of all enzymes involved in the process. It is therefore difficult to establish, whether MAAs are interconverted from a few precursor MAAs, such as shinorine and porphyra-334 or directly synthesized from mycosporine-glycine by the condensation of the respective amino compound to the C_1 atom of the ring structure. A parallel existence of both biosynthetic routes is also possible. Because amino acids are basic structural compounds, all MAAs with an amino acid conjugated to the C_1 atom might be synthesized directly from mycosporine-glycine catalysed by an amino acid-specific ligase, while the other MAAs may result from interconversion catalysed by different lyases.

Polychromatic response spectra for MAA formation were calculated for both field and laboratory experiments (Pub. IV and V). Because MAA accumulation occurs already in specimens exposed under PAR without the major part of blue light, the calculated response spectra can not be interpreted as the absorption spectrum of the photoreceptor triggering MAA formation (see Coohill 1992, 1994). In this case, polychromatic response spectra merely coincide with the wavelength dependence of enzyme induction/inhibition necessary for interconversion/biosynthesis of MAAs. Under high irradiance (field experiment; Pub. IV) both blue light and long-wavelengths UV-A stimulates the interconversion of shinorine to palythine, while UV-B radiation may have an inhibitory effect. This inhibitory effect may not be restricted to the enzymes catalysing MAA interconversion but may also affect enzymes involved in the biosynthesis of shinorine, resulting in lower MAA concentrations. Under low irradiance (laboratory experiments; Pub. V) blue light and long-wavelengths UV-A are also stimulants. In contrast to high UV-B radiation, low UV-B radiation may stimulate the interconversion of MAAs.

Once again, all enzymes involved in the biosynthesis of MAAs are still unknown and need to be identified.

4.3.2 Signal transduction in the initiation of MAA biosynthesis

Thus far the term "induction" has been avoided, because it is historically associated with gene regulation. As MAA accumulation mostly starts from initial low MAA concentrations, it is not quite clear whether enzymes necessary for MAA synthesis are constitutive or induced. An up regulation of MAA formation via an increased transcription of biosynthetic enzymes as a specific response to UV radiation is possible (Shick et al. 2000). Thus the transcription of genes coding DAHP synthase, the first enzyme of the shikimate pathway, increases under UV radiation (Logemann et al. 2000). Furthermore, the expression of other isoenzymes of the shikimate pathway is dependent on environmental conditions (Herrmann and Weaver 1999), one of which may be irradiance (Weaver and Herrmann 1997). This might be important, because it has been proposed that MAA biosynthesis is an early branch of the shikimate pathway (Shick et al. 1999, Shick and Dunlap 2002, Portwich and Garcia-Pichel 2003).

Nevertheless, photoreceptors for specific wavebands of UV radiation and PAR are possible, because a dose-dependent MAA formation was observed for UV radiation and blue light, but not for red light (Carreto et al. 1990 a). In *C. crispus* the existence of photoreceptors is probable because UV stimulated shinorine formation persists during darkness and low irradiance monochromatic light induces shinorine formation (Pub. VI).

Beside the photo induction of MAAs a stimulation of MAA formation through ROS has been discussed (see Shick et al. 2000 and references therein). Using the band pass filter UG5 that is transparent for UV radiation and wavelengths above 650 nm, evidence against ROS stimulation and for photo induction was found (Pub. V). Because production of ROS is dependent on photosynthetic electron transport during photosynthesis (Foyer and Noctor 2003), it can be assumed that the concentration of ROS was low during the experiment. This is supported by results showing that malondialdehyde concentrations, which indicate production of ROS remained unchanged in *Ulva rotundata* exposed under the filter UG5 to natural radiation (Bischof et al. 2003). Because similar MAA concentrations were accumulated in algae exposed to either the full spectrum or UV radiation a photo induction of MAA formation can be assumed (Pub. V).

Nevertheless, the comparison of final MAA concentrations synthesized in algal thalli exposed to continuous light to those exposed to a light dark cycle may indicate an additional stimulation through ROS. Specimens exposed under the cut-off filters

WG345 to GG420 exhibited similar MAA concentrations irrespective of the duration of exposure, while specimens grown under filters WG320 and WG335 differed significantly in their final MAA concentration. Specifically, thalli exposed under filters WG320 and WG335 to continuous light accumulated 1.43- and 1.75-folds higher MAA concentrations, respectively, than those exposed under a light-dark cycle. Because ROS production is enhanced under UV radiation and stress in general (Foyer and Noctor 2003), this further increase in MAA concentration may be due to ROS stimulation. At this time, stimulation via ROS is regarded as key regulator of plant metabolism, acting as a reinforcement to withstand further stress (Foyer and Noctor 2003, Mahalingam and Fedoroff 2003).

4.3.3 Putative Photoreceptors triggering MAA synthesis

Polychromatic response spectra and experiments with band pass filters revealed a high quantum efficiency for MAA formation for two wavebands, UV-A radiation and blue light (Franklin et al. 2001; Pub. III, IV and V), indicating the presence of two photoreceptor chromophores. An action spectrum in the range of 280 to 750 nm was determined for MAA formation to obtain the absorption characteristics of the putative photoreceptors (Pub. VI). The action spectrum obtained indicated that the photoreceptor mediating shinorine photo induction might be an as yet unknown UV-A photoreceptor with absorption peaks at 320, 340 and 400 nm. This action spectrum, which should match the absorption spectrum of the photoreceptor chromophore (Schäfer and Fukshansy 1984, Coohill 1990, Holmes 1997), does not compare well with the absorption spectra of the UV-A/blue light type photoreceptors (Watanabe 1995) for which several flavoproteins (cryptochromes, phototropins, photo activated adanylyl cyclase, Appt and WC-1) have been identified (Watanabe 2004 and references therein). Therefore, one (or two) as yet unidentified UV-A photoreceptor(s) is responsible for triggering shinorine formation. Because pterins can form radicals, undergo redox reactions and absorb UV radiation, all necessary features of photochromophores, each of the action spectral peaks might well be attributable to the absorption spectral peaks of different pterin chromophores in these UV-A photoreceptor proteins (Galland and Senger 1988).

The absorption characteristics of the putative blue light photoreceptor chromophore (see also Carreto et al. 1990 a) could not be discovered, although blue light became inductive for fluence rates above 50 μmol m^{-2} s^{-1} (Pub. VI). This might be explained

through a lower sensitivity of this chromophore towards PAR than of the as yet unknown UV-A photoreceptor chromophore towards UV-A radiation (see also Karsten et al. 1998 a). Because blue light and UV-A can act synergistically, a flavin based cryptochrome may be the chromophore of the putative blue light photoreceptor (Franklin et al. 2001; Pub. III).

