Occurrence, induction and physiological importance of UV-absorbing substances in polar macroalgae

Vorkommen, Induktion und physiologische Bedeutung UV-absorbierender Substanzen in polaren Makroalgen

---

Kirsten Hoyer

Ber. Polarforsch. Meeresforsch. 440 (2003)
ISSN 1618 - 3193

Kirsten Hoyer
Alfred-Wegener-Institut für Polar- und Meeresforschung
in der Helmholtz-Gemeinschaft
Am Handelshafen 12
27570 Bremerhaven

Die vorliegende Arbeit ist die inhaltlich unveränderte Fassung einer kumulativen Dissertation, die in der Projektgruppe „Solare UV-Strahlung" bei Prof. Dr. Christian Wiencke und Prof. Dr. Ulf Karsten angefertigt und 2002 dem Fachbereich 2 (Biologie/Chemie) der Universität Bremen vorgelegt wurde.

Contents

ABBREVIATIONS

CIE	Commission Internationale de l´Eclairage
chl a	chlorophyll a
DAHP	3-deoxy-*D*-arabinoheptulosinate-7-phosphate
DHQ	3-dehydroquinate
DNA	desoxyribo nucleid acid
DW	dry weight
e.g.	for example
F_v/F_m	optiumum quantum yield of Photosystem II
HPLC	high performance liquid chromatography
i.e.	that is
MAA(s)	mycosporine-like amino acid(s)
p-334	porphyra-334 (MAA)
PAM	pulse-amplitude modulation fluorometer
PAR	photosynthetic active radiation
RNA	ribulose nucleic acid
ROS	reactive oxygen species
SD	standard deviation
UVA	ultraviolet A radiation
UVB	ultraviolet B radiation
UVR	ultraviolet radiation

ABSTRACT

The present study focuses on a putative passive sunscreen, the UV-absorbing mycosporine-like amino acids (MAAs) synthesised in macroalgae. MAAs as UV-defence mechanism are of particular interest in Antarctic and Arctic algae because deleterious UVB radiation (280-315 nm) increases due to the stratospheric ozone depletion over the polar regions. The survey examined MAA occurrence based on vertical distribution, individual MAAs were identified and quantified, and correlated with habitat and radiation climate in air and under water. Three physiologically different groups of polar algae were represented in terms of MAA-values: I) supra- and (upper) eulittoral species with an always relatively high MAA concentration, II) eu- and sublittoral species adjust MAA concentrations to the occurring environmental radiation, III) deep-water and understorey plants without MAAs. In addition, the physiological capability for inducing MAA synthesis/accumulation was investigated, as red algae were exposed to three different radiation conditions (PAR: 400-700 nm, PAR+UVA: 320-700 nm, PAR+UVA+UVB: 295-700 nm). Algal responses to irradiance could be divided into 3 physiological types based on their maximal MAA concentrations: a) species with highest total MAA values under the full radiation spectrum, b) species with highest MAA concentrations under PAR+UVA, and c) species with a strong MAA decrease under PAR+UVR.

Furthermore, the influence of other abiotic factors such as temperature and salinity on the inducibility of MAA synthesis/accumulation were examined as well as the interactive effects of radiation and temperature/salinity on MAAs and photosynthesis evaluated. Elevated temperature alone had no effect on MAA contents, but in combination with enhanced PAR and UVR MAA concentration became higher at 5 °C in Antarctic red algae (*Iridaea cordata*, *Palmaria decipiens*) compared with samples at 10 °C. In contrast, MAA contents of Antarctic green (*Prasiola crispa* ssp. *antarctica*) and Arctic red (*Palmaria palmata*) algae remained almost unchanged under both temperatures. Therefore it is suggested that the enzymatic processes for the MAA synthesis in Antarctic species are well adapted to their cold habitat whereas the Arctic plant has a broader tolerance to temperature, which is even more pronounced in the green alga. An additional indicator was found in its similar F_v/F_m values of photosynthesis at both temperatures.

Under the different salinity concentrations (15, 34 and 50 psu) the MAA content did not rise in Arctic *Devaleraea ramentacea* and *Palmaria palmata*, but in combination with UV radiation an increase could be obtained. While *D. ramentacea* exhibited euryhaline features and acclimated well to the UV radiation applied, *P. palmata* can be characterised as stenohaline plant because of its high mortality already under mild hyposaline conditions. An osmotic role of MAAs could not be confirmed for these macroalgal species.

The protection of photosynthesis under UV-stress due to MAAs was investigated in *Palmaria decipiens* and *Palmaria palmata* by a comparison between MAA-low and experimentally induced MAA-high concentrated samples. Usually, photosynthesis of algae from the latter condition was less affected under UVR, consequently recovery was accelerated and improved. Furthermore, the photosynthetic response to enhanced PAR and UVR of two deep-water algae (*Odonthalia dentata* and *Coccotylus truncatus*), lacking MAAs, were analysed. Samples covered by biofilter-containers filled with MAA extracts or by cut-off filters were compared with each other. The biofilter-treated isolates were protected against UVR in a similar way as the 320 nm cut-off-treated samples (PAR+UVA). This indicates that MAAs screen harmful UVB, strongly supporting the photoprotective role of the MAAs.

All data presented demonstrate distinct MAA induction patterns, indicating that induction, formation and accumulation of individual MAAs is a very flexible and species-specific mechanism. The sunscreen effect of MAAs was confirmed although its effectiveness was species-specific and concentration-dependent, assuming that algae gain an advantage by containing MAAs when ozone depletion i.e. increase in UVB radiation becomes worse.

ZUSAMMENFASSUNG

Der Schwerpunkt dieser Arbeit liegt in der Untersuchung UV-Strahlung absorbierender Substanzen, im besonderen der mycosporin-ähnlichen Aminosäuren (MAAs). Sie werden u. a. von aquatischen, autotrophen Organismen synthetisiert, denen eine mögliche Sonnenschutzeigenschaft zugeschrieben wird. Diese Eigenschaft ist von Interesse bei arktischen und antarktischen Makroalgen auf Grund des starken stratosphärischen Ozonabbaus über den Polen und der damit verknüpften Zunahme an für Organismen schädlicher UVB-Strahlung (280-315 nm). Das Vorkommen der MAAs wird in Algenarten unter Berücksichtigung ihrer Lebensräume untersucht. Während vor allem in Rotalgen MAAs in z.T. hohen Konzentrationen gebildet werden, zeigen sich in Grünalgen meist nur geringe MAA-Konzentrationen. Eine Ausnahme bilden Arten der Gattung *Prasiola*, in denen eine in ihrer molekularen Struktur noch nicht identifizierte UV-absorbierende Substanz mit einem Absorptionsmaximum bei 324 nm analysiert wurde. Für die Algen kann ein bestimmtes Verteilungsmuster von Arten mit unterschiedlichen MAA-Konzentrationen festgestellt werden, die in drei physiologische Gruppen eingeordnet werden: I) Arten, die das Supra- und obere Eulitoral besiedeln und eine meist hohe MAA-Konzentration aufweisen, II) Arten aus dem Eu- und Sublitoral, die MAAs flexibel, dem Strahlungsangebot angepaßt akkumulieren, und III) Arten, in denen keine MAAs nachgewiesen werden und die hauptsächlich in großen Wassertiefen vorkommen oder als Unterwuchsarten bekannt sind, beides Habitate, in denen Schwachlichtbedingungn vorherrschen. Ferner wird in dieser Arbeit die Induzierbarkeit der MAA-Synthese bzw. Akkumulation durch verschiedene Strahlungsbedingungen (PAR: 400-700 nm, PAR+UVA: 320-700 nm, PAR+UVA+UVB: 295-700 nm) in polaren Rotalgen untersucht. Obwohl die Ergebnisse darauf hindeuten, dass die MAA-Stimulierung durch die verschiedenen Spektralbereiche artspezifisch ist, lassen sich auch hier die Algen in physiologische Reaktionstypen einteilen, je nachdem welche Auswirkungen die angebotene Strahlung auf die MAA-Akkumulierung/Synthese hat. In Typ (a) werden höchste MAA-Konzentrationen im gesamten Strahlungsbereich ausgebildet. In Algen, die zum Typ (b) gehören, sind höchste MAA-Konzentrationen unter PAR+UVA zu finden, während Arten, deren MAA-Konzentration unter UV-Strahlung abnimmt, dem Reaktionstypen (c) zugeordnet werden.

Der Einfluß anderer abiotischer Faktoren und deren interaktive Effekte auf das MAA-Bildungsverhalten einzelner Algen wird anhand zwei antarktischer Rotalgenarten

(*Iridaea cordata*, *Palmaria decipiens*), einer antarktischen Grün- und einer arktischen Rotalge (*Prasiola crispa* ssp. *antarctica* bzw. *Palmaria palmata*) untersucht, die erhöhten Temperaturen (5 und 10 °C) ausgesetzt waren; dieses hatte allerdings keinen Einfluß auf den MAA-Gehalt. In Wechselwirkung mit zusätzlich erhöhter PAR-Intensität und UV-Strahlung reicherten die antarktischen Rotalgen jedoch mehr MAAs bei 5 °C als bei 10 °C an. Die MAA-Gehalte der beiden anderen Arten unterschieden sich bei diesen Temperaturen unwesentlich. Antarktische Algen haben vermutlich ihre enzymatischen Reaktionen der MAA-Produktion gut an das kalte Habitat angepaßt, während arktische Pflanzen eine größere Toleranz gegenüber Temperaturänderungen besitzen. Grünalgen sind in der Lage Standorte zu besiedeln, die starken Umweltschwankungen ausgesetzt sind, was sich in einer noch höheren physiologischen Toleranzbreite äußert. Bei verschiedenen Salzgehalten (15, 34 und 50) erhöht sich der MAA-Gehalt in der arktischen *Devaleraea ramentacea* und *Palmaria palmata* nicht. Es zeigt sich allerdings ein Konzentrationsanstieg bei der Kombination von Salzgehalts- und UV-Strahlungsänderung. Während *D. ramentacea* euryhaline Merkmale zeigt und sich gut auf die UV-Strahlung einstellt, verhält sich *P. palmata* eher stenohaline, da sie eine hohe Mortalität schon unter geringen hyposalinen Bedingungen hat.

Der Schutz der Photosynthese durch MAAs unter "UV-Stress" wird durch den Vergleich von *Palmaria decipiens* und *Palmaria palmata* bei niedriger MAA und induzierter höherer MAA-Konzentrationen untersucht. Es wurde eine artspezifische MAA Konzentrations-abhängigkeit festgestellt, bei der die Photosynthese der Proben mit höheren MAA-Konzentrationen weniger beeinträchtigt war und ihre Erholung nach Beendigung des Stresses schneller erfolgte sowie bessere Photosynthesewerte ergab als in den Proben mit geringeren MAA-Konzentrationen. Die Analyse der photosynthetischen Reaktion zweier Tiefenwasseralgen ohne MAAs (*Odonthalia dentata* und *Coccotylus truncatus*) auf verstärkte PAR und UV-Strahlung bei Bedeckung der Proben durch mit MAA-Extrakt gefüllten Biofiltern bzw. "cut-off"-Filtern zeigt, dass die mit dem Biofilter geschützten Proben in derselben Weise gegen UV-Strahlung geschützt waren, wie die durch den 320 nm "cut-off"-Filter. Dies deutet darauf hin, dass MAAs die schädliche UVB-Strahlung abschirmen und unterstützt die Annahme, dass MAAs eine photoprotektive Rolle im Organismus spielen.

Alle dargestellten Daten demonstrieren bestimmte MAA-Induktionsmuster und zeigen, dass die Induktion, Bildung und Ansammlung individueller MAAs sehr flexibel sind und es sich um einen artspezifischischer Mechanismus handelt. Der Sonnenschutz-Effekt der MAAs wurde bestätigt, obwohl die Effektivität artspezifisch und

konzentrationsabhängig ist. Für Algen ist es von Vorteil, MAAs zu besitzen, vor allem, wenn durch die Ozonabnahme die UVB-Strahlung zunimmt.

1 INTRODUCTION

Ultraviolet radiation (UVR) has both positive and negative effects on life on Earth. In particular, UVB radiation (280-315 nm) deleteriously affects biological organisms not only in terrestrial, but also in aquatic ecosystems particularly primary producers such as phytoplankton, macroalgae and microbenthic communities. The stratospheric ozone layer absorbs the high energetic short wavelengths of solar radiation and an increase in UVB radiation is regarded as a direct result of ozone depletion (Frederick and Snell 1988). However, in earlier times, when the ozone layer was in the process of building up and the concentration of atmospheric oxygen was lower than it is at present, UVB radiation levels were even higher. Consequently, ancient terrestrial and aquatic plants developed protection and repair mechanisms against the harsh radiation conditions (Rozema et al. 1997). The UVB-absorbing substances, mycosporine-like amino acids (MAAs) found in polar macroalgae, which are reportedly photoprotective compounds playing a role in the sunscreen mechanisms, are the main focus of this study. The introduction describes why the ozone layer decreases, resulting in an increase in UVB, as well as the effects of UVB on marine organisms and their defence strategies. A brief overview of MAAs is also given as well as some details of the polar regions.

1.1 Stratospheric ozone

The stratospheric gas ozone is found at altitudes of approximately 10 to 50 km. The maximum ozone density (90%) lies in the range of 15 to 25 km (Solomon 1990), commonly known as the ozone layer, and acts as a UV radiation (UVR) shield. The ozone concentration is expressed in Dobson Units (DU), i.e. 100 DU correspond to an ozone layer of 1 mm thickness at normal pressure and normal temperature.

Over the equator, where irradiance is highest, most ozone is produced by the action of solar radiation on atomic and molecular oxygen. The ozone is transported polewards via atmospheric circulation, producing a maximum at higher latitudes. Nevertheless, the thickness of the ozone layer undergoes natural variation, due to seasonal and daily changes and locally dynamic processes (Gathen et al. 1995). However, generally ozone levels over Antarctica are lower, with average values of about 320 DU compared with the Arctic, with values of about 450 DU before the 1980s, and lowest over the equator with 260 DU (WMO 1998).

Ozone formation and decomposition are natural processes occurring by the *Chapman* reaction, and by other homogenous chemical reactions. The latter are catalytic cycles involving free radicals such as NO, HO and halogen radicals, greatly enhancing the rate of ozone recombination (Langer 1999, Whitehead et al. 2000). Severe ozone depletion was initially described in the Antarctic in 1985 (Farman et al. 1985), and somewhat later for the midlatitudes of the Southern and Northern Hemisphere (Atkinson et al. 1998, Roy et al. 1990, and references therein) and the Arctic (Austin et al. 1992, Salawitch et al. 1993). It is thought that further depletion is mainly due to the release of anthropogenic/industrial pollution into the atmosphere, comprising compounds such as chlorofluorocarbons, bromines and nitrogen oxides (NO_x) (Solomon 1990). These molecules degrade as promoter ozone molecules in solar radiation-induced catalytic cycles. Polar stratospheric clouds (PSCs) are the precondition for these heterogeneous (gas-solid) chemical mechanisms, as they are the solid part of the surface catalysed reactions (Langer 1999, Whitehead et al. 2000).

The region in which the ozone shows a decrease to 30 % of concentration seen pre 1980, is commonly called ´ozone hole` (WMO 2002). They are formed during the Antarctic spring (September – November) and are normally most pronounced in October (Lubin and Frederick 1989). In the last few years, the Antarctic ozone hole has persisted for longer, starting in August, and continuing into December (WMO 2002). Furthermore, it is still increasing with a maximum size to date of 30 Million km² in October 2001 (WMO 2002). In the Arctic, the ozone hole occurs for a shorter time, appearing in late January and persisting until March, although also sometimes longer (Rex et al. 1997, Langer 1999) and is generally largest in March (WMO 1998).

1.2 UVB radiation

Ozone depletion results in an increase in ultraviolet radiation (UVR) at the Earth´s surface. UVR is subdivided into three components and defined as follows by the Commission Internationale de l'Eclairage (CIE); UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm). Under ´normal` stratospheric ozone conditions, UVC is completely absorbed by the ozone shield, oxygen and water vapor, while the largest portion of UVB is absorbed downward from about 310 nm. UVA and photosynthetically active radiation (PAR, 400-700 nm) pass through the ozone layer almost unaffected. However, a loss in ozone of around 10 % might lead to an increase in

spectral irradiance of about 50 % at 297 nm, 25 % at 303 nm and 0 % at 325 nm (Roy et al. 1990). In this case, the solar spectral distribution may shift to shorter wavelengths, which exhibits highest energy, resulting in a higher UVB:UVA:PAR ratio (Smith et al. 1992). It should be considered that other factors such as cloud cover, aerosols, solar zenith angle and precipitable water column may also influence the absorption and reflection of UVB (Lubin et al. 1998). Cirrus clouds, stratospheric and tropospheric particles, for example, attenuated UVBR by 12%, 6 % and 5 %, respectively (Whitehead et al. 2000, references therein), but, in contrast, cumulus clouds have been shown to enhance total sky UVBR by up to 30 % (Mims and Frederick 1994).

1.3 UVB radiation effects

Enhanced UVBR may not only affect organisms, but also materials and biogeochemical cycles. An increased morbidity from eye diseases and skin cancer has been observed in humans (Longstreth et al. 1995), and there are many effects seen in terrestrial plants, including inhibition of photosynthesis, DNA damage, changes in morphology, phenology and biomass accumulation. This in turn may result in an altered communitiy composition and in the weakening of the genomic stability (Caldwell et al. 1995, Rozema et al. 1997, Ries et al. 2000). Furthermore, UVB penetrates into the water column, affecting freshwater and marine organisms, even when scattered and absorbed by water, dissolved organic matter (DOM, Gelbstoff) or by particulate matter, such as phytoplankton. The radiation transmittance through the water column can be calculated by the vertical attenuation coefficient of downward irradiance (K_d), related to UVR as well as to PAR (Kirk 1994). In clear oceans such as the Southern Ocean, UVB can be measured down to depths of 60 – 70 m (Smith et al. 1992) with biologically harmful effects on marine organisms down to 10 – 30 m (Karentz and Lutz 1990, Boelen et al. 2000).