Cryptochromes are a diverse group of flavoprotein blue light photoreceptors, which share sequence homology with photolyase repair enzymes (Sancar 2003, Özgür and Sancar 2003). Together, cryptochromes and photolyases form the cryptochrome/photolyase protein family (Brudler et al. 2003). In contrast to photolyases, cryptochromes can not repair UV-induced DNA damage, but their crystal structure has similarities in DNA recognition and redox activity to those of cyclobutane-pyrimidine dimer photolyases (Brudler et al. 2003). Furthermore, cryptochromes of the subfamily "cryptochrome DASH" (isolated from Drosophila, Arabidopsis, Synechocystis and Homo) can bind DNA and act in the transcriptional regulation (Brudler et al. 2003). However, the photochemical mechanism of signal transduction of cryptochromes is still unknown (Sancar 2003).

Cryptochromes play a role in the regulation of the circadian clock and in many development processes (Hegemann et al. 2001, Sancar 2003). They contain FAD as the first chromophore and folate or deazaflavin as the second chromophore (Hegemann et al. 2001).

Taking the close relationship between photolyases and cryptochromes into consideration, a FAD/deazaflavin cryptochrome may be a more likely candidate for the blue light photoreceptor than a FAD/folate cryptochrome. Folate class photolyases have their absorption maximum between 370 and 420 nm, while all deazaflavin class photolyases have the same maximum at 440 nm (Sancar 2003). The latter would be a better fit for MAA induction, which occurs even in algae exposed to irradiance under the filter GG495 to irradiance (Pub. IV and V). Below 350 nm the absorption by the cryptochrome may sink into insignificance compared to the absorption by the unknown UV-A photoreceptor chromophore, which seems to be more sensitive to UV-A radiation.

However, it remains to be proved that a FAD/deazaflavin cryptochrome or a cryptochrome in general is in fact the blue light photoreceptor chromophore for MAA induction.

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MAA formation may, therefore, be mediated through a UV-A and a blue light-absorbing chromophore, which may act synergistically. Whether they are based in one or in individual photoreceptor protein(s) needs to be ascertained.

The advantage of two chromophores interacting is a broader screening of the radiation spectrum received by plants (Casal 2000). The UV-A chromophore may generate a "fast" response (within hours or days), while the response provided by the blue light chromophore might cover longer time scales (weeks). Therefore, signal transduction via the UV-A chromophore may be important for *C. crispus* growing in the intertidal as well as for subtidal specimens, which may be exposed to higher UV radiation during times of greater water transparency. The blue light chromophore may be advantageous if an increase in blue light preceded increased UV radiation (Franklin et al. 2001; Pub. III)

Furthermore, the blue light chromophore may also regulate the accumulated MAA concentration. After induction, high MAA concentrations block UV-A radiation before it reaches the UV-A photoreceptor by which the final MAA concentration may be set. Yet *C. crispus* adjusts the MAA concentration very flexibly to the given radiation conditions. Therefore, a signal for an active reduction of MAAs is needed in times of low irradiance, which can then only be sensed through the intensity of blue light. Thus, the assumed long scale response of the blue light chromophore may guarantee that the concentration of MAA molecules is only enzymatically reduced during long-term low irradiances. Accordingly, seasonal variations in MAA content may be regulated without an unnecessary waste of energy resources through the *de novo* synthesis of MAAs after short-term low irradiances. This is supported by the variation in MAA content of field specimens of *C. crispus* transferred to 25 μmol m^{-2} s^{-1} PAR. Initial MAA concentrations were maintained for approximately two weeks, after which a decline MAA concentration was observed (unpublished data).

4.4 Conclusion

MAA biosynthesis is a very complex process with many interacting parameters influencing its different steps or their biosynthesis in general. To split the process into different parameters either inducing, stimulating or even inhibiting biosynthesis, is therefore almost impossible. Based on the present results, a model for MAA biosynthesis is proposed (Fig. 4.1). Specific wavebands induce the MAA biosynthesis and regulate putative enzymes involved in the process (Pub. III, IV and V). In parallel with the high quantum efficiency for MAA induction by UV-A, these wavelengths may depress photosynthetic activity. However, an impaired photosynthetic activity provides lower concentrations of energy equivalents and less fixed carbon molecules, which may inhibit the biosynthesis of MAAs. On the other hand, blue light may stimulate both MAA induction and photosynthesis. Sufficient energy equivalents and carbon molecules of photosynthesis may further support an induced MAA synthesis. This may result in an additive or synergistic effect on accumulated MAA concentration. Furthermore, a UV- and/or stress-induced increase of ROS production may provide a further stimulation of MAA formation (Pub. V) and/or a bioconversion of the imino MAAs shinorine and porphyra-334 into mycosporine-glycine, which may act as an antioxidant (Pub. I). However, accumulated MAAs provide a UV-A sunscreen (Pub. II, III and IV), changing the interplay of the different metabolic processes once more.

It could be proved that MAAs are photo-inducible. The absorption characteristics of the putative blue light receptor could not be identified, but an, as yet unknown, UV-A photoreceptor with absorption peaks at 320, 340 and 400 nm may mitigate the photo induction of shinorine.

Further investigations should aim to identify the biosynthetic pathway of MAA biosynthesis and the enzymes catalysing both biosynthesis and interconversion of MAAs. Based on a detailed knowledge of the different compounds involved, different stress scenarios in vivo and in vitro may help to understand the interaction of abiotic influences and biotic responses. The generation of different MAA mutants may give further insight into the regulation of MAA biosynthesis. Furthermore, different taxa should be investigated under similar irradiance and/or stress conditions to provide more information about the general distribution of the regulating mechanisms. For example,
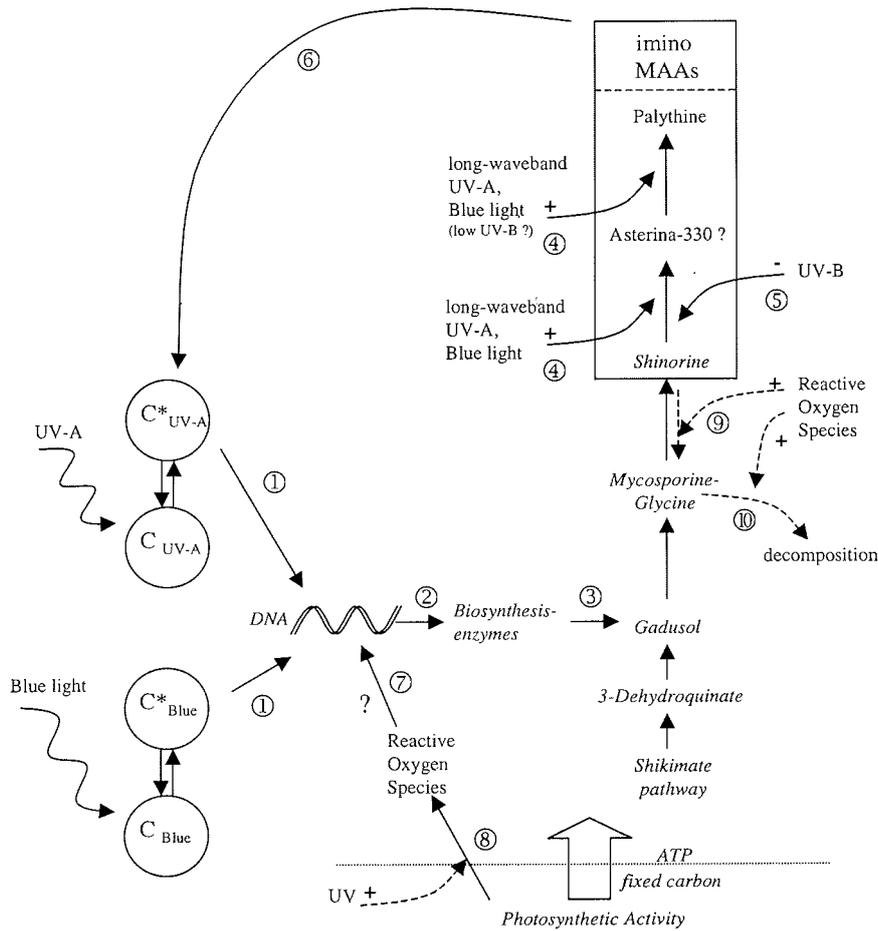
Figure 4.1: Model for MAA biosynthesis and regulating factors: UV-A radiation and blue light are sensed by the respective photoreceptor chromophores (C_{UV-A} or C_{Blue}). When activated they (C^*_{UV-A} or C^*_{Blue}) transmit the signal into the cell ①, inducing the gene expression ② for enzymes catalysing MAA biosynthesis ③. The interconversion of shinorine presumably via asterina-330 to palythine is stimulated by UV-A radiation and blue light ④ and inhibited by UV-B radiation ⑤. Accumulated imino MAA concentrations shade the UV-A chromophore ⑥. A further stimulation of MAA biosynthesis ⑦ may be due to an UV-induced increased formation of reactive oxygen species during photosynthetic activity ⑧. – Reactive oxygen species may induce the bioconversion of shinorine into mycosporine-glycine ⑨, which may act as antioxidant ⑩. – ATP and carbon molecules are provided by photosynthetic activity ⇨; solid arrows indicate regulatory mechanisms found for *Chondrus crispus*; dotted arrows those found for *Porphyra* ssp.; italic letters indicate literature results (Shick et al. 2000, Portwich and Garcia-Pichel 2003).

the putative UV-A and blue-light photoreceptors postulated to mitigate MAA synthesis in *C. crispus* might be species specific. In contrast to *C. crispus*, MAA formation in the cyanobacterium *Chlorogloeopsis* PCC6912 may be induced through a UV-B photoreceptor (Protwich and Garcia-Pichel 2000).

4.5 Ecological Outlook

MAAs provide a sunscreen against UV-A radiation (Pub. II, III, and IV; Neale et al. 1998 a). Some species, like *C. crispus,* are able to adjust both concentration and composition of MAAs flexibly to irradiance conditions (Pub. II, III, IV and V). The sunscreen potential of MAAs is especially important for intertidal species. Repair and mitigating processes are impaired in desiccated thalli, therefore MAAs confer an advantage by blocking UV radiation and thus preventing stress reactions within the algal metabolism, at least to some extent.

High concentrations of MAAs may also provide some protection against UV-B radiation (Pub. II). However, the localization of MAAs in macroalgae is, as yet, unknown. They may be homogeneously distributed in the cytoplasma of all cells, as suggested for cyanobacteria (Garcia-Pichel 1994), but a targeted localization in specific cell types, as recently discovered in ascidians (Maruyama et al. 2003), is also possible. In the case of targeted localization in the cortex, the screening factor for medulla lying directly beneath the cortex will be higher, and this is more advantageous for the organism in general than in the case of a homogeneous distribution. Findings that self-shaded basal parts of algae contain much lower MAA concentrations than exposed apical tips (Karsten et al. 1999, Karsten and Wiencke 1999) indicate that algal parts or even single cells adjust their MAA concentration to the given radiation conditions. This might further indicate that cells of the cortex have higher MAA concentrations than those of the medulla.

The putative photoreceptor(s) proposed for MAA formation in *C. crispus* enables a flexible adjustment of MAA concentration to variations of the spectral composition of irradiance caused through emersion, changes in the water transparency and/or seasonal variations. Nevertheless, they are not sensitive to UV-B radiation and are therefore unable to mitigate an additional increase in MAA content in the case of rising UV-B levels during ozone depletion.

Furthermore, high levels of UV-B inhibit the synthesis of MAAs in some species (Pub. IV and V; Hoyer et al. 2002). The target for the inhibition is yet unknown, but UV-B might affect multiple targets within the algal metabolism. UV-B impairs photosynthesis and enzymes, which may both result in a less effective MAA biosynthesis. In addition, other repair and mitigating processes, for example photolyase enzymes, antioxidants and antioxidant enzymes, may be preferred, redirecting energy and carbon flow.

Beside the effect of UV-B on algal metabolism in general, the ratio of UV-B:UV-A:PAR may influence the final MAA concentration in some ways. Thus, already accumulated MAA concentrations decrease when algae are exposed to the full light spectrum (Pub. V) although MAAs are photostable (Adams and Shick 1996, 2001, Conde et al.2000).

Therefore, ozone depletion may have a negative effect on MAA formation and thus on the organism in general. Under natural sunlight *C. crispus* grown under the full light spectrum accumulated 50 % less MAAs than specimens exposed under the cut-off filter WG305. Because UV-A and PAR intensities were similar, this may be due to a 1.5-fold higher UV-B radiation (Pub. IV). Under the experimental conditions neither photosynthesis nor pigment concentrations were affected. Nevertheless ozone depletion will shift the ratio between UV-B:UV-A:PAR towards shorter wavelengths, which may result in an impaired balance between damaging effects and repair and mitigating mechanisms (Smith et al. 1989, Halliwell and Gutteridge 1989).

Besides their photo protective function, MAAs may also act as osmolytes (Oren 1997, Karsten 2002), quenchers of the exited thymine residue, which results in DNA protection (Misonou et al. 2003) and antioxidants and precursors of antioxidants (Dunlap and Yamamoto 1995, Dunlap and Shick 1998, Nakayama et al. 1999). For the latter the knowledge of both metabolic rates and of the underlying mechanism is necessary to assess detected MAA concentrations and their impact for algal metabolism.