UVB can cause many negative effects at different levels, especially on marine sessile organisms such as macroalgae, e.g. at the molecular and cellular levels. These effects can be classified into direct and indirect. With direct effects, the main targets are biomolecules such as nucleic acids and proteins, that are degraded or transformed by absorbing UVB, resulting in disturbance or even elimination of their biological functions (Vincent and Neale 2000). UVB-induced DNA damage causes photoproducts such as cyclobutane pyrimidine dimers and (6-4) photoproducts as detected in

macroalgae and phytoplankton (Buma et al. 2000, Helbling et al. 2001, Poll et al. 2001) leading to mutations or to an immobilization of the RNA polymerase. Furthermore, proteins, functioning as enzymes, hormones and structural components of cells, can also be affected. For example, the destruction of phycobilisomes (Lao and Glazer 1996) and the reduction of the activity of the ribulose 1,5 carboxylase contributing to the inhibition of photosynthesis (Bischof et al. 2000a, 2002).

Indirect effects are caused by reactive oxygen species (ROS), whose resultant photoproducts may be more reactive and cytotoxic than the direct effects of UVR. ROS such as hydrogen peroxide, hydroxyl radicals and superoxide radicals are oxidants capable of damaging DNA, RNA, proteins and pigments. This may result in a complete change in metabolic effects on growth, reproduction and productivity (Dring et al. 1996, Aguilera et al. 1999, Wiencke et al. 2000, Makarov and Voskoboinikov 2001), genetic damage (Kuluncsics et al. 1999, Vincent and Neale 2000), depression of key physiological processes like photosynthesis (Larkum and Wood 1993, Hanelt et al. 1997b, Hanelt and Nultsch 2003, Bischof et al. 2000a, 2002, Gómez et al. 2001), and, in time, an altered community structure (Madronich et al. 1995).

Marine organisms such as macroalgae, have evolved biological defences against UV-damage. These mechanisms are able to repair, detoxify and screen, i.e. to minimize the deleterious effects of UVBR as far as possible (Roy 2000). Repair of DNA damage is feasable on the molecular level by photolyases and excision enzymes (Mitchell and Karentz 1993). UVR-induced reactive oxygen species can be eliminated by the expression of detoxifying enzymes and antioxidants (Dunlap and Yamamoto 1995, Collen and Davison 1999, Aguilera et al. 2002a). Additionally, there are also physiological and biochemical counteracting strategies such as the synthesis of UV-absorbing sunscreen compounds. The latter occur in all kingdoms, resulting in an important mechanism for reducing potential radiation damage (Cockell 2001). It has been suggested that these compounds played an important role in the evolution from aquatic to land plants (Rozema et al. 2002).

1.4 UV-absorbing compounds

UV-absorbing compounds are often secondary metabolites such as flavonoids in terrestrial plants, other polyphenolic substances, which also occur in brown algae

(reviewed in Schoenwaelder 2002) and coumarins, which have been found in the green algal family *Dasycladaceae* (Menzel et al. 1983). The UV-absorbing scytonemin, found in extracellular sheaths of cyanobacteria, is known to function as a passive sunscreen. This substance is a yellowish-brown, lipid-soluble pigment with an absoption maximum at 370 nm (García-Pichel and Castenholz 1991, Ehling-Schulz et al. 1997, Dillon et al. 2002). Finally, mycosporine-like amino acids (MAAs), which also absorb UVR, have been detected in the majority of aquatic organisms including vertebrates, invertebrates, coral reef organisms, bacteria, and algae in both marine and freshwater ecosystems (Cockell and Knowland 1999, reviewed in Karentz 2001). MAAs have been postulated to play a photoprotective role, contributing to the prevention of UV-induced damage, the main advantage of a passive sunscreen (Dunlap and Shick 1998). However, in order to be defined as a passive sunscreen, the substances must meet several prerequisites (García-Pichel et al. 1993). Firstly, the compounds must screen UVR at a high enough level to provide a noteworthy benefit to the organism and secondly, the compounds must be inducible. Furthermore, physiological efficiency of the UV-substance must be proved, i.e. that UV damage is really reduced and that survival is enhanced under UV-stress due to the sunscreen compared with same species lacking it. Under conditions of physiological inactivity, excluding other active photoprotective mechanisms, the correlation between the UV-absorbing substance and the resistence to UVR should still be present. Additionally, it should be proven that the protective effect is maximal at the wavelengths of maximal absorption of the respective UV-absorbing substance and negligible where it does not significantly absorb. Another approach is that after artificial removal of this compound the protection should be lost (García-Pichel et al. 1992, 1993, Cockell and Knowland 1999). Up till now, it is still not confirmed if MAAs satisfy all these requirements.

1.5 Molecular characteristics of the UV-absorbing mycosporine-like amino acids

MAAs are small, colorless, water-soluble molecules with an average molecular weight of 300 Da (g mol^{-1}). Due to their high molecular extinction coefficients (Table 1), they are very effective in absorbing the wavelengths in the range between 309 and 360 nm, resulting in a sharp absorption spectrum (Banderanayake 1998, Dunlap and Shick 1998, Cockell and Knowland 1999). Up to now, distinct molecular structures of 19 MAAs have been identified in marine organisms (Karentz 2001). These compounds have a

cyclohexenon or a cyclohexenimine structure in common, with a conjugated amino acid (mostly glycine) or another amine (Fig. 1). There are chemical exceptions, where glycine is replaced by threonine, serine or methylamin, and the mycosporine-taurine. The MAA absorption characteristics primarily depend on a system of conjugated double bounds in the core ring structure, in which the π-electron system effectively absorb UV radiation, causing an energetic transition of π-electrons to anti-bonding π*-electron orbitals. Hence, two of the MAAs, mycosporine-glycine and mycosporine-taurine, containing the chromophore cyclohexenon, clearly absorb in the UVB range. The other MAAs comprise a cyclohexenimine, showing their absorption maximum in the UVA range. In addition, the substitutions at C-3 (and C-1) are able to alter absorption properties of the ring structure. These factors not only result in a shift of the absorption maximum, but also in an altered extinction coefficient, which then determines the specific absorption maximum of the respective MAA (Banderanayake 1998, Cockell and Knowland 1999).

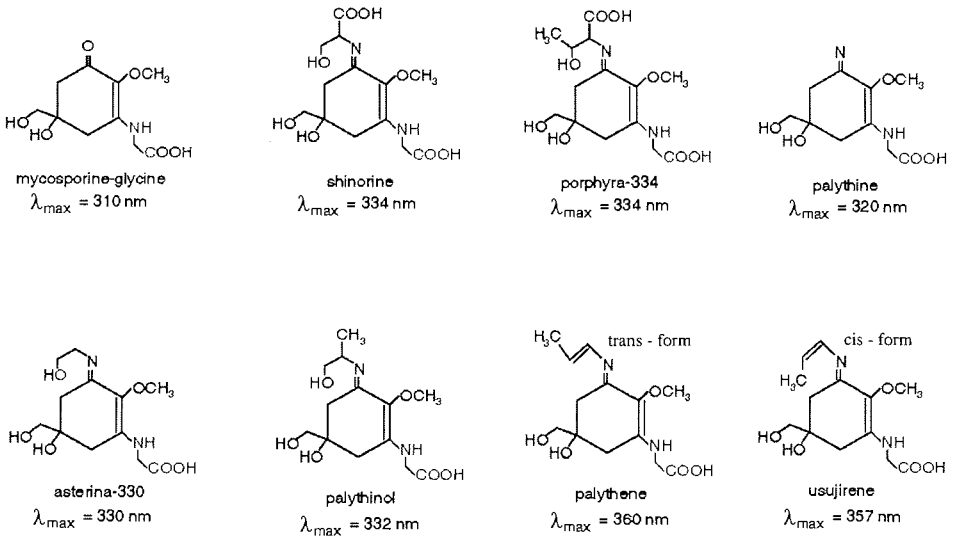


Figure 1: Structures of the most common MAAs in marine red algae

The absorbed UV-energy of a molecule may be emitted as fluorescence, heat dissipation, or by an interaction with another molecule. Contradictory statements have

been made about the capability of the MAA structure to fluoresce. The most recent studies have revealed that partially-purified shinorine did not show any fluorescent capabilities (Shick et al. 2000), and p-334 has only a very low quantum yield of fluorescence, but a strongly exothermic triplet energy level (Conde et al. 2000). Furthermore, radical production did not occur in a solution of shinorine nor was fluorescence detected, when irradiated with UVR (Shick et al. 2000). These are characteristics, which corroborate the hypothesis of the photoprotective role of MAAs (Shick and Dunlap 2002).

MAAs, which have a close relationship to fungal mycosporines in aquatic organisms (Bandaranayake 1998) and UV-absorbing compounds have been found and often identified in a plurality of autotrophic and heterotrophic organisms, such as red algae (Tsujino et al. 1978, Karsten et al. 1998b), dinoflagellates (Carreto 1990a,b), diatoms (Helbling et al. 1996, Riegger and Robinson 1997), cyanobacteria (García Pichel and Castenholz 1993, García-Pichel et al. 1993), freshwater zooplankton (Tartarótti et al. 2001), invertebrates such as corals (Shibata 1969, Dunlap and Chalker 1986), echinoderms (Karentz et al. 1991, Carrol and Shick 1996) and chordata such as ascidia and fish (Dunlap and Yamamoto 1995, McClintock et al. 1997). It has been suggested, that MAAs are synthesised via the shikimate pathway, which only occurs in plants and microorganisms (Herrmann and Weaver 1999, Shick et al. 1999). Favre-Bonvin et al. (1987) showed that the carbon ring structure of mycosporine is derived from 3-dehydroquinate (DHQ), an intermediate of the shikimate pathway, and further synthesised via gadusol (Shick and Dunlap 2002). Apart from the origin of the MAAs through the shikimate pathway, additional enzymatic interconversion amongst MAAs may be possible. The variable kinetics of increasing levels of individual MAAs, which can occur over several days for the suite of MAAs in red algae (Karsten et al. 1998a, Franklin et al. 1999) and over hours in dinoflagellates (Carreto et al. 1990a,b) support this view.

Table 1: Molar extinction coefficients, molecular weight and their citations for seven different MAAs, that typically occur in the macroalgae studied

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. 1978a
Asterina-330	43500	288.30	Gleason et al. 1993
Palythinol	43500	302.32	Takano et al. 1978b
Palythene	50000	284.31	Takano et al. 1978b

1.6 MAAs in macroalgae

MAAs are very common in marine macroalgae from the polar regions to the tropics. This is verified by several MAA-inventories from the polar (Arctic) via cold- and warm-temperate (Karsten et al. 1998b) to the tropic region (Banaszak and Lesser 1995, Banaszak et al. 1998, Karsten et al. 1998c, Karsten et al. 2000). Only a few examples for Antarctic macroalgal species exist (Karentz et al. 1991, Post and Larkum 1993, McClintock and Karentz 1997). The summerised results of those studies demonstrate that MAAs occur particularly often in high amounts in Rhodophyceae, whereas MAAs when at all present in members of the Phaeophyceae, are usually found in trace concentrations. In Chlorophyceae, MAAs were often detected in low concentrations (e.g. Xiong et al. 1999).

The most common MAAs found in macroalgae, often as principal MAAs are palythine, porphyra-334 and shinorine. Mycosporine-glycine, asterina-330, palythinol and palythene/usujirene usually occur in lower concentrations. Often a suite of at least three MAAs has been found, providing a broad spectral band and therefore a more effective optical filter than the presence of a sole MAA (Karentz 2001). Furthermore, these results and those of Karsten and West (2000) suggest that a correlation exists between MAA concentrations and latitudes, with respect to the solar radiation climate. The MAA concentrations were highest in plants from the tropics (< 12.8 mg g^{-1} DW), decreasing from warm temperatue species (< 7.8 mg g^{-1} DW) to cold temperate/Arctic algae (< 3.5 mg g^{-1} DW). In warm temperate species, comparable MAA concentrations were found to those of Antarctic macroalgae (< 9.4 mg g^{-1} DW, Publ. 1). Furthermore, seasonal effects on MAA concentrations have been reported for algae from the Arctic and Antarctic, with a trend to higher MAA concentrations in the (late) summer compared to the other seasons (Post and Larkum 1993, Karsten et al. 1999).

It must be emphasised, that MAA contents are strongly influenced by vertical distribution of the algae and the related radiation climate. Generally, a decrease in MAA concentrations and contents of UV-absorbing compounds is coupled with an increasing algal depth (Yakovleva et al. 1998, Karsten et al. 1998b, 1999, Karsten and Wiencke 1999). Stimulating irradiance effects on MAAs have been shown in transplantation experiments, in which algal samples were transferred from deep to shallower water and exposed to different solar radiation conditions. In these experiments, different spectral ranges with and without UVR were applied using cut-off filters. Laboratory experiments with artificial radiation sources to enhance PAR and UVR were also performed. It was demonstrated that algae responded differently to distinct radiation, conditions with or without enhanced MAA accumulation and contents of UV-absorbing compounds (Wood 1989, Karsten et al. 1998a, 1999, 2000, Karsten and Wiencke 1999, Franklin et al. 1999, 2001, Gröniger et al. 1999, Yakovleva and Titlyanov 2001). Recent studies have calculated polychromatic response spectra for several MAAs in the red alga *Chondrus crispus* (L.) Stackhouse (Kräbs et al. 2002), and for an unknown UV-absorbing substance-324 in the green alga *Prasiola stipitata* Suhr ex Jessen (Gröniger and Häder 2002). The latter author showed that UVB radiation strongly stimulated the accumulation of the unknown substance-324 in *P. stipitata*. However, these studies were all particular examples from one or two species from different regions, e.g. Arctic, cold-temperate and tropic regions. There is still a necessity for a comprehensive study of several algae from the same region in order to better characterise general physiological responses of MAA accumulation under different radiation scenarios. Nevertheless, a possible photoprotective role of MAAs was also suggested in later studies, in which macroalgae containing MAAs were seen to be better adapted and less affected to UVR (Bischof et al. 2000b, Karsten et al. 2001, Aguilera et al. 2002b).

1.7 MAAs in microalgae

MAAs and UV-absorbing compounds have been found in marine and freshwater phytoplankton assemblages (Vernet et al. 1989, Banaszak and Neale 2001, Sommaruga and García-Pichel 1999, Bracher and Wiencke 2000, Laurion et al. 2002), in benthic microalgae (Sundbäck et al. 1997, Wulff et al. 1999) and also in phytoplankton cultures (Riegger and Robinson 1997, Banaszak et al. 2000) throughout the world. A broad MAA-inventory in microalgae has been performed by Jeffrey et al. (1999). These

authors investigated 152 species from a total of 12 classes. It was found that all taxa absorbed in the UVA range, and over 10 % additionally in the UVB range. Members of the Bacillariophyceae, Dinophyceae and Prymnesiophyceae were the most represented by species, determining the ratio of absorbance intensity at the UV maxima between 280 and 390 nm to that of chlorophyll *a* at 665 nm (UV:chl *a*). Significant MAA concentrations were assumed when the ratio was found to be greater than 0.9. Dinoflagellates, especially the bloom-forming types, showed the highest UV:chl *a* ratio, and possessed a significant UV-absorbing suite. In contrast, many diatoms have been found to have a low UV:chl *a* ratio, with only two species of the group Pennales having a comparably high ratio. This situation mirrors samples of natural phytoplankton assemblages, in which a high UV:chl *a* ratio was detected when dinoflagellates predominated, whereas it was low in a diatom-rich samples (Jeffrey et al. 1999).

The stimulation of MAA accumulation or even synthesis under solar and artificial radiation has been investigated for several phytoplankton species (Carreto et al. 1990a,b, Helbling et al. 1996, Riegger and Robinson 1997, Moisan and Mitchell 2001, Zudaire and Roy 2001, Klisch and Häder 2002, Hernando et al. 2002). When an enhancement of MAAs occurred at all, then at different time scales, suggesting individual MAA-specific responses. A correlation between N-limitation in the medium and MAA decrease has been found in dinoflagellates, resulting in MAA concentrations similar to those cells grown under low light conditions (Litchman et al. 2002).

MAAs and UV-absorbing compounds also occur in marine, freshwater and terrestrial cyanobacteria (García-Pichel and Castenholz 1993, Karsten and García-Pichel 1996, Karsten et al. 1998d, Quesada and Vincent 1997, Sommaruga and García-Pichel 1999), in cyanobacterial lichens (Büdel et al. 1997) as well as those associated with coral reefs (Shibata 1969). Several investigations were performed to induce MAA synthesis and accumulation by different radiation treatments (García-Pichel et al. 1993, García-Pichel and Castenholz 1993, George et al. 2001, Sinha et al. 2001) and by different osmotic conditions (Portwich and García-Pichel 1999, Karsten 2002). In any case, the response of cyanobacteria exposed to different stress treatments varied and was species-specific.

García-Pichel (1994) developed a bio-optical model, in which he demonstrated that MAAs cannot be used as photoprotection in cells smaller than 1 μm, and only restricted in cells of 1 – 10 μm in size considering the short optical pathway and the high cost factor in terms of production as a high concentration of MAAs is necessary to be

efficient (> 10% of dry biomass). A good protective efficiency could be provided by MAAs in cells 10 - >100 μm, and for cells >200μm it may become a powerful (< 1% of dry biomass) photoprotective mechanism, assuming a homogeneous intracellular distribution of the MAAs. However, in the external sheath of the cyanobacterium *Nostoc commune*, a MAA covalently linked to oligosaccharides has been identified, whose concentration also could be enhanced under exposure to UVB (Böhm et al. 1995, Ehling-Schulz et al. 1997). It has also been suggested that MAAs were extracellularly embedded in the matrix of colonial cells in the prymnesiophyte *Phaeocystis*, as their concentrations appeared physiologically too high to be contained within the cell (Marchant et al. 1991, Riegger and Robinson 1997).

In many studies, MAAs have only been identified by their absorption maxima with no explicit differentiation between individual MAAs. When they have been carefully analysed, shinorine and mycosporine-glycine dominated.

1.8 MAAs in animals

Only primary producers/autotrophic organisms are able to synthesise the various MAAs. Despite this, many marine animals exhibit MAAs, as demonstrated in two general surveys of Antarctic species from 12 faunal clades (Karentz et al. 1991, McClintock et al. 1997). Different concentrations of MAAs are accumulated in different tissues for example; in skin, digestive-glands, ovaries and spawn of sea urchins and molluscs (Karentz et al. 1992, 1997, Ishikura et al. 1997, Carefoot et al. 2000), and also in coral mucus (Drollet et al. 1997, Teai et al. 1998). Furthermore, a negative correlation has been described between MAA contents and depth in coral reef organisms and invertebrates such as echinoderms and molluscs (Dunlap et al. 1986, Shick et al. 1995, Karentz et al. 1992, 1997). Stimulation of MAA accumulation under enhanced UVR has also been tested (Banaszak and Trech 1995, Shick et al. 1991, Dionisio-Sese et al. 1997, Gleason 2001), although the results of such studies were contradictory. Nevertheless, a photoprotective role or at least a partial reduction of UVR-induced damage due to MAAs was consistently postulated for most marine animals. Adams and Shick (1996, 2001) have clearly shown that in echinoderm eggs with different MAA contents due to controlled diet uptake, the cleavage delay and abnormalities up to the pluteus stage caused by UVR was significantly reduced in developing eggs with higher MAA contents.