5. References

Adams NL, Shick JM. 1996. Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of green sea urchin *Strongylocentrotus droebachiensis*. J Photochem Photobiol B 64:149-158

Adams NL, Shick JM. 2001. Mycosporine-like amino acids prevent UV-B induced abnormalities during early development of the green sea urchin *Strongylocentrotus dreobachiensis*. Mar Biol 138:267-280

Aguilera J, Karsten U, Lippert H, Vögele B, Philipp E, Hanelt D, Wiencke C. 1999. Effects of solar radiation on growth, photosynthesis and respiration of marine macroalgae from the Arctic. Mar Ecol Prog Ser 191:109-119

Aguilera J, Bischof K, Karsten U, Hanelt D, Wiencke C. 2002a. Seasonal variations in ecophysiological patterns in macroalgae from an Arctic fjord. II Pigment accumulation and biochemical defence systems against high light stress. Mar Biol 140:1087-1095

Aguilera J, Dummermuth A, Karsten U, Schriek R, Wiencke C. 2002b. Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. Polar Biol 25:432-441

Alscher RG, Donahue JL, Cramer CL. 1997. Reactive oxygen species and antioxidants: relationships in green cells. Physiol Plant 100:224-233

Banaszak AT, Lesser MP, Kuffner IB, Ondrusek M. 1998. Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAs) in marine organisms. Bull Mar Sci 63:617-628

Bandaranayake WM. 1998. Mycosporines: are they nature's sunscreens? Nat Product Reports 15:159-172

Batschauer A. 1993. A plant gene for photolyase: an enzyme catalyzing the repair of UV-light-induced DNA damage. Plant J 4:705-709

Bhalla US, Iyengar R. 1999. Emergent properties of networks of biological signalling pathways. Science 283:381-387

Bischof K, Kräbs G, Hanelt D, Wiencke C. 2000. Photosynthetic characteristics and mycosporine-like amino acids under UV radiation: a competitive advantage of *Mastocarpus stellatus* over Chondrus crispus at the Helgoland shoreline? Helgoland Mar Res 54:47-52

Bischof K, Kräbs G, Wiencke C, Hanelt D. 2002a. Solar ultraviolet radiation affects the activity of ribulose-1,5-bisphosphate carboxylase-oxygenase and the composition of photosynthetic and xanthophyll cycle pigments in the intertidal green alga *Ulva lactuca* L.. Planta 215:502-509

Bischof K, Peralta G, Kräbs G, van de Poll WH, Pérez-Lloréns JL, Breeman AM. 2002b. Effects of solar UV-B radiation on canopy structure of *Ulva* communities from southern Spain. J Exp Bot 53:2411-2421

Bischof K, Janknegt PJ, Buma AGJ, Rijstenbil JW, Peralta G, Breeman AM. 2003. Oxidative stress and enzymatic scavenging of superodide radiacals induced by solar UV-B radiation in *Ulva* canopies from southern Spain. Sci Mar 67:353-359

Bischoff-Bäsmann B, Wiencke C. 1996. Temperature requirements for growth and survival of Antarctic Rhodophyta. J Phycol 32:525-535

Bowler C, Montagu M, Inzé D. 1992. Superoxide dismutase and stress tolerance. Annu Rev Physiol Plant Mol Biol 43:83-116

Brudler R, Hitomi K, Daiyasu H, Toh H, Kucho K-I, Ishiura M, Kanehisa M, Roberts VA, Todo T, Tainer JA, Getzhoff ED. 2003. Identification of a new cryptochrome class: structure, function, and evolution. Molecular Cell 11:59-67

Carreto JI, Lutz VA, de Marco SG, Carignan MO. 1990 a. Fluence and wavelength dependence of mycosporine-like amino acid synthesis in the dinoflagellate *Alexandrium excavatum*. In: Granéli E, Sundström B, Edler L, Anderson DM, eds. Toxic Marine Phythoplankton; New York; Elsevier. p. 275-279

Carreto JI, Carignan MO, Daleo G, de Marco SG. 1990 b. Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? J Plankton Res 12:909-921

Carreto JI, Carignan MO, Montoya NG. 2001. Comparative studies on mycosporine-like amino acids, paralytic shellfish toxins and pigment profiles of the toxic dinoflagellates *Alexandrium tamarense*, *A. catenella* and *A. minutum*. Mar Ecol Prog. Ser 223:49-60

Casal JJ. 2000. Phytochromes, cryptochromes, phototropin: photoreceptor interactions in plants. Photochem Photobiol 71, 1-11

Cockell CS, Knowland J. 1999. Ultraviolet radiation screening compounds. Biol Rev 74:311-345

Cockell CS, Blaustein AR, eds. 2001. Ecosystems, evolution, and ultraviolet radiation. New York, Springer-Verlag

Collén J, Davison IR. 1999. Reactive oxygen production and damage in intertidal *Fucus* spp. (Phaeophyceae). J Phycol 35:54-41

Conde FR, Churio MS, Previtali CM. 2000. The photorprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. J Photochem Photobiol B 56:139-144

Coohill TP. 1989. Ultraviolet action spectra (280 to 380 nm) and solar effectiveness spectra for higher plants. Photochem Photobiol 50:451-457

Coohill TP. 1990. Action spectra again? Photochem Photobiol 54:859-870

Coohill TP. 1992. Action spectra revisited. J Photochem Photobiol B 13:95-100

Coohill TP. 1994. Exposure response curves action spectra and amplification factors. In: Biggs RH and Joyner MEB, eds Stratospheric Ozone depletion/UV-B Radiation in the Biosphere; Berlin; NATO ASI Series, Springer, Vol. 108 p. 57-62

Cullen JJ, Neale PJ. 1997. Biological weighting functions for describing the effect of ultraviolet radiation on aquatic systems. In: Häder D-P, ed. The effects of Ozone Depletion on Aquatic Ecosystems; Austin: R. G. Landes, p. 97-118

Daubeny C. 1836. On the action of light upon plant and plants upon the atmosphere. Phil Trans R Soc London 149-179

Day TA, Neale PJ. 2002. Effects of UV-B radiation on terrestrial and aquatic primary producers. Annu Rev Ecol Syst 33:371-396

de Mora SJ, Demers S, Vernet M, eds. 2000. The effects of UV radiation on marine ecosystems. Environ Chem Ser Cambridge, Cambridge Univ. Press

Draper JW. 1884. Note on decomposition of carbon acid by the leaves of plants under the influence of yellow light. Phil Mag 25:159-173

Dring MJ, Makarov V, Schoschina E, Lorenz M, Lüning K. 1996. Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages oh three species of *Laminaria* (Phaeophyta). Mar Biol 126:183-191

Droge W. 2002. Free radicals in the physiological control of cell function. Physiol Rev 82:47-95

Duarte CM. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41:87-112

Dudgeon SR, Davison IR, Vadas RL. 1989. Effect of freezing on photosynthesis of intertidal macroalgae: relative tolerance of *Chondrus crispus* and *Mastocarpus stellatus* (Rhodophyta). Mar Biol 101:107-114

Dudgeon SR, Davison IR, Vadas RL. 1990. Freezing tolerance in the intertidal red algae *Chondrus crispus* and *Mastocarpus stellatus*: relative importance of acclimation and adaptation. Mar Biol 106:427-436