However, the fact remains that heterotrophic organisms must take up the MAAs in their diet, either as MAA-enriched algae or particles (Carrol and Shick 1996, Newman et al. 2000), by the secondary consumer (Whitehead et al. 2001), or by endosymbionts such as zooxanthellae in corals (reviewed in Gleason 2001). How the reabsorption from the diet and the inclusion of individual MAAs in epidermal tissue actually functions is still subject to further investigation (Shick et al. 2000). It is possible that there are some enzymatic carriers or specific transport mechanisms as assumed for the selective accumulation of one MAA (shinorine) in the ovaries of a green sea urchin (Adams et al. 2001), or MAAs are able to simply diffuse through the cells (Adams and Shick 1996, Shick and Dunlap 2002). Interestingly, several isolated zooxanthellae were unable to synthesise any MAAs, or they synthesised different suites of MAAs than those detected in the host (Shick et al. 1999, Banaszak et al. 2000). This may indicate that some zooxanthellae need a special trigger from the host to be able to produce MAAs, or that the original MAAs may be used as precursors by the host or by its intestinal microflora and later become enzymatically converted (Shick and Dunlap 2002) as shown for the bacterium *Vibrio harveyii* (Dunlap and Shick 1998).

Many fish species, inhabiting the shallow photic zone, have UV-absorbing substances including MAAs in their occular tissues that block transmission of wavelengths < 400 nm (Shick and Dunlap 2002). They may also acquire MAAs from the diet, and tend to accumulate very specifically only palythine and asterina-330 via a specific transport mechanism located in their eyes. In contrast, mammals did not seem to be able to accumulate MAAs either in skin, eyes or liver after maintenance on an experimental diet (Mason et al. 1998).

1.9 Ecological role of macroalgae

Macroalgae play an important role in shallow, coastal water ecosystems (Klöser et al. 1994a, Gómez et al. 1997). They account for a large fraction of global primary productivity of about 5 % (Smith 1981). Directly eaten by herbivores and omnivores (Iken 1996) they not only serve as nutrient and energy sources but algal compounds such as the MAAs probably also bring benefit as a protective mechanism against UVR in animals. Additionally, fragmented drift algae function as food for benthic organisms and form a significant part of the sediment (Wiencke and Fischer 1992). In the Antarctic

and Arctic, macroalgae are an essential source of dissolved and particulate detritus, with the advantage of providing a year-round carbon source (Amsler et al. 1995 and references therein). Furthermore, the algae serve as spawning and nursery areas for juvenile animals such as fish and crustaceans. They are used as shelter, habitat and substrata for mobile and sessile invertebrates as well as microflora. They are also important in terms of biodiversity (Norton et al. 1996) and as source of useful natural products for the aquaculture industry (Lüning 1990). Within the context of expected global climate change and continuously occurring ozone depletion, it is of major interest to investigate the effects of enhanced UV radiation on macroalgae and their defence strategies against UV-damage. Possible changes in algal nutritive substances or even alterations in macroalgal communities due to enhanced UVR may influence other marine organisms via the food web. Therefore, it is timely to explore organisms from polar regions where the ozone depletion is severe.

1.10 Antarctic – Arctic

Antarctica is the coldest place in the world, with huge ice-covered areas and a resulting high albedo (~ 0.9). This is one cause for a strong polar vortex, which in combination with the formation of the polar stratospheric clouds contribute to the ozone depletion. Furthermore, the Southern Ocean surrounding continental Antarctica features some particular characteristics. The terrestrial impact is not so large, resulting in a high water transparency with very low diffuse attenuation coefficients (K_d) (Smith et al. 1992). The Antarctic surface water temperatures are stable ranging from – 1.7 to 2 °C. Finally, the macronutrient concentrations are not seasonally limited (Drew and Hastings 1992) although the primary productivity is quite low for phytoplankton during summer (0.05 to 0.7 g C m^{-2} d^{-1}), suggesting that the phytoplankton community is co-limited by iron concentrations and incident irradiance (Strutton et al. 2000).

In the Arctic, there is a greater atmospheric exchange with the lower latitudes than in the Antarctic. Therefore, the cooling of the atmosphere and also of the stratosphere is not so great and results in a weaker polar vortex than that of Antarctica, and hence, a less pronounced ozone depletion. The Arctic Ocean, which has a permanent ice-cover at its centre, is basically an inland sea, surrounded by the northern margins of mainlands and islands, and as a result is more influenced by terrestrial phenomena. This includes large input of soils, clays and huge masses of warm fresh water from rivers especially

over the Eurasian shelf (Stonehouse 1989). Nevertheless, it is still a clear ocean with relatively low K_d values (0.2 m^{-1}, Wängberg et al. 1996). Macronutrient concentrations vary seasonally and are limited in summer (Aguilera et al. 2002b), and common primary phytoplankton productivity values ranging from 1 to 4 g C m^{-2} day^{-1} have been found (Jensen et al. 1999, Springer and McRoy 1993).

Antarctica and the Arctic, are two of the harshest environments in the world, and their fauna and flora show a high level of adaptability. The most obvious situation that these organisms need to cope with are very low temperatures and large seasonal variation of solar radiation fluctuating between of 24 h daylength in summer and 24 h darkness in winter within the polar circles. The season of winter darkness can be even prolonged for marine organisms due to ice-cover and polar macroalgae exhibit some physiological characteristics that allows them to grow under these conditions (Kirst and Wiencke 1995). They are well adapted to low light conditions, especially the Antarctic species with very low initial light saturation points of photosynthesis (18.6 to 52.6 μmol photons $m^{-2}s^{-1}$) and very low light requirements for the completion of their life cycles (Wiencke 1990, Kirst and Wiencke 1995). Additionally, the kinetics of photoinhibition and recovery of photosynthesis in Antarctic species are very fast (Hanelt et al. 1994), and as a result they are able to withstand excessive light conditions, as well as to efficiently use low photon fluence rates. Furthermore, adaptation of polar algae to cold temperatures is remarkable, showing growth optima between 0 and 5 °C (Wiencke and tom Dieck 1989, Bischoff and Wiencke 1993), and again, this is most pronounced in Antarctic algae. These described differences in adaptation between Arctic and Antarctic algal species are most probably related to the different cold water histories of the two polar regions (Wiencke et al. 1994). The Antarctic species have had a longer evolutionary development, as compared with the Arctic. Whilst Antarctica became isolated by the Circumantarctic Current about 25 million years ago, the Arctic remains well connected by longitudinally oriented coastlines. Furthermore, the Antarctic low water temperatures have predominated for about the last 14 million years, whereas the low water temperature in the Arctic has been more or less stable for only three million years. This is evident in both the physiology of the organisms and in the degree of endemism in the two polar marine floras, which is higher in Antarctica with almost 40 % of macroalgal species, of which most belong to the red algae (Wiencke and Clayton 2002).

In summary, high latitude ecosystems have not only developed under cold temperatures but also under a lower UV regime, and may not be readily adaptable to large increases in UVR intensity and shifts in the spectrum due to the ozone depletion (Whitehead et al. 2000). Furthermore, the ecosystem of the Southern Ocean may be extremely sensitive to disturbance as a result of its long and independent development under comparatively constant environmental conditions (Arntz et al. 1997). Climate change is expected to be noticed first in the polar regions (Svendsen et al. 2002).

1.11 Aim of the study

The present study focuses on a putative passive sunscreen mechanism, the UV-absorbing mycosporine-like amino acids (MAAs) synthesised in macroalgae. These compounds may reduce UV-damage in the cell or increase the UV-tolerance, the latter being of particular importance in polar regions. The general aim of the present study is to gain an amplified knowledge about ecological and physiological characteristics of MAAs and attempt to present evidence about MAA occurrence and physiology in polar macroalgae. Therefore it was necessary to perform both field and laboratory experiments. Field experiments are useful in obtaining a broad survey of macroalgae containing MAAs and to achieve further understanding of ecological aspects by recording the radiation climate at the study sites and relating them to MAA content in macroalgal species. In laboratory experiments, it is possible to investigate physiological algal responses, i.e. MAA synthesis and accumulation under controlled and simulated stress conditions.

Hitherto, there are few studies on the occurrence of sunscreening-compounds in macroalgae from the Antarctic. Thus, in the first publication qualitative and quantitative distribution patterns from MAAs were evaluated in a comprehensive set of Antarctic macroalgae with a main focus on species collected in the field. This study was supplemented with data from macroalgal cultures. Furthermore, inter- and intraspecific differences in MAA concentrations were investigated in addition to the physiological capability to form MAAs after transplantation from deeper tor shallow water exposed to different (solar) radiation.

The second publication aimed to detect possible differences in the MAA concentrations between macroalgal species from the Antarctic and the Arctic, and to relate these to the

habitat-specific UVR conditions. Furthermore, irradiance as a possible primary abiotic factor to induce MAA formation and accumulation as a photoprotective strategy was studied under controlled conditions in the second and additionally more detailed in the third publication. The aim was to acquire a better understanding of the physiological capability of red macroalgae to synthesise and accumulate MAAs.

The question whether other abiotic factors such as temperature and salinity influence the inducibility of MAA synthesis and accumulation, and the evaluation of interactive effects of radiation (with/without UVR) and temperature/salinity on photosynthesis were the central topics in the fourth and fifth publications.

Last but not least, it is still not completely clear whether the MAAs accomplish all requirements concerning a passive sunscreen protection mechanism. In an attempt to answer this question a preliminary study was undertaken. In one set up, different MAA contents were used, in order to assess the protection of photosynthesis within the plant, when samples are exposed to enhanced UVR. In a second experiment, the protective efficiency of isolated MAAs used as a radiation absorbing filter on top of plants exposed to defined radiation (with/without UVR) was investigated measuring the photosynthetic performance.

2 MATERIALS AND METHODS

2.1 Study sites

The study site in the Antarctic was Potter Cove at King George Island (South Shetlands), where the Dallmann-Laboratory, with the Jubany Station is located (62°14´S, 58°40´W, Fig. 2). Potter Cove is a small fjord with an area of approximately 7.5 km² (Roese and Drabble 1998) divided in an outer and an inner sector. The inner part has a muddy bottom, and is not deeper than 50 m, bordered by a sill of about 30 m depth (Klöser et al. 1994b). The outer sector predominantly has hard substrata, which exceeds depths of 100 m and is bordered by steep inclines in the North and by a broad intertidal platform in the Southeast (Klöser et al. 1996). The South and West coasts are flat beaches made up of sand and gravel, with numerous rocks and several cliffs, whilst the Northern and Eastern coasts have glaciers (Roese and Drabble 1998, Wunderle et al. 1998). During the snowmelt, a substantial amount of fine silt material is transported into Potter Cove through drainage streambeds and two main creeks (Varela 1998, Wunderle et al. 1998). In the summer season 1995/96 the surface water temperature in the inner cove ranged from 0.2 to 1.4 °C and the salinity from 33.2 to 34.0 psu (Schloss et al. 1998). The tide is predominantly semidiurnal with amplitudes of about average 135 cm (Schöne et al. 1998). Generally, in South Shetland region, a maritime climate predominates with summer air temperatures varying mainly between 0 and 5 °C, exhibiting maxima and minima at 15 and –7°C, respectively (Winkler et al. 1998, Wunderle et al. 1998). Moreover, these authors observed an intensifying snow smelt due to an increase of the shorter wavelengths because of the ozone depletion in late spring combined with higher air temperatures. Further descriptions of the environmental parameters of this inlet are given by Klöser et al. (1993, 1994b, 1996) and Roese and Drabble (1998).

In the Arctic, the study site was Kongsfjord, Ny-Ålesund, Spitsbergen (78°55´N, 11°55´E, Fig. 3), which is roughly 20 times bigger than Potter Cove, King George Island. It is a glacial fjord system which is 20 km long and 4 to 10 km wide, presenting an inner fjord with relatively shallow water less than 100 m and a deeper fjord estimating a total volume of 29.4 km³ (Svendsen et al. 2002, references therein). During summer, a coastal climate prevails because the fjord is open, relatively ice-free and strongly influenced by the ocean. The break up and melting of the sea ice occurs between April and July, with a high interannual variation. In contrast, a continental climate is observed during winter.

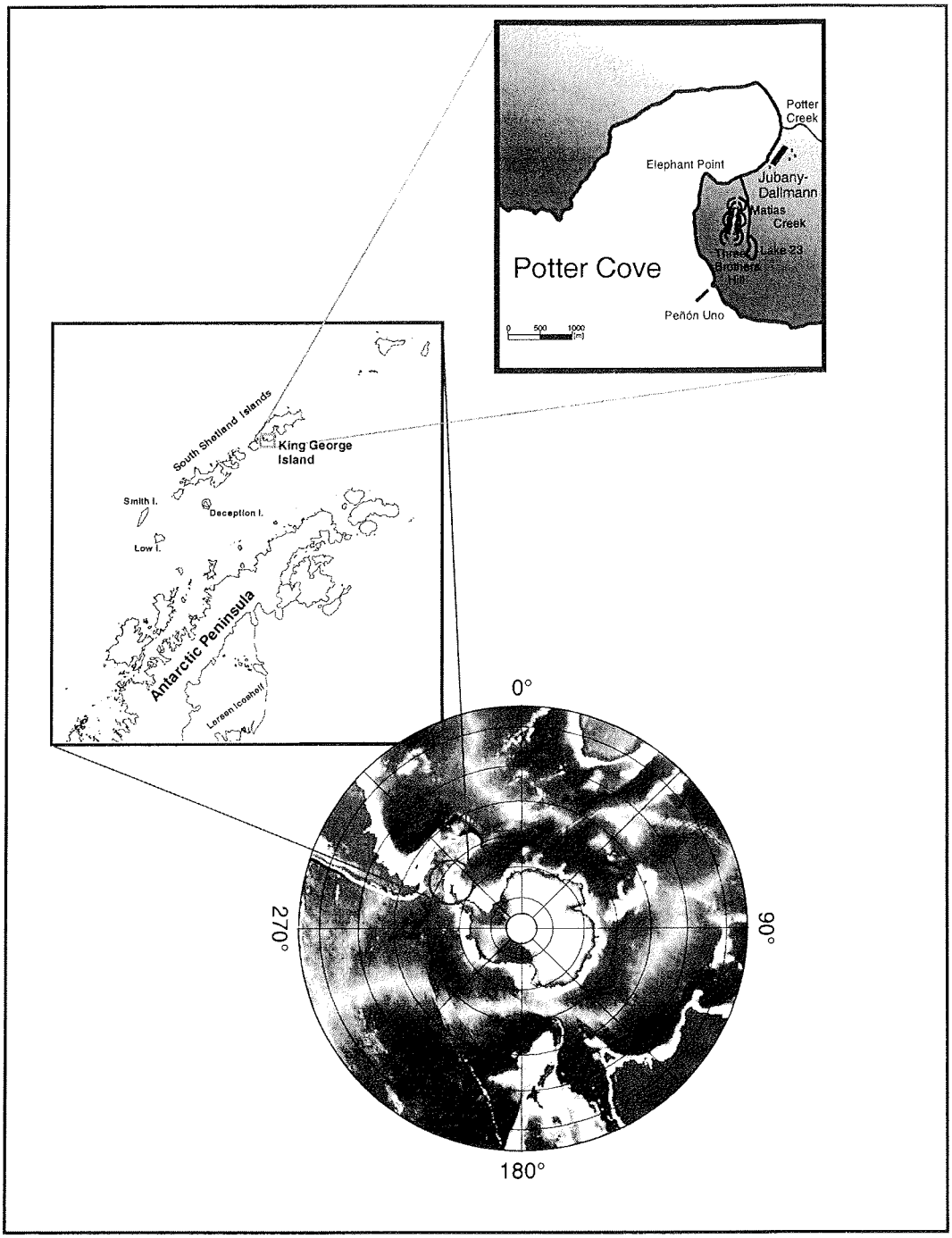


Figure 2: The Antarctic; in detail the Antarctic Peninsula and the Shetland Islands; the study site Potter Cove on King George Island with the Dallmann Laboratory at the Jubany Station

In relation to the freshwater supply and temperatures, the fjord features characteristics specific to Arctic fjords with marked seasonal patterns (Hanelt et al. 2001). The main driving forces acting on the upper water masses are fall winds, glacier ablation, ice calving, snowmelt and rainfall. These factors result in the surface water salinity drops to 30 psu and even below 28 psu near the tidewater glaciers, of which, two are located at the head of Kongsfjord and two at the northern side (Svendsen et al. 2002). The surface water temperatures in Kongsfjord range from –1 to 5 °C and the water salinity from 32 to 34.8 psu in summer. Furthermore, the tide is semidiurnal with amplitudes of 92 cm (Norwegian Hydrographic Service 2001, personal communication).