Dudgeon SR, Kübler JE, Vadas RL, Davison IR. 1995. Physiological response to environmental variation in intertidal red algae: does thallus morphology matter? Mar Ecol Prog Ser 117:193-206

Dummermuth AL, Hoyer K, Karsten U, Wiencke C. 2003. Seasonal variation in ecophysiological patterns in two red macroalgae from Artic Konsfjord (Spitsbergen, Norway) in a long-term culture study with emphasis on UV protective mechanisms I. *Palmaria palmate* (L.) Greville. In: Dummermuth AL, Antioxidative properties of marine macroalgae from the Arctic, Ber Polarforsch 458:118-146

Dummermuth AL, Hoyer K, Karsten U, Wiencke C. 2003. Seasonal variation in ecophysiological patterns in two red macroalgae from Artic Konsfjord (Spitsbergen, Norway) in a long-term culture study with emphasis on UV protective mechanisms I. Devaleraea ramentacea (L.) Guiry. In: Dummermuth AL, Antioxidative properties of marine macroalgae from the Arctic, Ber Polarforsch 458:146-168

Dunlap WC, Chalker BE. 1986. Identification and quantification of near-UV absorbing compounds (S-320) in hermatypic scleractinian. Coral Reefs 5:155-159

Dunlap WC, Chalker BE, Oliver JK. 1986. Bathymetric adaptions of the reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UB-B absorbing compounds. J Exp Mar Biol Ecol 104:239-248

Dunlap WC, Yamamoto Y. 1995. Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. Comp Biochem Physiol 112B:105-114

Dunlap WC, Shick MJ. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspective. J Phycol 34:418-430

Dunlap WC, Shick JM, Yamamoto Y. 2000. UV protection in marine organisms. I. sunscreens, ocidative stress and antioxidants. In: Yoshikawa S, Toyokuni S, Yamamoto Y, Naito Y, eds. Free Radicals in Chemistry, Biology and Medicine; London: OICA Int. p. 200-214

Dunton KH, Schell DM. 1987. Dependence of consumers on macroalgal (*Laminaria solidungula*) carbon in an Arctic kelp community: 13C evidence. Mar Biol 93:615-625

Engelmann TW. 1882. Über Sauerstoffausscheidung von Pflanzenzellen im Microspectrum. Bot Zeit 40:419-426

Ermark G. Davies KJ. 2002. Calcium and oxidative stress: from cell signaling to cell death. Mol Immunol 38:713-721

Favre-Bonvin J, Bernillon J, Salin N, Arpin N. 1987. Biosynthesis of mycosporines: mycosporine glutaminol in *Trichothecium roseum*. Phytochemistry 26:2509-2514

Foyer CH, Lopez-Delago H, Dat JF, Scott IM. 1997. Hydrogen peroxide- and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. Physiol Plant 100:241-253

Foyer CH, Noctor G. 2003. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119:355-364

Franklin LA, Forster RM. 1997. The changing irradiance environment: consequences for marine mycophyte physiology, productivity and ecology. Eur J Phycol 32:207-232

Franklin LA, Yakovleva I, Karsten U, Lüning K. 1999. Synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae) and the consequences for sensitivity to ultraviolet B radiation. J Phycol 35:682-693

Franklin LA, Kräbs G, Kuhlenkamp R. 2001. Blue light and UVA radiation control the synthesis of mycosporine-like amino acids in *Chondrus crispus* (Florideophyceae). J Phycol 37:257-270

Frederick JE, Snell HE, Haywood EK. 1989. Solar ultraviolet radiation at the earth's surface. Photochem Photobiol 50:443-450

Fridovich I. 1996. Biological effects of the superoxide radical. Arch Biochem Biophys 247:1-11

Galland P, Senger H. 1988. The role of pterins in the photoreception and metabolism of plants. Photochem Photobiol 48:811-820

Garcia-Pichel F, Castenholz RW. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J Phycol 27:395-409

Garcia-Pichel F. 1994. A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol Oceanogr 39:1704-1717

Garcia-Pichel F. 1998. Solar ultraviolet and the evolutionary history of cyanobacteria. Origins of Life and Evolution of the Biosphere 28:321-347

Gleason DF. 1993. Differential effects of ultraviolet radiation on green and brown morphs of the Carribean coral *Porites asteroids*. Limnol Oceanogr 38:1452-1463

Gómez I, Weykam G, Klöser H, Wiencke C. 1997. Photosynthetic light requirements, metabolic carbon balance and zonation of sublittoral macroalgae from King George Island (Antarctica). Mar Ecol Prog Ser 148:281-293

Gómez I, Pérez-Rodrígez E, Viñegla B, Figueroa FL, Karsten U. 1998. Effects of solar radiation on photosynthesis, UV-absorbing compounds and enzyme activities of the green alga *Dasycladus vermicularis* from southern Spain. J Photochem Photobiol B 47:46-57

Gómez I, Figueroa FL, Sousa-Pinto I, Viñegla B, Pérez-Rodrígez E, Maestre C, Coelho S, Felga A, Pereira R. 2001. Effects of UV radiation and temperature on photosynthesis as measured by PAM fluorescence in the red alga *Glidium pulchellum* (Turner) Kützing. Bot Mar 44:9-16

Goncalves RJ, Villafañe VE, Helbling EW. 2002. Photorepair activity and protective compounds in two freshwater zooplankton species (*Daphnia menucoensis* and *Metacyclops mendocinus*) from Patagonia, Argentina. Photochem Photobiol Sci 1:996-1000

Gröniger A, Häder D-P. 2002. Induction of the synthesis of an UV-absorbing substance in the green alga *Prasiola stipitata*. J Photochem Photobiol B 66:54-59

Häder D-P, Tevini M. 1987. General Photobiology. Oxford: Pergamon Press

Halliwell B, Gutteridge JMC. 1989. Free radicals in biology and medicine. Oxford, Clarendon Press

Hanelt D, Melchersmann B, Wiencke C, Nultsch W. 1997. Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255-266

Hanelt D. 1998. Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar Biol. 131:361-369

Hanelt D, Nultsch W. 2003. Photoinhibition in Seaweeds In: Heldmaier G, Werner D, eds. Environmental signals, processing and adaptation. Heidelberg, New York, Springer, p 141-167

Hegemann P, Fuhrmann M, Kateriya S. 2001. Algal sensory photoreceptores. J Phycol 37:668-676

Hellingwerf KJ, Hoff WD, Crielaard W. 1996. Photobiology of microorganisms: how photosensors catch a photon to initialize signaling. Mol Microbiol 21:683-693

Hernando M, Carreto JI, Carignan MO, Ferreyra GA, Groß C. 2002. Effects of solar radiation on growth and mycosporine-like amino acids content in *Thalasiosaria* sp., an Antarctic diatom. Polar Biol 25:12-20

Herrmann KM, Weaver LM. 1999. The shikimate pathway. Annu Rev Plant Physiol Plant Mol Biol 50:473-503

Hester RE, Harrison RM, eds. 2000. Causes and environmental implications of increased U.V.-B. radiation, Vol.14. Cambridge, R. Soc. Chem.

Hoekstra FA, Golovina EA, Buitink J. 2001. Mechanisms of plant desiccation tolerance. Trends in Plant Science 6:431-438

Holmes MG. 1997. Action spectra for UV-B effects on plants: monochromatic and polychromatic approaches for analyzing plant response. In: Lumsden PS, ed. Plant and UV-B: Response to Environmental Changes; Cambridge; Cambridge Univ Press, pp. 31-50

Hoyer K, Karsten U, Wiencke C. 2002. Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions. Mar Biol 141:619-627

Iken K. 1996.Trophische Beziehungen zwischen Makroalgen und Herbivoren in der Potter Cove (King-George-Insel, Antarktis). Ber Polarforsch 201

Iken K, Barrera-Oro ER, Quartino ML, Casaux RJ, Brey T. 1997. Grazing by the Antarctic fish *Notothenia coriiceps*: evidence for selective feeling on macroalgae. Antarctic Sci 9:386-391

Ito S, Hirata Y. 1977. Isolation and structure of a mycosporine from the zoanthid *Palythoa tuberculosa*. Tetrahedron Letters 28:2429-4230

Jeffrey SW, MacTavish HS, Dunlap WC, Vesk M, Groenwoud K. 1999. Occurence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae. Mar Ecol Prog Ser 189:35-51

Karentz D, McEuen FS, Land MC, Dunlap WC. 1991. Survey of mycosporine-like amino acid compounds in Artic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108:157-166

Karsten U, Franklin LA, Lüning K, Wiencke C. 1998 a. Natural ultraviolet radiation and photosynthetically active radiation induce formation of mycosporine.like amino acids in the amrine macroalga *Chondrus crispus* (Rhodophyta). Planta 205:257-262

Karsten U, Sawall T, Hanelt D, Bischof K, Figueroa FL, Flores-Moya A, Wiencke C. 1998 b. An inventory of UV-absorbing mycosporine-like amino acids in macroalgae from polar to warm temperate regions. Botanica Marina 41:443-453

Karsten U; Sawall T, Wiencke C. 1998 c. A survey of the distribution of UV-absorbing substances in tropical macroalgae. Phycol Res 46:271-278

Karsten U, Wiencke C. 1999. Factors controlling the formation of UV-absorbing mycosporine-like amino acids in the marine red alga *Palmaria palmata* from Spitsbergen (Norway). J Plant Physiol 155:407-415

Karsten U, Bischof K, Hanelt D, Tüg H, Wiencke C. 1999. The effects of ultraviolet radiation on photosynthesis and ultraviolet-absorbing substances in the endemic Arctic marcoalga *Devaleraea ramentacea* (Rhodophyta). Physiol Plant 105:58-66

Karsten U, West J. 2000. Living in the intertidal zone – seasonal effects on heterosides and sunscreen compounds in the red alga *Bangia atropurpurea* (Bangiales). J Exp Mar Biol Ecol 254:221-234

Karsten U, Sawall T, West J, Wiencke C. 2000. Ultraviolet sunscreen compounds in epiphytic red algae from mangroves. Hydrobiologia 432:159-171

Karsten U. 2002. Effects of salinity and ultraviolet radiation on the concentration of mycosporine-like amino acids in various isolates of the benthic cyanobacterium Microcoleaus chtonoplates. Phyol Res 50:129-134

Karsten U, Dummermuth A, Hoyer K, Wiencke C. 2003. Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters. Polar Biol 26:249-258

Kleczkowski A. 1972. Action spectra in their interpretation. In: Gallo U, Santamaria L, eds. Research Progress in Organic Biological and Medicinal Chemistry; New York: North Holland Publishing Company, American Publishing Company Inc.. Vol. 3 p. 48-70

Klöser H, Mercuri G, Laturnus F, Quartino ML, Wiencke C. 1994. On the competitive balance of macroalgae at Potter Cove (King George Island, South Shetlands). Polar Biol 14:11-16

Klöser H, Quartino ML, Wiencke C. 1996. Distribution of macroalgae and macroalgal communities in gradients of physical conditions in Potter Cove, King George Island, Antarctica. Hydrobiologia 333:1-17

Kraft GT, Wölkerling WJ. 1990. Rhodophyta. In: Clayton MN, King RJ. Eds. Biology of marine plants. Melbourne, Longeman, p. 41-85

Kräbs G, Bischof K, Hanelt D, Karsten U, Wiencke C. 2002. Wavelength-dependent induction of UV-absorbing mycosporine-like amino acids in the red alga *Chondrus crispus* under natural solar radiation. J Exp Mar Biol Ecol 268:69-82

Kuffner IB, Ondrusek ME, Lesser MP. 1995. Distribution of mycosporine-like amino acids in the tissues of Hawaiian *Scleractinia*: a depth profile. In: Gulko D, Jokiel PL, eds. Ultraviolet Radiation and Coral Reefs, Hawaii Institute of Marine Biology Tech Report 41, University of Hawaii, Honolulu, p. 77-85

Kuhlenkamp R, Franklin LA, Lüning K. 2001. Effects of solar ultraviolet radiation on growth in the marine macroalgae *Dictyota dichotoma* (Phaeophyceae) at Helgoland and its ecological consequences. Helgol Mar Res 55:77-86

Kulunscics Z, Perdiz D, Brulay E, Muel B, Sage E. 1999. Wavelength dependence of ultraviolet-induced DNA damage distribution: involvement of direct and indirect mechanisms and possible artifacts. J Photochem Photobiol B 49:71-80

Lao K, Glazer AN. 1996. Ultraviolet-B photodestruction of a light-harvesting complex. Proc Nat Acad Sci USA 93:5258-5263

Lesser MP. 1996. Elevated temperatures and ultraviolet radiation cause oxidative stress and inhibit photosynthesis in symbiotic dinoflagellates. Limnol Oceanogr 41:271-283

Lesser MP. 2000. Depth-dependent photoacclimatization to solar ultraviolet radiation in the Caribbean coral *Montastraea faveolata*. Mar Ecol. Prog Ser 192:137-151

Lesser MP, Barry TM. 2003. Survivorship, development, and DNA damage in echinoderm embryos and larvae exposed to ultraviolet radiation (290-400 nm). J Exp Mar Biol Ecol 292:75-91

Litchman E, Neale P, Banaszak A. 2002. Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: photoprotection and repair. Limnol Oceanogr 47:86-94

Logemann E, Tavernaro A, Schulz W, Somssich IE, Hahlbrock K. 2000. UV light selectively coinduces supply pathways from primary metabolism and flavonoid secondary product formation in parsley. PNAS 97:1903-1907

Lozán JL, Kausch H. 1998. Angewandte Statistik für Naturwissenschaftler. Berlin Parey,. 287 p.