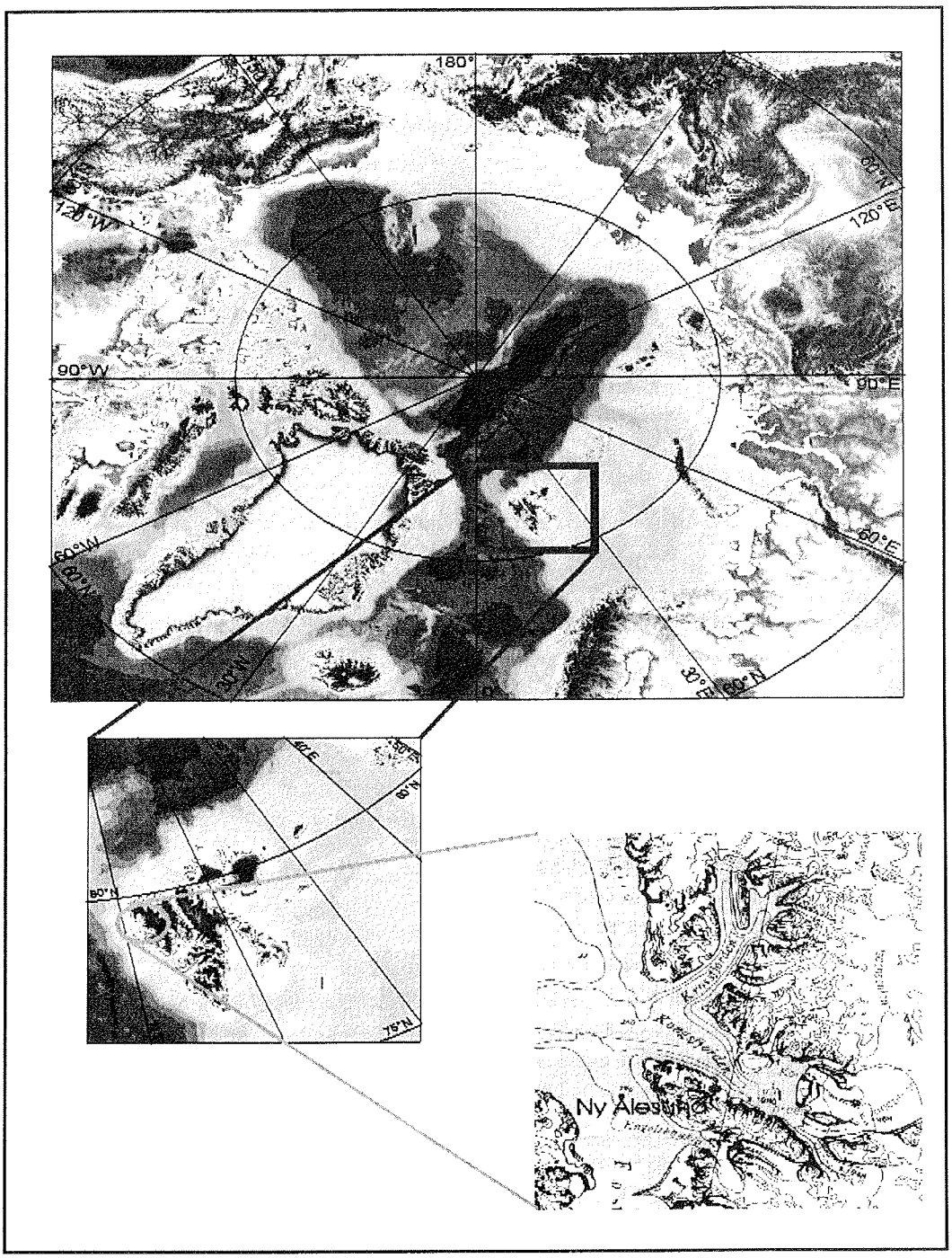
At both study sites, important macroalgal communities have become established, with about at least 62 species in Potter Cove (Quartino et al. 1998) and about 70 species in Kongsfjord (Hop et al. 2002).

2.2 Radiation measurements and conditions

In this study, a slightly modified division of the UV spectrum range compared to the definition of the CIE norms (see Introduction) was taken; UVA: 320 – 400 nm, UVB: 280(295)-320 nm. This division is normally used by photobiologists, because wavelengths shorter than 320 nm are generally more photobiologically active than longer wavelength UV radiation (Diffey 2002). In all laboratory experiments, daylight fluorescents lamps (Lumilux Deluxe, OsramL 36 W/12-950) and special fluorescent tubes (UVA 340, Q-Panel, USA), emitting a spectrum below 340 nm similar to the solar spectrum, were mainly used together. But it has to be considered that the ratio of PAR to UVR is lower in the experimental set-ups compared with the solar radiation spectrum. An artificial radiation spectrum is depicted in Publ. 4, Fig. 1.

2.3 HPLC-analysis

The analysis of the MAAs via high perfomance liquid chromatography (HPLC) is presently an established method. Due to a lack of purified standards, no calibration curve could be performed. Therefore, the molar extinction coefficient of each MAA (see Introduction) is used for the estimation of the MAA quantity via the Beer-Lambert law. The molar extinction coefficient for usujirene is still not identified therefore the one for palythene is taken as they are isomeric forms. Identification was done by spectra, retention time, and by co-chromatography with standards extracted from the marine red macroalgae *Chondrus crispus* and *Porphyra umbilicalis* from Helgoland, Germany as well as from ocular lenses of the coral trout *Plectropomus leopardus* (kindly obtained from Dr D. Bellwood, James Cook University, Townsville, Australia). A typical HPLC-chromatogram for known MAAs and one unknown UV-absorbing substance is shown in Publ. 1, Fig. 2.



Figures 3: The Arctic; the archipelago Spitsbergen; the scientific village Ny Ålesund at the Kongsfjord

2.4 MAAs as sunscreen

Protective effects of MAAs on algal photosynthesis (degree of photoinhibition) under UV treatment were investigated in algae with experimentally induced higher MAA concentrations and in algae below biofilter-containers filled with a MAA extract.

– MAAs as a cellular protection mechansim

In this experiment, the algal species *Palmaria decipiens* (Reinsch) R.W. Ricker from the Antarctic and *Palmaria palmata* (Linnaeus) Kuntze from the Arctic cultured at the Alfred Wegener Institute, Bremerhaven, Germany, under the conditions described in publication 1 were used to investigate the influence of UVR on photosynthetic activity in MAA-enriched samples, compared to control plants with low MAA concentrations. MAA accumulation was stimulated by enhancement of PAR from 15 to 38 μmol $m^{-2}s^{-1}$. *P. decipiens* was exposed for 5 days and *P. palmata* for 4 days under a 16:8 h light:dark cycle. MAAs were analysed as described in publication 4. Both MAA-enriched algal samples and those with low MAA contents were exposed to 1 h of UVR (7.1 W m^{-2} UVA and 0.66 W m^{-2} UVB) and a slight background of 5 μmol $m^{-2}s^{-1}$ PAR. During the UVR exposure, the photosynthetic performance was determined after 0.5 and 1 hour using a PAM 2000 chlorophyll fluorometer (Walz, Germany) as described in publication 4. Afterwards the samples were immediately transferred to recovery conditions of 20 μmol $m^{-2}s^{-1}$ PAR and the photosynthetic activity (F_v/F_m) was measured after 1, 2, 4 and 6 hours. This experiment was repeated, but the UVR-exposure was extended to 5 h, and F_v/F_m determined after 0.5, 1, 3 and 5 hours.

- MAAs as external biofilter

In the following experiments, the algal response of photosynthesis to UVR exposure when external MAA extracts cover the plants was studied. Therefore, the so-called biofilters were constructed. These biofilters were 0.9 cm high and made from UVR-transparent plexiglass boxes, filled with a purified methanolic shinorine extract or an isolated methanolic porphyra-334 (p-334) solution, which were prepared as follows.

Shinorine as sole MAA was extracted from *Mastocarpus stellatus* (Stackhouse) Guiry and p-334 from *Porphyra umbilicalis* (Linnaeus) Kützing, which also contains other MAAs. Both algal species originate from Helgoland. The extraction of the MAAs has been done as described in the publications but with modifications listed in table 2.

The *Mastocarpus*-extract was used directly after the extraction. But p-334 still had to be isolated from the *Porphyra*-extract using a cation exchange resin (Dowex 50W-X8, H^+-form, Sigma). Unbound compounds were removed with 2.5 fold column volume of

purified water. The MAAs were eluted from the column with a gradually increasing NaCl gradient (0.3–0.75 mM NaCl) at a flow rate of 2.5 ml. MAAs were detected with a photodiode detector at 334 nm. The main peak was collected, neutralized and afterwards evaporated to dryness under reduced pressure. The pellet was redissolved in purified water. In a second step the compound of the main peak were separated on a Äkta Purifier (Amersham pharmacia biotech) using a reversed phase column (RPC-15, Pharmacia).

Table 2: MAA extraction for the use in the biofilters (modified MAA extraction)

- 50 g dried algal samples were extracted in 700 ml 25 % aqueous methanol (v/v) over night in a water bath at 45 °C
- 2nd extraction in 500 ml 25 % methanol (v/v) for 6 h in a water bath at 45 °C
- both extracts were filtered, pooled and then evaporated under reduced pressure (rotatory evaporator) to a viscous residue
- resuspension in a minimum of water, then filled up with methanol to a final concentration of 99 % (v/v)
- centrifugation for 20 min at 10,000 rpm (3K10, Sigma)
- evaporation of the supernatant under reduced pressure
- redissolution in a small volume (20 ml) of 5 % methanol (v/v)
- filtration through a Waters C-18 Sep-Pak eluted with 5 % methanol (v/v) of a maximal 10 fold column volume
- evaporation of the elutants under reduced pressure and resuspension of the pellet in 100 % methanol

The mobile phase contained 0.1 % 2,2,3,3,3 – pentafluoropropionic acid (v/v) and 0.25 % ethanol (v/v) in water, running isocratically at a flow rate of 2 ml min^{-1}. Once again MAAs were detected with a photodiode detector at 334 nm, the p-334-peak was collected separately, again evaporated and redissolved in a small volume of (almost) 100 % methanol. The purification of both, the *Mastocarpus* extract and p-334-solution, was checked via HPLC analysis (Fig. 4) as described in Publ. 4.

The MAA solution was diluted until the transmittance was minimal at the respective absorption maximum (Fig. 5) measured in a photometer (Spectra Max 190). These solutions had an estimated concentration of 0.025 mM for the *Mastocarpus* extract and 0.026 mM for the p-334 solution used in the plexiglas boxes. The incident radiation, penetrating through the filters, shows no spectral changes in the PAR range only the intensity is slightly reduced by about 12.5 % for both biofilters compared with the solar radiation (Fig. 6). In contrast, in the UV range the intensity is much more reduced by

33.6% for UVA and 87.6 % for UVB for the *Mastocarpus* extract (shinorine-biofilter) and by 33.4 % for UVA and 80.0 % for UVB, in the case of the p-334 solution. From the wavelength 365 nm down to 310 nm the spectrum changes, with a marked depression occurring at the beginning of 353 nm. Despite this the harmful UVBR is not totally quenched. The radiation transmittance through these biofilters is shown in Fig. 6 recorded with the Sonsi spectrometer (Isitec, Germany) equipped with a cosine diffusor.

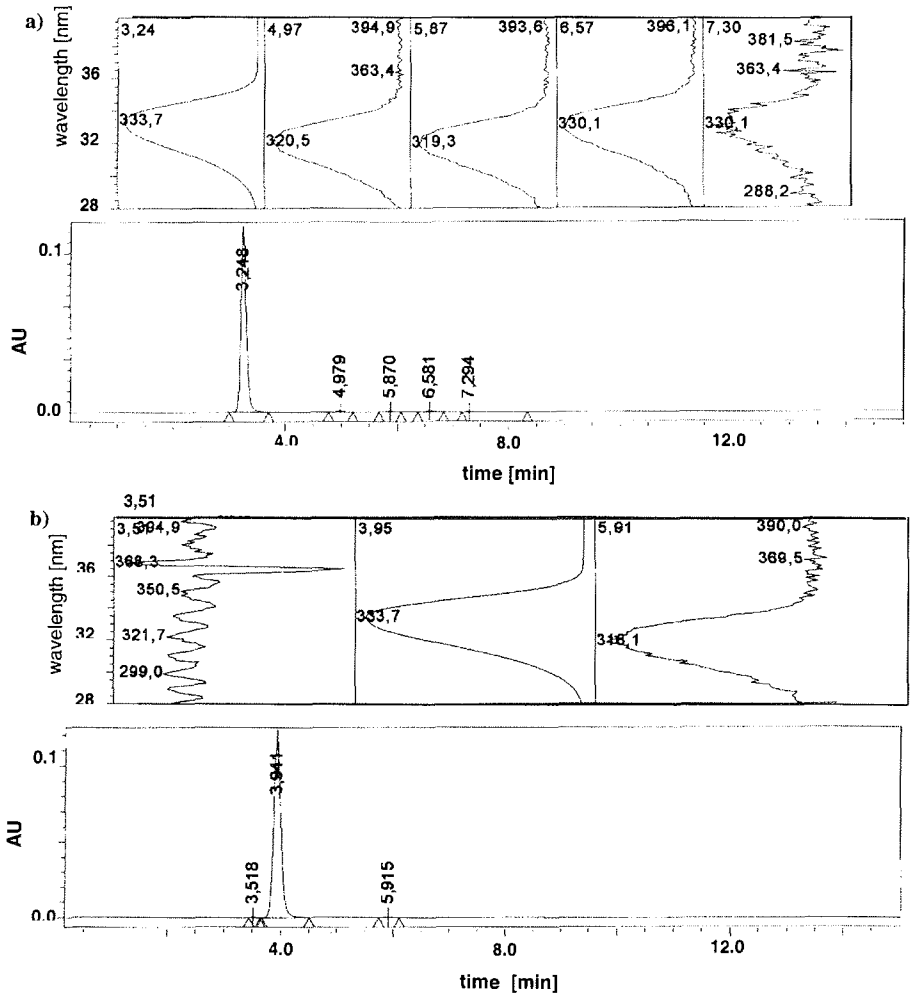


Figure 4: HPLC chromatograms of; a) purified *Mastocarpus* extract, peak with the absorption maximum at 333.7 nm and retention time of 3.2 min is shinorine with the respective absorption spectrum between 280 and 400 nm (above). This main peak contains an area of 95 % of the whole extract. b) purified p-334-solution, peak with the absorption maximum at 333.7 nm and retention time of 3.9 min is porphyra-334 with the respective absorption spectrum between 280 and 400 nm (above). This main peak contains an area of 99 % of the solution. In both chromatograms, only small contaminations were left, indicated by the other very small peaks. Mobile phase: 5 % aqueous methanol (v/v). AU: absorption units at 330 nm

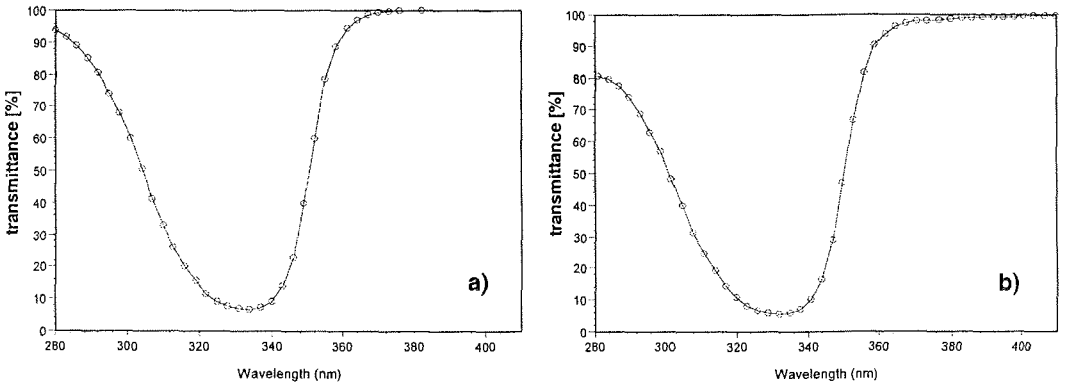


Figure 5: Transmittance spectrum of a) p-334 solution and b) *Mastocarpus* extract used in the biofilter

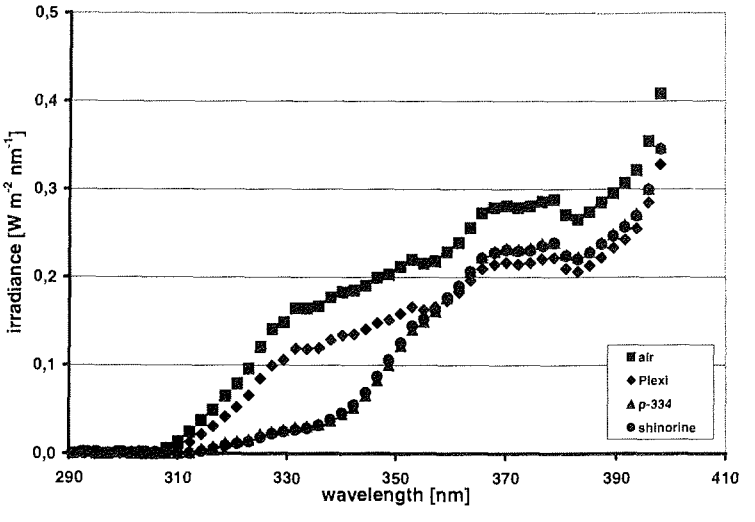


Figure 6: Solar radiation spectrum (air) from the 26 of July 2001 at 10.30 h in Ny Ålesund (Kongsfjord, Arctic) and the radiation transmittance through the empty plexiglas box (Plexi) and through the biofilter filled with the p-334 solution (p-334) and *Mastocarpus* extract (shinorine) in the range from 300 to 400 nm.

In an experiment with two Arctic red algal species (*Odonthalia dentata* (Linnaeus) Lyngbye and *Coccotylus truncatus* (Pallas) M.J. Wynne & J.N. Heine) lacking MAAs the biofilters were set in to investigate algal responses in terms of photosynthetic activity under UVR. The samples were collected by SCUBA Diving in the Kongsfjord at 11 – 13 m depth. Thalli were cut into equal sized pieces and kept for two days under

dim light conditions (<10 μmol $m^{-2}s^{-1}$) in seawater at 5 °C, aiding wound healing. Thereafter samples of *O. dentata* were transferred to following radiation conditions; 1 – only PAR (400-700 nm); 2 – PAR +UVA (320-700 nm); 3 – PAR+UVA+UVB (295-700), 4 – PAR+UVA+UVB with 34 % UVA and 88 % UVB attenuation and 5 – PAR+UVA+UVB with 33 % UVA and 80 % UVB attenuation. The radiation conditions 1 to 3 were obtained using cut-off filters (400, 320 and 295 nm cut-off foils, respectively, detailed described in publ. 3) and the conditions 4 and 5 by covering the samples with the shinorine- and p-334-biofilter, respectively. Algae were exposed to 24 h 10.6 W m^{-2} PAR, simulating Arctic summer conditions. In addition, UVR was applied for 10 h per day with 6.6 W m^{-2} UVA and 0.53 W m^{-2} UVB. Radiation was measured with the Sonsi spectrometer equipped with a cosine diffusor (Isitec, Germany). Before exposing algae to the different radiation conditions the photosynthetic activity was measured with the DIVING PAM (see Publ. 5). At day 1, 2, 3 and 5 F_v/F_m was determined each time after 9 h of UVR and after 12 h of recovery.

Samples of *C. truncatus* were irradiated with 11.5 W m^{-2} PAR, 7.4 W m^{-2} UVA and 0.62 W m^{-2} UVB, and additionally covered with a black gauze, which generally reduces all irradiance by 30 % to prevent a quick bleaching of the thalli. Furthermore, in addition to the three cut-off filters, only the p-334-biofilter was used. Photosynthetic activity was determined after 9 h of UVR and after 12 h of recovery at day 1, 2, 5, 8 and 9.