Lüder UH, Wiencke C, Knoetzel J. 2002. Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta): a study to simulate Antarctic winter sea ice cover. J Phycol 38:904-913

Lüning K. 1990. Seaweeds: Their environment, biogeography and ecophysiology. John Wiley, New York

Madronich S, McKenzie RL, Caldwell MM, Björn LO. 1995. Changes in ultraviolet radiation reaching earth's surface. Ambio 24:143-152

Madronich S, McKenzie RL, Björn LO, Caldwell MM. 1998. Changes in biologically active ultraviolet radiation reaching the earth's surface. J Photochem Photobiol B 46:5-19

Mahalingam R, Fedoroff N. 2003. Stress response, cell death and signaling: the many faces of reactive oxygen species. Physiol Plant 119:56-68

Makarov MV, Voskoboinikov GM. 2001. The influence of ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina*. Bot Mar 44:89-94

Mann KH. 1973. Seaweeds: their productivity and strategy for growth. Science 182:975-981

Maruyama T, Hirose E, Ishikura M. 2003. Ultraviolet-Light-Absorbing Tunic Cells in Didemnid Ascidians Hosting a Symbiotic Photo-oxygenic Prokaryote, Prochloron. Reference Biol Bull 204:109-113

Menzel D, Kazlauskas R, Reichelt J. 1983. Coumarins in the siphonalean green algal family *Dasycladaceae* Kützing (Chlorophyceae). Bot Mar 16:23-29

Michalek-Wagner K. 2001. Seasonal and sex-specific variations in levels of photo-protecting mycosporine-like amino acids (MAAs) in soft corals. Mar Biol 139:651-660

Misonou T, Saitoh J, Oshiba S, Tokitomo Y, Maegawa M, Inoue Y, Hori H, Sakurai T. 2003. UV-absorbing substance in the red alga *Porphyra yezoensis* (Bangiales, Rhodophyta) block thymine photodimer production. Mar Biotechnol 5:194-200

Molina JM, Rowland FS. 1974. Stratospheric sink for chlorofluoromethanes: chlorine atom-catalysed destruction of ozone. Nature 249:810-812

Molina X, Montecino V. 1996. Acclimation to UV irradiance in *Gracialaria chilensis* Bird, McLachlan & Oliveira (Gigartinales, Rhodophyta). Hydrobiologia 326/327:415-420

Montzka SA, Butler JH, Elkins JW, Thompson TM, Clarke AD et al. 1999 Present and future trends in the atmospheric burden of ozone-depleting halogens. Nature 398:690-694

Muszynski FZ, Bruckner A, Armstrong RA, Morell JM, Corredor JE. 1998. Within-colony variations of UV absorption in a reef building coral. Bull Mar Sci 63:589-594

Nakajima S, Sugiyama M, Iwai S, Hitomi K, Otoshi E, Kim ST, Jiang CZ, Todo T, britt AB, Yamamoto K. 1998. Cloning and characterization of a gene (UVR3) required for photorepair of 6-4 photoproducts in Arabidopsis thaliana. Nucleic Acid Res 26:638-644

Nakayama R, Tamura Y, Kikuzaki H, Nakatani N. 1999. Antioxidant Effect of the Constituents of Susabinori (Porphyra yezoensis). JAOCS 76:649-653

National Academy of Sciences. 1979. Protection against depletion of stratospheric ozone by chlorofluorocaebons. Washington, DC, Natl Acad Sci

Neale PJ, Banaszak AT, Jarriel CR. 1998a. Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. J Phycol 34:928-938

Neale PJ, Cullen JJ, Davis RF. 1998b. Inhibition of marine photosynthesis by ultraviolet radiation: variable sensitivity of phytoplankton in the Weddell-Scotia Confluence during the austral spring. Limnol Oceanogr 43:433-448

Neale PJ. 2000. Spectral weighting functions for quantifying the effects of ultraviolet radiation in marine ecosystems. In: de Mora SJ, Demers S, Vernet M, eds. The effects of UV radiation on marine ecosystems. Environmental Chemistry Series; Cambridge: Cambridge Univ. Press. p. 73-100

Niyogi KK, Björkman O, Grossman AR. 1997. The role of specific xanthophylls in photoprotection. Proc Nat Acad Sci USA 94:14162-14167

Norton T, Melkonian M, Anderson R. 1996. Algal biodiversity. Physiologia 35:308-326

Oren A. 1997. Mycosporine-like amino acids as osmotic solutes in a community of halopilic cyanobacteria. Geomicrobiology Journal 14:231-240

Özgür S, Sancar A. 2003. Purification and properties of human blue-light photoreceptor cryptochrome 2. Biochemistry 42:2926-2932

Peak MJ, Peak JG. 1990. Hydroxyl radical quenching agents protect against DNA breakage caused by both 365-nm and gamma radiation. Photochem Photobiol 51:649-652

Pogson BJ, Niyogi KK, Björkman O, DellaPenna D. 1998. Altered xanthophylls composition adversely affect chlorophyll accumulation and nonphotochemical quenching in Arabidopsis mutans. Proc Nat Acad Sci USA 95:13324-13329

Portwich A, Garcia-Pichel F. 1999. Ultraviolet and osmotic stresses induce and regulate the synthesis of mycosporines in the cyanobacterium *Chlorogoeopsis* PCC 6912. Arch Microbiol 172:187-192

Portwich A, Garcia-Pichel F. 2000. A novel prokaryotic UVB photoreceptor in the cyanobayterium *Chlorogloeopsis* PCC 6912. Photochem Photobiol 74:493-498

Portwich A, Garcia-Pichel F. 2003. Biosynthetic pathway of mycosporines (mycosporine-like amino acids) in the cyanobacterium *Chlorogloeopsis* sp. Strain PCC 6912. Phycologia 42:384-392

Post A, Larkum AW. 1993. UV-absorbing pigments, photosynthesis and UV exposure in Antarctica: comparison of terrestrial and marine algae. Aquatic Botany 45:231-243

Potterat O. 1997. Antioxidants and free radical scavengers of natural origin. Current Organic Chemistry 1:415-440

Rasmus J. 1992. Productivity of seaweeds. In: Falkoski PG, Woodhead AD, eds. Primary Productivity and Biogeochemical Cycles in the Sea. Plenum Press, New York

Raven JA. 1991. Response of aquatic photosynthetic organisms to increased solar UVB. J Photochem Photobiol B 9:239-244

Riegger L, Robison D. 1997. Photoinduction of UV-absorbing compounds in Antarctic diatoms and *Phaeocystis antarctica*. Mar Ecol Prog Ser 160:13-25

Roy CR, Gies HP, Elliott G. 1990. Ozone depletion. Nature 347:235-236

Roy S. 2000. Strategies for the minimization of UV-induced damage. In: de Mora S, Demers S, Vernet M, eds. The effects of UV radiation in the marine environment. Cambridge: Cambridge University. p. 177-205

Rüdiger W, López-Figueroa F. 1992. Photoreceptors in algae. Photochem Photobiol 55:949-954

Rundel RD. 1983. Action spectra and estmation of biologically effective UV radiation. Physiol Plant 58:360-366

References

Sancar A. 2003. Structure and function of DNA photolyase and cryptochrome blue-light photoreceptors. Chem Rev 103:2203-2237

Sauer H, Wartenberg M, Heschler J. 2001. Reactive oxygen species as intracellular messsengers during cell growth and differentiation. Cell Physiol Biochem 11:173-186

Schäfer E, Fukshansky L. 1984. Action spectroscopy. In: Smith H,Holmes MG Techniques in photomorphogenesis. London; Academic Press; p. 109-129

Schoenwaelder ME. 2002. The occurrence and cellular significance of physodes in brown algae. Phycologia 41:125-139

Shibata K. 1969. Pigmenta and a UV-absorbing substances in corals and a blue-green alga living in the Great Barrier Reef. Plant and Cell Physiol 10:325-335

Shick JM, Lesser MP, Jokiel PL. 1996. Effects of ultraviolet radiation on corals and other coral reef organisms. Global Change Biol 2:527-545

Shick JM, Romaine-Lioud S, Ferrier-Pagès C, Gattuso J-P. 1999. Ultraviolet-B radiation stimulates shikimate pathway-dependent accumulation of mycosporine-like amino acids in the coral Stylophora pistillata despite decreases in its populationof symbiotic dinoflagellates. Limnol Oceanogr 44:1667-1682

Shick J, Dunlap W, Buettner G. 2000. Ultraviolet (UV) protection in marine organisms II. Biosynthesis, accumulation, and sunscreening function of mycosporine-like amino acids. In: Yoshikawa T, Toyokuni S, Yamamoto Y, Naito Y, eds. Free radiacals in chemistry, biology and medicine. London: OICA Int.. p. 215-228

Shick J, Dunlap W. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation and UV-protective functions in aquatic organisms. Annual Reviews Physiology 64:223-262

Shindell DT, Rind D, Lonergan P. 1998. Increased polar stratospheric ozone losses and delayed eventual recovery owing to increasing greenhouse-gas concentrations. Nature 392:589-592

Shropshire W. 1972. Action spectroscopy. In: Mitrakos K, Shropshire W, eds. Phytochrome; London: Academic Press. p. 161-181

Sinha RP, Sinha JP, Gröniger A, Häder D-P. 2002. Polychromaic action spectrum for the induction of a mycosporine-like amino acid in a rice-field cyanobacterium, *Anabaena* sp.. J Photochem Photobiol B 66:47-53

Sivalingam PM, Ikawa T, Nisizawa K. 1976. Physiological roles of a substance-334 in algae. Bot Mar 19:9-21

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol 125:27-58

Smith IK, Vierheller TL, Thome CJ. 1989. properties and functions of glutathione reductase in plants. Physiol Plant 77, 449-456

Smith S. 1981. Marine macrophytes as global carbon sink. Science 211:838-840

Solomon S. 1990. Progress towards a quantitative understanding of Antarctic ozone depletion. Nature 347:347-354

Takano S, Uemura D, Hirata Y. 1978 a. Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa*. Tetrahedron Letters 26:2299-2230

Takano S, Uemura D, Hirata Y. 1978 b. Isolation and structure of two new amino acids, palythinol and palythene, from the zoanthid *Palythoa tuberculosa*. Tetrahedron Letters 49:4909-4912

Takano S, Nakanishi A, Uemura D, Hirata Y. 1979. Isolation and structure of a 334 nm UV-absorbing substance, porphyra-334, from the red alga *Porphyra tenera* Kjellmann. Chemistry Letters: 419-420

Thomas D. 2002. Seaweeds. The Natural History Museum, London

Tsujino I, Yabe K, Sekikawa I. 1980. Isolation and structure of a new amino acid, shinorine, from the red alga *Chondrus yendoi* Yamada et Mikami. Bot Mar 23:65-68

Vass I. 1997. Adverse effects of UV-B light on the structure and function of the photosynthetic apparatus. In: Pessarakli M, ed., Handbook of photosynthesis, New York, Marcel Dekker Inc, p. 931-949

References

Vincent WF, Neale PJ. 2000. Mechanisms of UV damage to aquatic organisms. In: de Mora, S, Demers, S, Vernet, ,. eds., The effects of UV radiation in the marine environment. Cambridge, Cambridge University Press, p. 149-176

Watanabe M., Furuya M., Miyoshi Y., Inoue Y, Iwahashi I. Matsumoto K. (1982) Design and performance of the Okazaki Large Spectrograph for photobiological reseach. *Photochem. Photobiol.* 36, 491-498.

Watanabe M. 1995. Action spectroscopy: Photomovement and Photomorphogenesis Spectra. In: Horspool W, Song PS, eds. Handbook of organic photochemistry and photobiology; Boca Raton; CRC Press; p. 1276-1288

Watanabe M. 2004. Action Spectroscopy for Photosensory Processes. In: Horspool WM, Lenci F, eds. Handbook of Organic Photochemistry and Photobiology, 2nd ed., CRC Press, Boca Raton, pp. 115.1-115.16

Weaver LM, Herrman KM. 1997. Dynamicy of the shikimate pathway in plants. TRENDS in plant Science 2:346-351

Weng G, Bhalla US, Iyengar R. 1999. Complexity in Biological Signalling Systems. Science 284:92-96

Whitehead K, Karentz D, Hedges JI. 2001. Mycosporine-like amino acids (MAAs) in phytoplankton, a herbivorous pteropod (*Limacina helicina*), and its pteropod predator (*Clione Antarctica*) in McMurdo Bay, Antarctica. Mar Biol 139:1013-1019

Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa F. 2000. Impact of UV radiation on viability, photosynthesis characteristics and DNA of brown algal zoospores: Implications for depth zonation. Mar Ecol Prog Ser 197:217-229

Wood WF. 1989. Photoadaptive response of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultra-violet radiation. Aquat Bot 33:41-51

Yakovleva IM, Dring M, Titlyanov EA. 1998. Tolerance of North Sea algae to UV and visible radiation. Russian Journal of Plant Physiology 45:45-54

Yordanov I, Velikova V, Tsonev T. 2000. Plant responses to drought, acclimation, and stress tolerance. Photosynthetica 38:171-186

Zudaire L, Roy S. 2001. Photoprotection and long-term acclimation to UV radiation in the marine diatom *Thalassiosira weissflogii*. J Photochem Photobiol B 62:26-34

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