- *Statistics*

Five replicates were used for the MAA analysis. Measurements of photosynthesis were replicated 3 times in the first and 5 to 10 times in the other experiments. Mean values and standard deviations were calculated. Significant differences between MAA concentrations and between photosynthesis activity under the different radiation treatments were tested by ANOVA followed by a multiple comparison test (Tukey-Kramer HSD-test). When no homogeneity of variance could be obtained a non-parametric Kruskal-Wallis test was assessed followed by Dunn´s multiple comparison test. Significances occurred when the probability was at $p \leq 0.05$. The calculations were performed with the programs InStat (Graph Pad, San Diego, USA) and StatView (Abacus Concepts, USA).

Publication 1

Hoyer, K., Karsten, U., Sawall, T., Wiencke, C.

Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages

Marine Ecology Progress Series (2001) 211: 117-129

Copyright: Inter-Research, reprinted with kind permission

Vol. 211: 117–129, 2001 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published February 14

Photoprotective substances in Antarctic macroalgae and their variation with respect to depth distribution, different tissues and developmental stages

Kirsten Hoyer*, Ulf Karsten, Thomas Sawall, Christian Wiencke**

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

ABSTRACT: In this study the distribution pattern of UV-absorbing mycosporine-like amino acids (MAAs) was identified and quantified in Antarctic macroalgae and correlated with habitat as well as with the radiation climate in air and under water. In addition, specific amounts of MAAs from selected species collected at different depths, from different parts of the thallus and developmental stages were investigated. Seven different MAAs were detected in 17 out of 28 red algal species, whereas in all brown and 2 green algal species only traces of MAAs were found. In the green alga *Prasiola crispa* ssp. *antarctica* a high concentration of an unknown UV-absorbing substance with an absorption maximum at 324 nm was detected. MAA content was negatively correlated with water depth. Higher concentrations of UV-absorbing substances were found in the marginal tissues of thalli than in the basal parts. Tetrasporophytes and gametophytes exhibited similar MAA values. After transplantation from deep to shallow water, the MAA content remained unchanged for 8 d after transplantation. The data presented indicate 3 physiologically different groups of algae in terms of MAA values: (1) species with no capability for MAA biosynthesis; (2) species with a basic MAA concentration which is adjusted relative to changes in environmental radiation; (3) species with a constant relatively high MAA composition and concentration irrespective of environmental conditions.

KEY WORDS: Antarctica · Macroalgae · Mycosporine-like amino acids · UV-absorbing compounds · UV radiation

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Due to the depletion of the stratospheric ozone layer, solar ultraviolet-B radiation (UVBR) is increasing on the earth's surface particularly over the Antarctic region. The decrease in the ozone concentration during the Antarctic spring (September to November) is normally most pronounced in October (Lubin & Frederick 1989). The lowest ozone concentration ever recorded was measured in September 1998. This value is only about 25% of the mean concentration measured in the late 1960s (World Meteorological Organization 1998). Consequently, surface erythemal UV doses in spring had increased by about 130% compared to the 1960s (Madronich et al. 1998).

UVBR is known to harm many biological processes, and may induce direct mutagenic and lethal effects by damage to DNA, changes in enzymatic activity, and reduction of photosynthetic efficiency (Strid et al. 1994). Besides terrestrial biota, aquatic organisms are also strongly affected because UVBR can penetrate the water column to depths of 10 to 30 m (Karentz 1989, Bischof et al. 1998a), in Antarctic waters even down to 60–70 m (Smith et al. 1992).

Benthic macroalgae play an important role in Antarctic (coastal) shallow-water ecosystems (Klöser et al. 1994, Gómez et al. 1997). These plants serve as a habitat for mobile and sessile invertebrates such as gastropods and bryozoans, as a food source for herbivores and detrivores, as well as nursery areas for juve-

*E-mail: khoyer@awi-bremerhaven.de
**Present address: University of Rostock, Institute of Aquatic Ecology, 18051 Rostock, Germany

© Inter-Research 2001

nile animals such as fishes and crustaceans (Iken et al. 1999).

UVBR has many negative effects on macroalgae, such as reduction of growth, reproduction and productivity (Dring et al. 1996, Aguilera et al. 1999), inhibition of photosynthetic activity especially in deep-water species (Larkum & Wood 1993, Hanelt et al. 1997, Bischof et al. 1998b, Gómez et al. 1998), and on the quantity and quality of photosynthetic pigments (Wood 1987). The main molecular targets are DNA, RNA and proteins, which strongly absorb UVBR. However, macroalgae have developed protective mechanisms which counteract the effects of UVBR stress. At the molecular level there is the capability to repair DNA damage by photolyases and excision enzymes. In addition, there are also physiological and biochemical counteracting strategies such as the expression of detoxifying enzymes for the elimination of UVR-induced reactive oxygen species (Collén & Davison 1999).

Moreover, many marine primary producers synthesize UV-absorbing substances, known as mycosporine-like amino acids (MAAs) (Riegger & Robinson 1997, Dunlap & Shick 1998, Karsten et al. 1998a, Franklin et al. 1999, Jeffrey et al. 1999, Karsten & Wiencke 1999). These compounds are considered as a passive sunscreen mechanism which protects against damaging UV radiation through absorption of these photons followed by heat and fluorescence emission (Cockell & Knowland 1999). To date, 19 structurally distinct MAAs have been identified in marine organisms. MAAs are water-soluble compounds characterized by a cyclohexenone or cyclohexenimine chromophore conjugated with the nitrogen substituent of an amino acid or amino alcohol and typically exhibiting UV-absorption maxima in the range of 310 to 360 nm (Dunlap & Shick 1998).

Examination of the qualitative and quantitative distribution patterns of MAAs among tropical and warm-temperate to Arctic macroalgae revealed the occurrence of 9 different MAAs, of which 7 could be identified in red algae (Karsten et al. 1998a,b).

Hitherto, only 2 surveys on MAAs in Antarctic organisms have been conducted: by Karentz et al. (1991) and by McClintock & Karentz (1997). However, these authors focused mainly on marine fauna. Consequently, there are few data available on the occurrence of sunscreening-compounds in macroalgae from the Antarctic region. Therefore, in the present study the patterns and amounts of MAAs were evaluated in a comprehensive set of Antarctic macroalgae, focusing mainly on species collected in the field, supplemented with data on macroalgal cultures. In addition, intraspecific differences in MAA content were correlated with collection depth, as already done for some Arctic red algae (Karsten & Wiencke 1999, Karsten et al. 1999). In some selected species, MAA concentrations in different tissues of vegetative and reproductive plants were measured, as well as the physiological capability to form MAAs after transplantation from deeper to shallow waters. Compared to the Arctic Ocean, the Antarctic has some characteristics such as a very small riverine influx that result in low concentrations of DOM and humic substances (gelbstoff) and are reflected in a higher water transparency and thus a higher penetration of UVBR. Therefore, the radiation climate at our study site was characterized and related to MAA content in macroalgal species growing at different depths.

MATERIALS AND METHODS

Study site and algal material. The investigations were performed at Potter Cove, King George Island, South Shetlands (62° 14' S, 58° 40' W), near the Dallmann Laboratory/Jubany Station during austral summer 1997/98 (Fig. 1). Potter Cove is a small fjord divided into an outer and an inner sector. The inner sector has

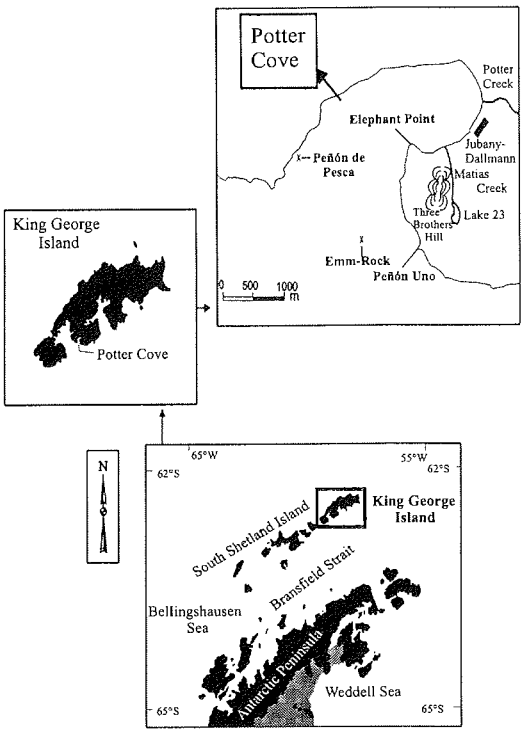


Fig. 1. South Shetland Islands (Antarctica), King George Island, and the study area Potter Cove, with sampling sites indicated

a muddy bottom and is no deeper than 50 m; the outer area is bordered by steep inclines to the north and by a broad intertidal platform to the southeast. Further descriptions of the environmental parameters of this inlet are given by Klöser et al. (1996 and references therein), and Roese & Drabble (1998).

Macroalgal species were collected by SCUBA diving at 3 sublittoral stations (Emm Rock [Klöser et al. 1996], Peñón de Pesca [PdP], and Elephant Point) and 1 eulittoral station (Peñón Uno [P1], characterized by rocky flats) (Fig.1). The locations, habitats, and some physiological data on the macroalgal species studied are listed in Table 1, as well as details of the cultured Rhodophyceae investigated. The latter were isolated from Potter Cove in 1994 according to Wiencke (1988) and established as a permanent growth culture in the laboratory under the following conditions: culture medium (Starr & Zeikus 1987), salinity of 30 to 32 ppt, aerated with membrane-filtered air (pore size 0.2 µm), temperature of 0°C, illumination of 10 to 20 µmol photons m^{-2} s^{-1} provided by daylight fluorescent lamps (Lumilux Deluxe, Osram, Germany). Daylength varied between 5 (winter) and 20 h (summer), simulating fluctuating Antarctic daylengths (Wiencke 1990). The harvested plants were oven-dried at 50°C overnight, and then stored in sealed plastic bags under dry and dark conditions prior to MAA analysis.

Radiation measurements. During the summer period, photosynthetically active radiation (PAR, 400 to 700 nm) in the atmosphere was measured continuously with a Li-Cor data-logger (LI-1000, Li-Cor, Lincoln, USA) equipped with a flat-head sensor (LI-190). The instantaneous PAR data (µmol photons m^{-2} s^{-1}) were plotted against time of day (h), and the total daily photon exposure (mol m^{-2} d^{-1}) was calculated by integrating the area under the light-time curves. Underwater light measurements were monitored using an underwater spherical quantum sensor (LI 193 SA). Additionally, underwater spectra of ambient radiation of the wavelength from 327 to 700 nm were recorded at various depths with a spectroradiometer (Ingenieurbüro M. Kruse, Stubben, Germany). Water transmittance was determined by measuring irradiance at different depths and calculating diffuse vertical attenuation coefficients of downward irradiance (K_d) according to the formula:

$$K_d = 1/(z_2 - z_1) \times \ln E_{d(z_1)}/E_{d(z_2)}$$

where $E_{d(z_1)}$ and $E_{d(z_2)}$ = irradiances at depths z_1 and z_2, respectively (Kirk 1994).

UVBR (280 to 320 nm) in the air was measured using a 32-channel single-photon counting spectroradiometer developed at the Physics Department of the Alfred Wegener Institute and installed on the roof of the Dallmann Laboratory. The spectroradiometer was computer controlled, allowing on-line recordings of the radiation data.

MAA concentrations in individuals from different depths and in different tissues and developmental stages. To study intraspecific variations of quantitative and qualitative MAA contents in relation to depth zonation, the red algae *Iridaea cordata*, *Palmaria decipiens*, *Myriogramme mangini* and *Plocamium cartilagineum* were collected at depths of 0 to 20 m at Emm Rock and P1 (Fig. 1). *Kallymenia antarctica* and *Gigartina skottsbergii* were collected at PdP at depths of 10 and 15 m, respectively.

Quantitative and qualitative variations in MAA contents of different tissues were determined in different parts of selected species as well as in different stages of development, in tetrasporophytic and gametophytic plants.

Two species, *Kallymenia antarctica* and *Gigartina skottsbergii*, were collected at 20 m depth and transplanted into floating UV-transparent XT Plexiglas tubes (300 × 110 mm outer diameter; Weissig, Berlin, Germany) fixed at 0.3, 5, 10, and 20 m water depth. Plexiglas tubes were fastened to a plastic tube of 5 m length. A buoy was attached to each end of the plastic tube; 1 buoy was fixed with an anchor to the sea bottom. For each species and depth, 3 UV transparent Plexiglas tubes were mounted together, one of which was wrapped with a specific filter foil to cut-off UV-B (320 nm cut-off: Ultraphan URUV, Digefra, München, Germany) and another one to cut off UV-B + UV-A (400 nm cut-off: Folex PR, Folex, Dreieich, Germany). After 8 d exposure at the respective depths the specimens were harvested for MAA analysis.

MAA extraction and analysis. Samples of about 10 to 20 mg dry weight (DW) were extracted for 1.5 to 2 h in screw-capped centrifuge vials filled with 1 ml 25% aqueous methanol (v/v) and incubated in a waterbath at 45°C. This procedure was sufficient to obtain >99.5% of MAAs in solution. After centrifugation at 5000 × *g* for 5 min, 800 µl of the supernatants were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were re-dissolved in 800 µl 100% methanol and vortexed for 30 s. Samples were analysed with a Waters high-performance liquid chromatography (HPLC) system according to the method of Karsten & Garcia-Pichel (1996), modified as follows. The MAAs were separated on a stainless-steel Knauer Spherisorb RP-8 column (5 µm, 250 × 4 mm i.d.) protected with an RP-8 guard cartridge (20 × 4 mm i.d.). The mobile phase was 5 to 25% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at a flow rate of 0.7 ml min^{-1}. The MAAs were detected with a photodiode detector at 330 nm, and absorption spectra (290 to 400 nm) were recorded each second directly on to the HPLC-separated peaks. Identification was by spectra, retention time, and by co-chromatography with standards extracted from the

Table 1. Investigated field and cultured macroalgae with the respective photosynthetic light compensation (I_c) and initial light saturation (I_k) values of photosynthesis, and details of collection site and habitat. I_c and I_k values were from material cultured in the laboratory under Antarctic conditions according to Weykam et al. (1996), and are expressed in µmol m^{-2} s^{-1}. Samples were collected between 26 November 1997 and 28 January 1998. P1: Peñón Uno; PdP: Peñón de Pesca

Species	Field/Culture	I_c	I_k	Collection site (depth); habitat (depth)
RHODOPHYCEAE				
Audouinella purpurea (Lightfoot) Woelkerling (Acrochaetiales)	Culture			Lower eulittoral
Antarcticothamnion polysporum Moe & Silva (Ceramiales)	Culture			Sublittoral (15–25 m)
Ballia callitricha Kützing (Ceramiales)	Culture	<12	<25	Eulittoral, sublittoral (0–45 m)
Bangia atropurpurea (Roth) C. Agardh (Bangiales)	Culture			Supralittoral
Curdiea racovitzae Hariot (Gracilariales)	Field	<8	<50	Eulittoral, P1; tide pools, sublittoral (0–35 m)
Georgiella confluens (Reinsch) Kylin (Ceramiales)	Field	<13	<40	Eulittoral, P1; sublittoral (5–25 m)
Gigartina skottsbergii Setchell & Gardner (Gigartinales)	Field/Culture	<12	<40	Eulittoral, P1, sublittoral, PdP, Emm Rock (9, 12–15, 20 m); tide pools, sublittoral (0–30 m)
Gymnogongrus antarcticus Skottsberg (Gigartinales)	Field	<25	<40	Eulittoral, P1; tide pools, sublittoral (0–15 m)
Gymnogongrus turquetii Hariot (Gigartinales)	Culture	<7	<35	Eulittoral, sublittoral (0–30 m)
Hymenocladiopsis crustigena Moe (Rhodymeniales)	Field/Culture	<9	<25	Sublittoral, Emm Rock (12–15 m); sublittoral (2–30 m)
Iridaea cordata (Turner) Bory de Saint Vincent (Gigartinales)	Field	<7	<50	Eulittoral, P1, sublittoral, Emm Rock, (5, 10, 15 m); tide pools, sublittoral (0–20 m)
Kallymenia antarctica Hariot (Cryptonemiales)	Field/Culture	<8	<45	Sublittoral, Emm Rock (12, 20 m); sublittoral (5–35 m)
Myriogramme mangini (Gain) Skottsberg (Ceramiales)	Field	<6	<25	Sublittoral, Emm Rock, (5, 10 m, 15 m); sublittoral (2–45 m)
Myriogramme smithii (Hooker fil. et Harvey) Kylin (Ceramiales)	Field	<12	<25	Sublittoral, Emm Rock (15, 20 m); sublittoral (8–45 m)
Neuroglossum ligulatum (Reinsch) Skottsberg (Ceramiales)	Field/Culture			Sublittoral, Emm Rock (15 m); sublittoral (1–10 m)
Notophycus fimbriatus Moe (Gigartinales)	Field			Eulittoral, P1; eulittoral tide pools, sublittoral (0–20 m)
Pachymenia orbicularis (Zanardini) Setchell & Gardner (Cryptonemiales)	Field			Sublittoral, P1 (15 m); lower eulittoral, sublittoral (0–20 m)
Palmaria decipiens (Reinsch) Ricker (Palmariales)	Field/Culture	<7	<55	Eulittoral, P1, sublittoral, Emm Rock (15, 20 m); eulittoral to sublittoral (0–30 m)
Pantoneura plocamioides Kylin (Ceramiales)	Culture	<20	<30	Sublittoral (2–45 m)
Phycodrys austrogeorgica Skottsberg (Ceramiales)	Field /Culture			Sublittoral, Emm Rock (20 m); sublittoral (2–45 m)
Phycodrys quercifolia (Bory) Skottsberg (Ceramiales)	Culture	<15	<20	Eulittoral – sublittoral (0–25 m)
Phyllophora ahnfeltioides Skottsberg (Gigartinales)	Culture		<20	Sublittoral (0–30 m)
Picconiella plumosa (Kylin) de Toni (Ceramiales)	Field	<16	<30	Sublittoral, Emm Rock (15, 20 m); sublittoral (2–50 m)
Plocamium cartilagineum (Linnaeus) Dixon (Plocamiales)	Field/Culture	<12	<25	Eulittoral, P1, sublittoral, Emm Rock (5, 10, 15, 20 m); sublittoral (2–40 m)
Porphyra endiviifolium Chamberlain (Bangiales)	Field/Culture	<14	<65	Supralittoral, eulittoral, P1; upper eulittoral
Rhodymenia subantarctica Ricker (Rhodymeniales)	Culture			Sublittoral (5–25 m)
Sarcothalia papillosa (Bory) Leister (Gigartinales)	Field			Eulittoral, P1, sublittoral, Emm Rock (12, 15 m); rock pools, sublittoral (0–16 m)
PHAEOPHYCEAE				
Adenocystis utricularis (Bory) Skottsberg (Dictyosiphonales)	Field	<11	<95	Eulittoral, P1; eulittoral, tide pools, sublittoral (0–10 m)
Ascoseira mirabilis Skottsberg (Ascoseirales)	Field	<16	<45	Eulittoral, P1; eulittoral, sublittoral (0–12 m)
Desmarestia menziesii Agardh (Desmarestiales)	Field	<19	<35	Eulittoral, P1; eulittoral to sublittoral (5–45 m)
Himanthothallus grandifolius (Gepp et Gepp) Zinova (Desmarestiales)	Field	<9	<30	Sublittoral, Pdp (20 m); sublittoral (7–70 m)
Phaeurus antarcticus Skottsberg (Desmarestiales)	Field	<10	<140	Eulittoral, P1; tide pools, sublittoral (0–10 m)
CHLOROPHYCEAE				
Enteromorpha bulbosa (Suhr) Montagne (Ulvales)	Field	<70	<13	Eulittoral, P1; tide pools
Prasiola crispa ssp. *antarctica* (Kützing) Knebel (Prasiolales)	Field			Supralittoral, nearby Penguin colony; supralittoral
Monostroma hariotii Gain (Ulvales)	Field	<30	<9	Eulittoral, P1; tide pools, sublittoral (0–20 m)

marine red macroalgae *Chondrus crispus* (Karsten et al. 1998a) and *Porphyra umbilicalis* (kindly provided by Dr L. A. Franklin, Australian National University, Canberra, Australia). Quantification was performed using the molar extinction coefficients listed in Karsten et al. (1998c). Some smaller amounts of unknown UV-absorbing compounds were summarized and, together with the unknown peaks in *Prasiola crispa* ssp. *antarctica*, quantified using an average molar extinction coefficient of all published values. All amounts are given as means of 3 replicates (±SD) randomly collected from the respective habitat or from cultures and expressed as concentration on a dry weight basis (mg g^{-1} DW).

RESULTS

Radiation data

Between 1 November 1997 and 31 January 1998, ozone concentration over Antarctica (Neumayer Station) ranged from 227 to 316 Dobson units (DU) (Table 2). During this period, cloud cover was mostly 80% (6.4 octas) on King George Island, hence influencing surface irradiance. The lowest PAR-dose was 17.4 mol photons m^{-2} d^{-1} measured on 2 January 1998 at a daylength of 20 h. However, on 1 December 1997 with a cloud cover of only 60% (4.8 octas) the maximum daily PAR-dose reached 67.2 mol photons m^{-2} d^{-1}. Instantaneous PAR values occasionally even exceeded 1700 µmol photon m^{-2} s^{-1} and 1.8 W m^{-2} for UVBR. The maximum daily dose of UVBR in the air was 52.4 kJ m^{-2} on 23 December 1997, with an average daily dose of 40.8 kJ m^{-2}, whereas the average daily dose in January 1998 was 29.9 kJ m^{-2}. In general in December 1997, PAR and UVR were higher than in January 1998. The light transmittance in the water was much lower in the middle of the bay at Elephant Point than in the outer part at Emm Rock (Fig. 1). The K_d value for PAR at Elephant Point was 0.45 m^{-1}, and UV (327 to 399 nm) = 1.1 m^{-1}, while at Emm Rock $K_{d\ PAR}$ = 0.17 m^{-1} and $K_{d\ UV}$ = 0.19 m^{-1}, as measured on 30 December 1997. Radiation data for the period of the field study are summarized in Table 2.

Table 2. Radiation measurements and ozone data for the expedition period (22 November 1997 to 31 January 1998). Ozone data taken from Meterology Observatory of Neumayer Station, Antarctica (70° 37' S, 8° 22' W). DU: Dobson units

	Date	Units
Ozone		
Max. ozone	8 December 1997	316 DU
Min. ozone	16 November 1997	227 DU
Ozone mean value	November 1997–January 1998	260 DU
Atmosphere		
PAR (400–700 nm)		
Daily dose	Average for December 1997	50.1 mol photons m^{-2} d^{-1}
Daily dose	Average for January 1998	32.9 mol photons m^{-2} d^{-1}
Max. irradiance	20 January 1998	1748 µmol photons m^{-2} s^{-1}
UVB (280–320 nm)		
Daily dose	Average for December 1997	40.8 kJ m^{-2}
Daily dose	Average for January 1998	29.9 kJ m^{-2}
Max. radiation	23 December 1997	1.80 W m^{-2}
Attenuation coefficient (K_d)		
$K_{d\ PAR}$	30 December 1998, Emm Rock	0.17 m^{-1}; 1% depth: 26.9 m
$K_{d\ UV\ (327-399\ nm)}$	30 December 1998, Emm Rock	0.19 m^{-1}; 1% depth: 24.2 m
$K_{d\ PAR}$	30 December 1998, Elephant Point	0.45 m^{-1}; 1% depth: 10.3 m
$K_{d\ UV\ (327-399\ nm)}$	30 December 1998, Elephant Point	1.1 m^{-1}; 1% depth: 4.2 m

MAA inventory

Eighteen different species of Rhodophyceae were collected, mostly from the sublittoral or from eulittoral locations (tide pools) at Potter Cove. In the investigated algae, 7 different MAAs were detected. These were identified as mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol and palythene. Furthermore, some smaller amounts of unknown UV-absorbing compounds with retention times between 4.5 and 6.0 min and absorbance maxima of 332 to 334 nm were summarized as Unknown[1] and some with retention times between 6.1 and 15.0 min and absorbance maxima of 321 to 337 nm as Unknown[2] (Table 3). Generally, in 70% of the field-grown algal species studied, shinorine and palythine were the most abundant MAAs, followed by porphyra-334 and asterina-330, which occurred in half the investigated species. However, in *Porphyra endiviifolium*, porphyra-334 was quantitatively the most abundant MAA (7.7 mg g^{-1} DW). This species also exhibited the highest total MAA content (9.0 mg g^{-1} DW), followed by *Curdiea racovitzae* with a total amount of 4.0 mg g^{-1} DW. MAAs were completely absent from 4 species (*Hymenocladiopsis crustigena*, *Myriogramme smithii*, *Phycodrys austrogeorgica* and *Picconiella plumosa*). These red algae all originated from the sublittoral. Only traces of 3 MAAs were detected in the sublittoral *Georgiella confluens*.

In 9 of 18 cultivated red algae investigated, no trace of any MAA could be detected. In the remaining plants, the most abundant and the quantitatively dominant MAA was porphyra-334 followed by shinorine (Table 3). *Bangia atropurpurea* contained the highest

Table 3. Concentrations of mycosporine-like amino acids (MAAs) (mg g^{-1} dry wt) in Antarctic macroalgae collected from the field (in **bold**) and from culture (in normal print). Values are means ±SD (n = 3). Unknown[1]: presumed but unknown MAA substances in low concentration with a retention time between 0 an 6.5 min; Unknown[2]: presumed but unknown MAA substances in low concentration with a retention time between 6.5 an 15 min; n: number of known MAA; MAA total: total MAA concentration for each species. See Table 1 for full genus names

Species	Mycosporine-glycine	Shinorine	Porphyra-334	Unknown[1]	Palythine	Asterina-330	Palythinol	Palythene	Unknown[2]	n	MAA total
RHODOPHYCEAE											
Aud. purpurea	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Ant. polysporum	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Bal. callitricha	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Ban. atropurpurea	0.000	0.000	5.800 ± 0.113	0.000	0.000	0.000	Trace	0.000	0.000	2	5.800 ± 0.113
Cur. racovitzae	0.029 ± 0.012	0.505 ± 0.146	0.002 ± 0.003	0.035 ± 0.011	3.876 ± 1.117	0.295 ± 0.088	0.000	0.000	0.239 ± 0.086	5	4.981 ± 1.462
Del. lancifolia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Georg. confluens	0.000	0.002 ± 0.004	0.013 ± 0.007	0.000	0.002 ± 0.002	0.000	0.000	0.000	0.000	3	0.017 ± 0.009
Gig. skottsbergii	0.000	0.120 ± 0.027	0.000	0.021 ± 0.005	1.162 ± 0.202	0.004 ± 0.003	0.000	0.000	0.253 ± 0.040	3	2.021 ± 0.264
Gig. skottsbergii	0.000	0.000	0.428 ± 0.0512	0.000	0.134 ± 0.012	0.007 ± 0.001	0.000	0.000	0.000	3	0.569 ± 0.064
Gym. antarcticus	0.000	2.129 ± 0.693	0.000	0.000	0.006 ± 0.002	0.002 ± 0.002	0.000	0.000	0.000	3	2.137 ± 0.697
Gym. turquetii	Trace	0.106 ± 0.029	0.000	0.000	0.000	0.000	0.000	0.000	0.000	2	0.108 ± 0.030
Hym. crustigena	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Hym. crustigena	0.000	0.000.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Iri. cordata	0.009 ± 0.009	1.033 ± 0.753	0.000	0.007 ± 0.008	1.823 ± 0.648	0.000	0.000	0.000	0.265 ± 0.140	3	3.138 ± 0.624
Kal. antarctica	0.089 ± 0.080	0.593 ± 0.504	0.000	0.000	1.805 ± 0.551	0.101 ± 0.049	0.028 ± 0.010	0.000	0.007 ± 0.006	5	2.590 ± 1.162
Kal. antarctica	Trace	0.000	0.501 ± 0.156	0.000	0.021 ± 0.005	0.000	0.000	0.000	0.000	3	0.523 ± 0.161
Myr. manginii	0.000	1.153 ± 1.622	1.755 ± 1.681	0.000	0.009 ± 0.017	0.003 ± 0.005	0.000	0.000	0.004 ± 0.006	4	2.925 ± 1.290
Myr. smithii	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Neu. ligulatum	0.000	2.550 ± 0.673	0.072 ± 0.021	0.024 ± 0.007	0.004 ± 0.004	0.000	0.000	0.000	0.000	3	2.649 ± 0.703
Neu. ligulatum	0.000	0.372 ± 0.046	0.012 ± 0.005	0.000	0.000	0.000	0.000	0.000	0.000	2	0.384 ± 0.047
Not. fimbriatus	0.005 ± 0.004	0.216 ± 0.070	0.846 ± 0.367	0.000	0.644 ± 0.210	0.026 ± 0.007	0.000	0.000	0.012 ± 0.005	5	1.750 ± 0.661
Pach. orbicularis	0.005 ± 0.000	0.000	0.892 ± 0.082	0.000	0.003 ± 0.001	0.000	0.000	0.000	0.000	3	0.845 ± 0.083
Pal. decipiens	0.035 ± 0.010	0.305 ± 0.004	2.548 ± 0.020	0.000	0.604 ± 0.028	0.213 ± 0.011	0.085 ± 0.039	0.139 ± 0.031	0.000	7	3.927 ± 0.118
Pal. decipiens	0.000	0.035 ± 0.023	0.414 ± 0.069	0.000	0.000	0.000	0.000	0.0181 ± 0.009	0.000	3	0.45 ± 0.092
Pan. plocamioides	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Phy. austrogeorgica	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Phy. austrogerorgica	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Phy. quercifolia	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Phyll. ahnfeltioides	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Pic. plumosa	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Plo. cartilagineum	0.000	0.233 ± 0.109	0.004 ± 0.006	0.000	0.789 ± 0.520	0.003 ± 0.005	0.002 ± 0.004	0.000	0.000	5	1.031 ± 0.643
Plo. cartilagineum	0.000	0.000	0.000	0.000	0.120	0.014 ± 0.005	0.000	0.000	0.000	2	0.134 ± 0.031
Por. endivilifolium	0.013 ± 0.010	1.722 ± 0.298	7.717 ± 0.833	0.244 ± 0.027	0.018 ± 0.004	0.006 ± 0.002	0.014 ± 0.008	0.000	0.000	6	9.734 ± 1.137
Por. endivilifolium	0.000	0.472 ± 0.047	3.796 ± 0.24	0.000	0.000	0.000	0.000	0.000	0.000	2	4.268 ± 0.280
Rho. subantarctica	0.000	0.000	0.193 ± 0.039	0.000	0.000	0.000	0.000	0.000	0.000	1	0.193 ± 0.0389
Sar. papillosa	0.000	0.046 ± 0.014	0.000	0.000	1.436 ± 0.172	0.000	0.000	0.000	0.133 ± 0.026	2	1.615 ± 0.208
PHAEOPHYCEAE											
Ade. utricularis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Asc. mirabilis	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Des. menziesii	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
Him. grandifolius	0.000	0.000	Trace	0.000	Trace	0.000	0.000	0.000	0.000	2	Trace
Pha. antarcticus	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	–	0.000
CHLOROPHYCEAE											
Ent. bulbosa	0.000	0.000	Trace	0.000	0.000	0.000	0.000	0.000	0.000	1	Trace
Pra. crispa ssp. ***antarctica***	0.038 ± 0.015	0.000	0.000	3.681 ± 0.515	0.000	0.000	0.000	0.000	0.000	1	3.719 ± 0515
Mon. hariotii	0.000	Trace	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1	Trace

quantity of total MAAs (5.8 mg g^{-1} DW), mainly due to the presence of porphyra-334. The MAA content in cultivated plants was generally only 15 to 25% of values found in algae collected in the field. One exception was *Porphyra endiviifolium*, which in culture exhibited 50% of the total MAA value of the field sample. In contrast, cultivated *Plocamium cartilagineum* contained only 10% of the total MAAs found in field material. *Hymenocladiopsis crustigena* did not contain any MAA, either as field-collected alga or as cultured plant (Table 3).

Traces of MAAs were found in 1 Antarctic field-collected phaeophyceaen, and all 3 tested chlorophyceaens contained MAAs. *Enteromorpha bulbosa* contained low concentrations of porphyra-334, and *Monostroma hariotii* a low amount of shinorine. In *Prasiola crispa* ssp. *antarctica*, traces of mycosporine-glycine and a high quantity of an unknown UV-absorbing substance (3.7 mg g^{-1} DW) with an absorption maximum at 324 nm and a retention time of 3.2 min were detected, appearing during HPLC analysis before shinorine (retention time = 3.4 min) and porphyra-334 (retention time = 4.0 min) (Fig. 2).

The initial light saturation (I_k) value of red algal photosynthesis (Table 1) correlated well with the MAA concentration (Table 3), as indicated by a correlation coefficient of r = 0.831 (Fig. 3). The higher the I_k, the higher the MAA content.

Depth profile

In *Iridaea cordata*, *Palmaria decipiens* and *Plocamium cartilagineum*, MAA concentrations continuously decreased with increasing collection depth. Generally, the MAA contents of these species were 1.5 to 20 times higher in shallow-water isolates than in the respective deep-water samples (Fig. 4). While in samples of *I. cordata* from shallow-water depths palythine was the quantitatively dominant MAA, in sublittoral plants the concentration of palythine decreased so that shinorine and palythine displayed almost the same concentration (Fig. 5a). When collected from shallow water, *Palmaria decipiens* exhibited 7 different MAAs; when collected from 20 m it showed, only 3, and in very low amounts (Fig. 5b). In contrast to these species, *Kallymenia antarctica* and *Gigartina skottsbergii* collected at different depths did not show significant differences in MAA concentrations (data not shown).

Tissue gradient

The MAA content was measured in different tissues of vegetative algal thalli of *Iridaea cordata*, *Palmaria decipiens* and *Curdiea racovitzae*. The algae exhibited a clear difference in MAA content between the young margin and the older base of the thallus: the margin tissue always contained a 1.5- to 3.6-fold higher MAA concentration (Fig. 6). The content of the quantitatively dominant MAA palythine in the margin tissue of *I. cordata* was 6 times, and in *C. racovitzae* 1.4 times, higher than that of basal tissue, whereas porphyra-334, the main compound in *P. decipiens*, increased 4-fold (data not shown).

Developmental stages

The tetrasporophytes and gametophytes of *Gigartina skottsbergii* and *Iridaea cordata* collected from the sublittoral contained nearly equal MAA concentrations. While the different development stages of the former contained MAA values between 2 and 2.5 mg g^{-1} DW, the latter species contained between 3.1 and 3.3 mg g^{-1} DW MAA.

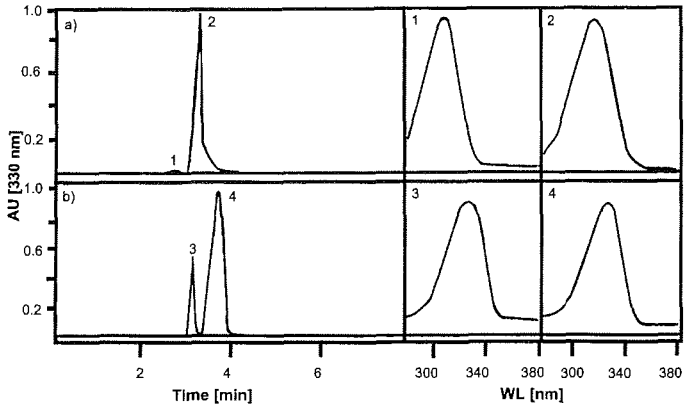


Fig. 2. (a) *Prasiola crispa* ssp. *antarctica* and (b) *Porphyra ediviifolium*. High-performance liquid chromatography chromatograms of (a) methanolic *P. crispa* ssp. *antarctica* extract recorded at 330 nm and the respective absorption spectra between 280 and 400 nm for each separated peak (peak identities are: 1, mycosporine-glycine [retention time, RT, = 2.8 min]; 2, unknown MAA [RT = 3.2 min]) and (b) *Porphyra endiviifolium* showing 2 typical peaks (1, shinorine [RT = 3.4 min], 2, porphyra-334 [RT = 4.0 min]). Mobile phase: 5% aqueous methanol (v/v). AU: absorption units, WL: wavelength

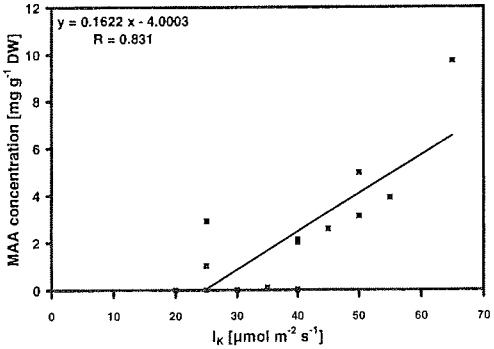


Fig. 3. Initial light-saturation points (I_k) of red algal photosynthesis according to Weykam et al. (1996) plotted against mycosporine-like amino acid (MAA) concentration (mg g^{-1} dry wt, DW) measured in the red algae from the field. Regression analysis: $y = 0.162x - 4.0$, with $r = 0.831$

Transplantation experiment

That total MAA content of *Kallymenia antarctica* collected from 20 m was about 2.6 mg g^{-1} DW (Fig. 7a). After transplantation to 0.3, 5, 10, and 20 m water depth and exposure for 8 d to solar radiation without UVAR + UVBR, solar radiation without UVBR and full solar radiation, the quantitative and qualitative MAA composition was measured. Although visual evaluation of the data pointed to a slight stimulation of MAA synthesis after transplantation from deeper to shallow water, in most cases statistical treatment (ANOVA, *F*-test) did not reveal significant differences, with a high standard deviation indicating high variability. Similar results were obtained for *Gigartina skottsbergii*, in which the total MAA concentration varied from 1 to 2.4 mg g^{-1} DW under the above-mentioned conditions (Fig. 7b).

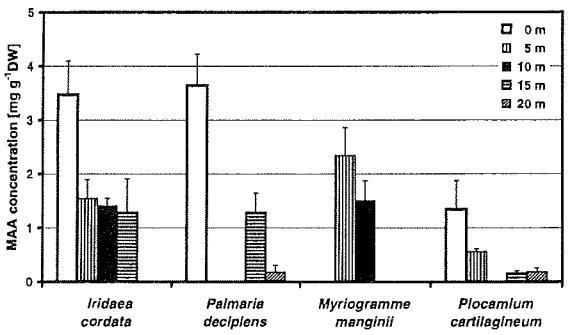


Fig. 4. Effect of increasing collection depth on total MAA content in 4 Antarctic red algae species. Means ± SD (n = 3)

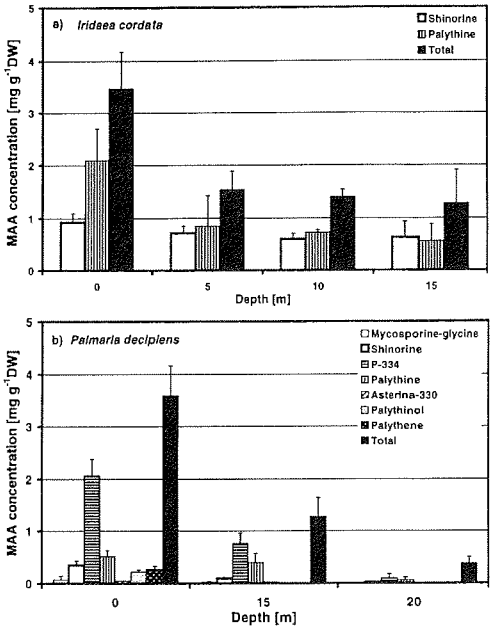


Fig. 5. (a) *Iridaea cordata*, (b) *Palmaria decipiens*. Effect of increasing collection depth on concentrations of individual MAAs. Means ± SD (n = 3)

DISCUSSION

Radiation data

The light regime on King George Island changes drastically over the course of the year due to fluctuating daylengths and atmospheric factors such as solar declination and cloud cover. Even during the relatively short study period in the southern summer of 1997/98 radiation data were highly variable. The daily average photon-fluence rate in December 1997 was quite high, at 50.1 mol m^{-2} d^{-1}, compared to other years where maxima of up to 37.9 mol m^{-2} d^{-1} were determined (Schloss et al. 1998). Consequently, the daily average of UVBR was also high, at 40.8 kJ m^{-2}, even compared with the values in January 1998 (29.9 kJ m^{-2}). However, the measurements for January 1998 are in good accordance with data given by Schloss et al. (1998). The maximum PAR value of 1700 µmol m^{-2} s^{-1} measured in the present investigation corresponds very well to values reported for King George Island by Hanelt et al. (1994) and Gómez et al. (1997).

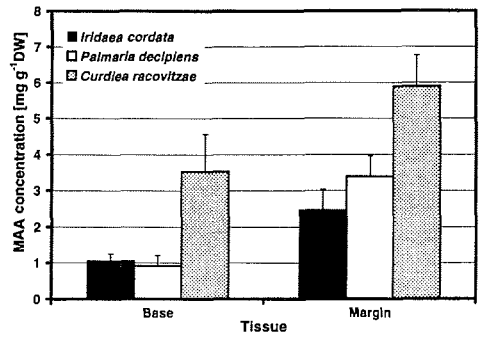


Fig. 6. Total MAA content in different tissues of thallus parts in Antarctic red algae species. Means ± SD (n = 3)

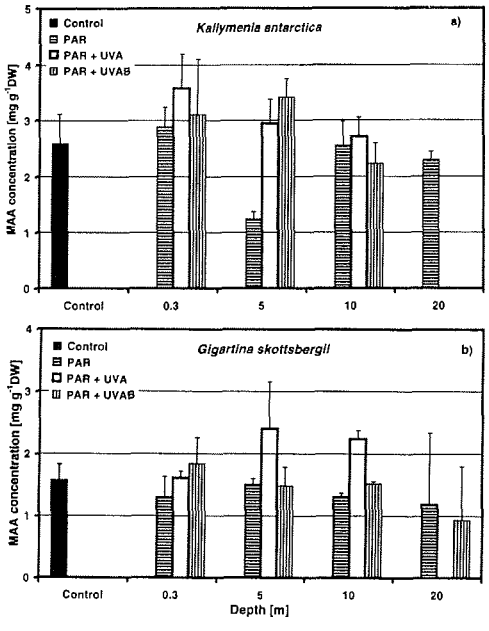


Fig. 7. (a) *Kallymenia antarctica*, (b) *Gigartina skottsbergii*. Total MAA concentration after transplantation from 20 m to shallow water followed by 1 wk exposure under various radiation conditions using specific cut-off filter foils. Means ± SD (n = 4)

However, the UVB maximum radiation in air of 1.8 W m^{-2} is considered to be high for such a high-latitude (62° S) area compared to data from (e.g.) Southern Spain (38° N) with values between 1.8 and 2.4 W m^{-2} (Gómez et al. 1998), and to data from Spitsbergen (79° N), with a value of 1.1 W m^{-2} (Bischof et al. 1998b), and may be explained by the ozone depletion over Antarctica.

Water transparency differed markedly between the stations Elephant Point and Emm Rock. The 1% depth for PAR-irradiance was restricted to 10 m at Elephant Point (end of December) because of the increasing discharge of melting water and the suspension of sediment leading to high turbidity of the water column. In contrast, Emm Rock station was only slightly affected by terrestrial influence because of its location in the outer Potter Cove. Here, the measured 1% depth of 26.9 m for visible light and 24.2 m for UV-irradiance (327 to 399 m) are comparable with the 1% PAR depth of 40 m reported by Gómez et al. (1997), all of these values are characteristic of clear oceanic waters (Smith & Baker 1981). The data presented are in good agreement with those of Wängberg et al. (1996) and Smith & Baker (1981), which indicated local very high transparency of Anctarctic waters for UVR, with possible consequential effects for sessile macroalgae, particularly in the upper and mid-sublittoral.

MAA inventory

UV-absorbing compounds have mainly been reported for macroalgal species of the Rhodophyceae (Karentz et al. 1991, McClintock & Karentz 1997, Karsten et al. 1998a,b,c). While shinorine and palythine are the predominant MAA compounds of subtidal Antarctic species, in eulittoral plants porphyra-334 is dominant (see Table 3). The highest total MAA concentrations (due to the presence of porphyra-334) were found in the eulittoral and supralittoral species *Porphyra endiviifolium* (field-collected alga) and *Bangia atropurpurea* (cultured sample). Both taxa belong to the order Bangiales, which is considered to be primitive due to its simple vegetative and reproductive organisation (Kraft & Woelkerling 1990), and hence are more ancestral relative to the other 'advanced' Rhodophyceae (Garbary & Gabrielson 1990). It may be speculated that these genera had to cope with high UVR typical for palaeozoic times and thus developed MAAs as protection mechanism. In our opinion, the capability to synthesize and accumulate such high concentrations of MAAs, as found particularly in *P. endiviifolium* and *B. atropurpurea*, is a conservative trait that allows the species to grow and successfully reproduce today in the intertidal zone of exposed rocky shores where extreme environmental conditions, including UVR stress, prevail.

Most Antarctic macroalgae are adapted to low light conditions, as reflected in the low I_c (light-compensation) and I_k values of the photosynthetic irradiance-

light curves (Wiencke et al. 1993, Weykam et al. 1996). Among the field-collected algae, the species *Hymenocladiopsis crustigena*, *Myriogramme smithii*, *Phycodrys austrogeorgica*, and *Picconiella plumosa* of the deeper sublittoral are characterized by low I_k values <30 µmol m^{-2} s^{-1} and lack MAAs (Tables 1 & 3, Fig. 3). They typically inhabit deep waters, grow as understorey species underneath the canopy, and, hence, are well protected from harmful UVBR. Consequently, there is no physiological need to synthesize and accumulate UV-absorbing substances with the concomitant outlay in energy, carbon and nitrogen. In contrast, canopy algae especially in shallow waters, exhibit I_ks of >40 µmol m^{-2} s^{-1}, and usually contain high MAA concentrations (Fig. 3).

The few previously published data on MAAs in Antarctic macroalgae (Karentz et al. 1991, McClintock & Karentz 1997) are in a similar range to those determined here. Small discrepancies can be explained by different collecting times resulting in a seasonal effect, different collecting depths, different hydrographic and atmospheric conditions, as well as by natural species variability.

Compared to the Antarctic Rhodophyceae of the present study, red algae from the Arctic exhibit lower MAA concentrations (Karsten et al. 1998b). Moreover, Antarctic species are characterized by a higher percentage of species capable of synthesizing MAAs. This obvious difference between both floras may be explained by the higher PAR and UVR at King George Island, Antarctica (1748 µmol m^{-2} s^{-1} PAR, 1.8 W m^{-2} UVBR), compared to Arctic Spitsbergen (1300 µmol m^{-2} s^{-1} PAR, 1.1 W m^{-2} UVBR [Bischof et al. 1998b]) as well as by the much clearer water conditions of the Southern Ocean and, thus, higher UV penetration. It is interesting to note that the MAA concentrations detected in Antarctic red algae are similar to those in cold-temperate to warm-temperate species (Karsten et al. 1998b), which are typically exposed to higher solar radiation than polar species. The MAA contents usually correlate with the biogeographic origin and the respective light regime, and hence the proportionally higher MAA values of Antarctic macroalgae may reflect an acclimation to the seasonally enhanced UVR due to ozone depletion. The relatively low MAA concentrations in the cultured algae compared to the field samples can be explained by the low-light conditions in the culture, which did not stimulate biosynthesis and accumulation of MAAs (Karsten et al. 1998a).

In contrast to the Rhodophyceae, extracts of the Phaeophyceae and Chlorophyceae did not show strong absorption in the UV range of the spectrum. The traces of MAAs measured in field samples of the Phaeophyceae might be due to contamination with epiphytic diatoms. These results are in good agreement with those of other studies (Karentz et al. 1991, Karsten et al. 1998 a,b). While Chlorophyceae usually contain only traces of MAAs, the green macroalga *Prasiola crispa* ssp. *antarctica* contains high concentrations of an unknown UV-absorbing compound. The occurrence of UV-absorbing substances in *P. crispa* from Antarctica has been reported earlier (Post & Larkum 1993, Jackson & Seppelt 1995). However, all authors extracted their samples in organic solvents, and recorded only absorption spectra on crude extracts obtained by spectrophotometry. Although with this methodology data of ecological importance were obtained, for example seasonally changing absorption patterns, the sunscreen substances involved could not be determined. By using HPLC technology, the present study demonstrates the occurrence of only 1 compound with strong UV-absorbance at 324 nm in *P. crispa* ssp. *antarctica* (Fig. 2).

Prasiola crispa ssp. *antarctica* is exposed to very extreme environmental conditions in melting or rainwater pools, salt-spray zones of the supralittoral, and even in penguin-rookeries (Jacob et al. 1991), and thereby must cope with large gradients of abiotic factors such as freezing, desiccation, changes in salinity, and high irradiance. This species has developed morphological, physiological and biochemical protective mechanisms such as thick cell walls as a measure against dehydration, temperature-tolerant photosynthetic activity, and the capacity for osmotic acclimation by using sucrose and sorbitol as osmolytes (Jacob et al. 1991, Jackson & Seppelt 1995). The chemically unknown UV-absorbing substance is considered as photoprotective strategy against UV stress because of its absorbance maximum at 324 nm, i.e. strong absorbance in the UVA/B range.

Depth-dependent MAA accumulation

The presence of increasing MAA contents with decreasing depth in macroalgae has already been documented for a few species (Franklin et al. 1999, Karsten & Wiencke 1999, Karsten et al. 1999). These observations are also valid for most of the red macroalgal species investigated in this study. Therefore, the data presented strongly support the hypothesis that MAAs are formed as sunscreen compounds in response to a more stressful situation in shallow waters where they are exposed to increasing UVBR and higher PAR. The 2 exceptions, *Gigartina skottsbergii* and *Kallymenia antarctica*, with unchanged MAA concentrations at different depths, can be explained by their already possessing high steady-state MAA values. *G. skottsbergii* typically occurs in eulittoral pools and in the sublittoral down to 30 m. Although shade-adapted (Wiencke et al. 1993, Weykam et al. 1996), this species can easily acclimate to high irradiances. *K. antarctica* occurs in the sublittoral in a depth range of

5 to 33 m only, usually as a canopy plant. This alga has a physiologically high and stable MAA inventory also. In both species, a high basic MAA composition seems to guarantee photoprotection under increasing radiation conditions. Based on photosynthetic studies, Bischof et al. (1998a) reported that UVR is one important factor affecting the vertical distribution of Antarctic macroalgae. The results of these authors are in good agreement with the present study, indicating a negative correlation between MAA contents and depth zonation. However, the transplantation of 2 Antarctic algae (*G. skottsbergii* and *K. antarctica*) from 20 m to shallow waters and different, enhanced light conditions did not induce further accumulation of total MAA concentration. This may be simply explained by the relatively high steady-state MAA values in both species and by the relative high water transparency during the experiment. Moreover, the study period (8 d) may have been too short to induce stronger changes in MAA contents. This contrasts to the situation in Arctic waters, where transplantation experiments showed a clear enhancement of MAA concentration in red algae after transplantion from deeper to shallower water (Karsten & Wiencke 1999, Karsten et al. 1999).

Tissue gradient

Karsten & Wiencke (1999) measured different MAA concentrations along the algal thallus of *Palmaria palmata* from Spitsbergen. Young apical tips of this alga showed 6- to 8-fold higher MAA amounts than the older basal region. In the 3 Rhodophyceae investigated here, a clear quantitative MAA difference in tissue samples taken from the base and margin of blades was also detected. Marginal tissue exhibiting higher MAA contents is more exposed to solar radiation and usually shows higher growth activities than basal tissue. The cell structure between marginal and basal tissue mostly differs as well, probably resulting in different degrees of cell-wall thickness and vacuolisation. These physiological and structural factors may explain MAA gradients between tissues. The measured high MAA concentrations in marginal or apical thallus regions are in good accordance with the presumed photoprotective function of these compounds.

MAA accumulation in different developmental stages

Spores are the most sensitive developmental stages against UV stress in the life history of macroalgae, as shown for various brown algae (mostly Laminariales) (Wiencke et al. 2000). The UVR-protective capacity of MAAs on spore-germination and gametophyte development has recently been demonstrated in the brown alga *Laminaria religiosa* incubated in the MAA palythine (Makino et al. 1999). Similar studies on red algae are still lacking. Initial data on MAA contents in different developmental stages of Antarctic Rhodophyceae, however, indicate similar MAA concentrations between their isomorphic gametophytes and tetrasporophytes. More pronounced differences can be expected in species with a heteromorphic life history.

The data presented here fully support the proposed sunscreening function of MAAs against the harmful effects of UVR (Dunlap & Shick 1998). However, the results also show that biosynthesis of MAAs in Antarctic red algae can be not only a plastic physiological process controlled by radiation conditions, as demonstrated in earlier experiments (Franklin et al. 1999, Karsten & Wiencke 1999, Karsten et al. 1999). The presence or absence of MAAs can also be determined at the genetic level. The capability for MAA biosynthesis may be present in one species and absent from another species of the same genus. Therefore we propose to classify the Rhodophyceae studied into 3 physiological groups. Members of the first group lack any trace of MAA (e.g. *Delesseria lancifolia*, *Phycodrys austrogeorgica*). These are mostly deep-water algae with low I_k values (Wiencke et al. 1993, Weykam et al. 1996). Moreover, photosynthesis of these species was shown to be particularly sensitive to UVR (Bischof et al. 1998a). Members of the second group (e.g. *Palmaria decipiens*) always contain MAAs at variable concentrations, depending on environmental conditions. However, in these plants 1 or even several MAAs may be species-specifically involved (Karsten & Wiencke 1999, Karsten et. al. 1999). Members of the third group (e.g. *Kallymenia antarctica*) always contain a high suite of MAAs almost irrespective of the environment, particularly of radiation conditions. Species of the last 2 groups are markedly less sensitive to UV exposure than plants of the first group, as indicated by a less strong inhibition of their photosynthesis and a good recovery after exposure to UVR (Bischof et al. 1998a). Phaeophyceae contain only traces of MAAs, and may use other UV-absorbing substances such as polyphenolic compounds to shield UV radiation (Pavia et al. 1997).

In conclusion, the Antarctic macroalgae studied seem to be well adapted to resist UV stress or can acclimate to it through their physiological capability to synthesize MAAs or other sunscreen substances.

Acknowledgements. This project was financially supported by the Deutsche Forschungsgemeinschaft (Ka 899/3-1/-2/-3). The authors would like to thank M. Schwanitz for diving support, C. Gross for processing and providing UVBR data, G. Koenig-Langlo for the ozone data, as well as K. Bischof for technical support and discussion.

LITERATURE CITED

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

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 PEM, Wiencke C (1998b) Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). Polar Biol 20:388–395

Cockell CS, Knowland J (1999) Ultraviolet radiation screening compounds. Biol Rev 74:311–345

Collén J, Davison IR (1999) Reactive oxygen production and damage in intertidal *Fucus* spp. (Phaeophyceae). J Phycol 35:54–61

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 of three species of *Laminaria* (Phaeophyta). Mar Biol 126:183–191

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

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

Garbary DJ, Gabrielson PW (1990) Taxonomy and evolution. In: Cole KM, Sheath RG (eds) Biology of the red algae. Cambridge University Press, Cambridge, p 477–498

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-Rodríguez 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. Photochem Photobiol 47:46–57

Hanelt D, Jaramillo JM, Nultsch W, Senger S, Westermeier R (1994) Photoinhibition as a regulative mechanism of photosynthesis in marine algae of Antarctica. Ser Cient INACH 44:67–77

Hanelt D, Wiencke C, Nultsch W (1997) Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. J Photochem Photobiol (Ser B) 38:40–47

Iken K, Quartino ML, Wiencke C (1999) Histological identification of macroalgae from stomach contents of the Antarctic fish *Notothenia coriiceps* using semi-thin sections. PSZN I: Mar Ecol 20:11–17

Jackson AE, Seppelt RD (1995) The accumulation of proline in *Prasiola crispa* during winter in Antarctica. Physiol Plant 94:25–30

Jacob A, Kirst GO, Wiencke C, Lehmann H (1991) Physiological responses of the Antarctic green alga *Prasiola crispa* ssp. *antartica* to salinity stress. J Plant Physiol 139:57–62

Jeffrey SW, MacTavish HS, Dunlap WC, Vesk M, Groenwoud 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 (1989) Report on studies related to the ecological implications of ozone depletion on the Antarctic environment. Antarct J US 24:175–176

Karentz D, McEuen FS, Land MC, Dunlap WC (1991) Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108:157–166

Karsten U, Garcia-Pichel F (1996) Carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcolens* (Cyanobacteria): a chemosystematic study. Syst Appl Microbiol 19:285–294

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, 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, Sawall T, Wiencke C (1998c) A survey of the distribution of UV-absorbing substances in tropical macroalgae. Phycol Res 46:271–279

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

Kirk JTO (1994) Optics of UV-B radiation in natural waters. E Schweizerbart'sche, Stuttgart

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, Woelkerling WJ (1990) Rhodophyta. In: Clayton MN, King RJ (eds) Biology of marine plants. Longman Melbourne, p 41–85

Larkum AWD, Wood WF (1993) The effect of UV-B radiation on photosynthesis and respiration of phytoplankton, benthic macroalgae and seagrass. Photosynth Res 36:17–23

Lubin D, Frederick JE (1989) The ultraviolet monitoring program at Palmer Station, spring 1988. Antarctic J US 24: 172–174

Madronich S, McKenzie RL, Björn LO, Caldwell MM (1998) Changes in biologically active ultraviolet radiation reaching the earth's surface. J Photochem Photobiol 46:5–19

Makino M, Suzuki M, Yabe K (1999) The influence of ultraviolet irradiation on marine organisms. 3. Effects of ultraviolet absorbing substance, palythine from *Chondrus yendoi*. Jpn J Phycol (Sôrui) 47:173–177

McClintock JB, Karentz D (1997) Mycosporine-like amino acids in 38 species of subtidal marine organisms from McMurdo Sound, Antarctica. Antarct Sci 9:392–398

Pavia H, Cevin G, Lindgren A, Åberg P (1997) Effects of UV-B radiation and stimulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. Mar Ecol Prog Ser 157:139–146

Post A, Larkum AWD (1993) UV-absorbing pigments, photosynthesis and UV exposure in Antarctica: comparison of terrestrial and marine algae. Aquat Bot 45:231–243

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

Roese M, Drabble M (1998) Wind-driven circulation in Potter Cove. Ber Polarforsch 299:40–46
Schloss I, Ferreyra G, Klöser H (1998) Seasonal variation of the conditions for phytoplankton growth in Potter Cove. Ber Polarforsch 299:59–66
Smith RC, Baker KS (1981) Optical properties of the clearest natural waters. Appl Optics 20:177–184
Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959
Starr RC, Zeikus JA (1987) UTEX. The culture collection of algae at the University of Texas at Austin. J Phycol (Suppl) 23:1–47
Strid Å, Chow SW, Anderson JM (1994) UV-B damage and protection at the molecular level in plants. Photosynth Res 39:475–489
Wängberg SA, Selmer JS, Eklund NGA, Gustavson K (1996) UV-B effects on nordic marine ecosystem. Tema Nord 1996. Nordic Council of Ministers, Copenhagen
Weykam G, Gómez I, Wiencke C, Iken K, Klöser H (1996) Photosynthetic characteristics and C:N ratios of macroalgae from King George Island (Antarctica). J Exp Mar Biol Ecol 204:1–22
Wiencke C (1988) Notes on the development of some benthic marine macroalgae of King George Island, Antarctica. Ser Cient INACH 37:23–47
Wiencke C (1990) Seasonality of red and green macroalgae from Antarctica—a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601–607
Wiencke C, Rahmel J, Karsten U, Weykam G, Kirst GO (1993) Photosynthesis of marine macroalgae from Antarctica: light and temperature requirements. Bot Acta 106:78–87
Wiencke C, Gómez I, Pakker H, Flores-Moya A, AM, Hanelt D, Bischof K, Figueroa F (2000) Impact of UV radiation on viability, photosynthetic characteristics and DNA of brown algal zoospores: implications for depth zonation. Mar Ecol Prog Ser 197:217–229
Wood WF (1987) Effect of solar ultraviolet radiation on the kelp *Ecklonia radiata*. Mar Biol 96:143–150
World Meteorological Organization (1998) Press release on 1 October 1998. World Meteorological Organization, Geneva

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: March 8, 2000; Accepted: June 22, 2000
Proofs received from author(s): January 15, 2001

Publication 2

Hoyer, K., Karsten, U., Wiencke, C.

Inventory of UV-absorbing mycosporine-like amino acids in polar macroalgae and factors controlling their content

In: Huiskes, A.H., Gieskes, W.W., Rozema, J., Schorno, R.M., van der Vies, S.M., Wolff, W.J., eds., Antarctic Biology in a Global Context, Proceedings of the International 8th SCAR Biology Symposium 2001, Amsterdam. Leiden: Backhuys (2003) (in press)

Copyright: Backhuys Publisher, reprinted with kind permission

Inventory of UV-absorbing mycosporine-like amino acids in polar macroalgae and factors controlling their content

Kirsten Hoyer[1], Ulf Karsten[2], Christian Wiencke[1]

[1]Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

[2]Institute of Aquatic Ecology, University of Rostock, Albert-Einstein-Str. 3, D-18057 Rostock, Germany

Author for correspondence:

Kirsten Hoyer

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12

D- 27570 Bremerhaven, Germany

E-mail: khoyer@awi-bremerhaven.de

ABSTRACT

In the Arctic and Antarctic, a quantitative survey of mycosporine-like amino acids (MAAs) in benthic macroalgal species based on their vertical distribution was performed. Differerences in MAA concentrations were related to the distinct habitats and to the underwater radiation climate which was measured, in addition to the atmospheric irradiance at both field stations. Red algae from the eulittoral generally contained higher MAA concentrations than those of the lower and upper sublittoral. The dependency of MAA synthesis on radiation conditions was also found in induction experiments in algae of both regions. The presence and type of trigger mechanisms for MAA biosynthesis is discussed as well as the possible photoprotective role of MAAs.

KEYWORDS

macroalgae • irradiance • mycosporine-like amino acids • UVR • vertical distribution

INTRODUCTION

The Antarctic and Arctic are polar regions exhibiting both similarities and differences. The Arctic Ocean, an almost enclosed ocean basin, is characterized by limited water exchange with the Atlantic and Pacific Oceans. There is large freshwater input from river systems, including large input of soils and clays, and it is often nutrient limited, especially with respect to inorganic nutrients (Wängberg *et al.* 1996). In contrast, the Southern Ocean is connected to the adjacent seas. The sediments are formed from a mosaic of muds, sands, and boulders of glacial origin. In addition, the Southern Ocean generally has higher nutrient levels and is colder as compared to the Nordic waters (Wängberg *et al.* 1996, references therein).

However, these two polar regions also share similarities, particularly with respect to ice cover and irradiance trends. Solar radiation reaches the extremes of 24 h daylength in summer and 24 h darkness in winter within the polar circles, resulting in a large seasonal variability. Ozone depletion in the stratosphere has also been reported in both polar regions (Holm-Hansen *et al.* 1993, Groß *et al.* 2001). Although reduction in ozone

levels in the Arctic atmosphere is less than reported for Antarctica, the depletion is still high enough to cause an increase in deleterious UVB radiation (320 – 280 nm) in this region. Both terrestrial, as well as marine environments are affected, as ultraviolet radiation (UVR, 400 – 280 nm) can penetrate the water column in clear Antarctic waters even down to 60 – 70 m (Smith *et al.* 1992).

UVB can cause many negative effects on marine organisms, such as genetic damage (Vincent & Neale 2000), inhibition of photosynthesis (Hanelt *et al.* 1997), and reduction of growth, reproduction and productivity (Aguilera *et al.* 1999, Wiencke *et al.* 2000). Therefore, it is vital, particularly for benthic organisms to develop strategies to cope with increased UVR in order to reduce UV-induced damage. This includes DNA and protein repair at the molecular level, or physiological and biochemical counteracting strategies such as the expression of detoxifiying enzymes to protect against UV-induced reactive oxygen compounds (Dunlap & Yamamoto 1995). The synthesis of UV-absorbing sunscreen compounds is also an important mechanism to reduce potential damage (Cockell 2001). In particular, mycosporine-like amino acids (MAAs), with their absorbance maxima ranging from 309 to 360 nm, are postulated to have a photoprotective role against damaging UVR in the cells (Dunlap & Shick 1998). These passive sunscreens have been detected in vertebrates, invertebrates, coral reef organisms, bacteria, and algae throughout the oceans of the world (Cockell & Knowland 1999, references therein). To date, 19 distinct molecular structures of MAAs have been identified. They are made up of free amino acids either, with a cyclohexenone or cyclohexenimine chromophore, conjugated with a nitrogen substitution of an amino acid (Dunlap & Shick 1998). It has been suggested that MAAs are synthesized via the shikimate pathway, which is found only in microorganisms and plants, but not in animals (Shick *et al.* 1999). Animals, therefore, must aquire MAAs through diet or symbiotic associations with microorganisms or microalgae (Carroll & Shick 1996). MAA-containing macroalgae may be an important food resource for herbivores and detrivores not only as a nutritional source but also for protection against UVB damage (Adams & Shick 2001). In all studies conducted in different geographical regions it has been demonstrated, that MAAs are found mainly in red algae. Only few green algae contain MAAs, whereas in most brown algae, these compounds are completely absent (McClintock & Karentz 1997, Karsten *et al.* 1998, Hoyer *et al.* 2001).

The aim of our study was to detect possible differences in the MAA content of macroalgal species from the Antarctic and the Arctic, and relate these to the habitat-

specific UVR conditions. Finally, irradiance as a possible primary abiotic factor inducing MAA formation was studied under controlled conditions, in an attempt to elucidate the trigger for MAA biosynthesis in species from both polar regions.

MATERIALS AND METHODS

Study sites and algal material

The samples of macroalgae were collected from different habitats by SCUBA diving from the sublittoral and the eulittoral in Antarctica at Potter Cove, King George Island, South Shetlands (Jubany Station, Dallmann Laboratory, 62°14´S, 58°40´W) during the austral summer of 1997/98 and in the Arctic at Kongsfjord, Ny-Ålesund, Spitsbergen (78°55´N, 11°55´E) during several summers (details of sample locations and collecting dates are listed in Table 1).

Red algae were isolated for culture purposes from Potter Cove 1994 and established as permanent growth culture in the laboratory. The light regime was varied between 5 h (winter) and 20 h (summer), simulating fluctuating Antarctic daylengths (Wiencke 1990).

Table 1: Investigated field macroalgae with details of sampling location and date.

Species	Sample location (depth)	Sampling date
RHODOPHYCEAE	**Potter Cove, King George Island, Antarctica**	
Curdiea racovitzae Hariot (Gracilariales)	eulittoral, inner fjord	28.01.1998
Georgiella confluens (Reinsch) Kylin (Ceramiales)	eulittoral, inner fjord; sublittoral, outer fjord (-7 m, -15 m)	01.01.1998, 29.12.1997, 05.01.1998
Gigartina skottsbergii Setchell & Gardner (Gigartinales)	outer fjord, (-9 m)	20.01.1998, 24.12.1997
Gymnogongrus antarcticus Skottsberg (Gigartinales)	inner fjord (-1-0 m)	01.12.1997
Hymenocladiopsis crustigena Moe (Rhodymeniales)	outer fjord (-15 m)	05.01.1998
Iridaea cordata (Turner) Bory de Saint Vincent (Gigartinales)	eulittoral, inner fjord, outer fjord, (-5 m, -10 m)	15.12.1997, 17.12.1998
Kallymenia antarctica Hariot (Cryptonemiales)	outer fjord (-20 m)	24.12.1997

Table 1: continued

Species	Sample location (depth)	Sampling date
Myriogramme smithii (Hooker fil. et Harvey) Kylin (Ceramiales)	outer fjord (-15 m)	05.01.1998
Neuroglossum ligulatum (Reinsch) Skottsberg (Ceramiales)	outer fjord (-15 m)	10.12.1997
Notophycus fimbriatus Moe (Gigartinales)	inner fjord (-1-0m)	26.11.1997
Pachymenia orbicularis (Zanardini) Setchell & Gardner (Cryptonemiales)	inner fjord (-15 m)	15.01.1998
Palmaria decipiens (Reinsch) Ricker (Palmariales)	eulittoral, inner fjord, outer fjord (-15 m)	05.01.1998
Phycodrys austrogeorgica Skottsberg (Ceramiales)	outer fjord (-20 m)	13.01.1998
Picconiella plumosa (Kylin) de Toni (Ceramiales)	outer fjord (-20 m)	13.01.1998
Plocamium cartilagineum (Linnaeus) Dixon (Ploeamiales)	eulittoral, inner fjord, outer fjord (-6, -15 m)	01.01.1998, 17.12.1997, 05.01.1998
Porphyra endiviifolium Chamberlain (Bangiales)	eulittoral, inner fjord	26.11.1997
Sarcothalia papillosa (Bory) Leister (Gigartinales)	inner fjord (-1-0m), outer fjord (-15 m)	16.12.1997, 09.12.1997
CHLOROPHYCEAE		
Prasiola crispa ssp. ***antarctica*** (Kützing) Knebel (Prasiolales)	supralittoral, in penguin rockeries	10.01.1998
RHODOPHYCEAE	**Kongsfjord, Spitsbergen, Arctica**	
Coccotylus truncatus (Pallas) M.J. Wynne & J.N. Heine (Gigartinales)	inner fjord (-11 m)	27.06.2001
Devaleraea ramentacea (Linnaeus) Giury (Palmariales)	inner fjord (-2 m, -8 m)	10.6.1998, 03.07.2000
Palmaria palmata (Linnaeus) Kuntze (Palmariales)	inner fjord (-2 m, -10 m)	10.6.1998, 03.07.2000
Odonthalia dentata (Linnaeus) Lyngbye (Ceramiales)	inner fjord (-9 m)	22.08.1995
Phycodrys rubens (Linnaeus) Batters	inner fjord (-15 m)	25.05.1996
Polysiphonia arctica J. Agardh (Ceramiales)	inner fjord (-1 m, -12 m)	22.08.1995, 21.06.2000
Porphyra spec. C. Agardh (Bangiales)	inner fjord (-10 m)	04.06.2001
Ptilota gunneri P.C. Silva, C.A. Maggs & L.M. Irvine (Ceramiales)	inner fjord (-13 m)	24.05.1996
Ptilota serrata Kützing (Ceramiales)	inner fjord (-10 m)	18.08.1995
Rhodomela confervoides (Hudson) P.C. Silva (Ceramiales)	inner fjord (-12 m)	08.06.2000
CHLOROPHYCEAE		
Prasiola crispa (Lightfoot) Kützing (Prasiolales)	supralittoral, underneath a coastal bird-cliff (seagull)	13.06.2000

Radiation measurements

Underwater spectra of ambient radiation of wavelengths from 327 to 700 nm were recorded at various depths with a spectroradiometer (Ingenieurbüro M. Kruse, Stubben, Germany). Water transmittance was determined by measuring irradiance at different depths and then by calculating diffuse vertical attenuation coefficients (Kd) of downward irradiance (Kirk 1994).

UVB radiation (UVB, 280-320 nm) in air was measured using a 32-channel single photon counting spectroradiometer equipped with a cosine diffuser, developed at the Physics Department of the Alfred Wegener Institute. The instrument was installed on the roof of the Dallmann Laboratory (Antarctic) as well as on the roof of the NDSC building in Ny-Ålesund (Arctic). The spectroradiometer was computer controlled allowing on-line recordings of the radiation data. Photosynthetically active radiation (PAR, 400-700 nm) in the atmosphere was measured with a Li-Cor datalogger (Li-1000, LI-Cor. Lincoln, USA) equipped with a flat head sensor (LI-190).

Induction experiments

For the induction experiments, low-light (PAR, 15 μmol photons $m^{-2}s^{-1}$) adapted Antarctic cultured (*Gymnogongrus turquetii, Kallymenia antarctica, Neuroglossum ligulatum, Palmaria decipiens*) and Arctic field algae (*Devaleraea ramentacea, Palmaria palmata, Polysiphonia arctica, Rhodomela confervoides*) were transferred to two different radiation conditions; PAR (400 to 700 nm, 25 μmol photons $m^{-2}s^{-1}$) and PAR + UVR (295 to 400 nm, 4.6 W m^{-2}). Daylight fluorescent lamps (Lumilux Deluxe, Osram, Germany), in combination with Q-Panel UVA-340 fluorescent tubes, (Cleveland, USA) emitting a spectrum similar to solar radiation in the UVR range, were used. Spectra emitted by these artificial radiation sources were measured with a Spectro 320 D spectroradiometer (Instrument Systems, Germany).

During the experiment, algae were kept in glass beakers filled with filtered nutrient-enriched sea water with an additional 2.1 mM sodium hydrogen carbonate, as an additional inorganic carbon source. The experiment with the Antarctic algae was performed in a constant temperature room at 0°C under PAR +UVR/dark cycles of 16:8 h. The Arctic algae were investigated at 5 °C under continuous white light with an addition exposure of 11 hours UVR. Glass vessels were covered with specific filters to cut-off UVB + UVA radiation (400 nm cut-off: Folex PR, Folex, Dreieich, Germany),

or with a filter with no transmission under 295 nm (Ultraphan UBT, Digefra, München, Germany).

After 12 days of exposure to the artificial radiation the Antarctic plants were harvested. The Arctic algae were incubated for 6 days, *P. arctica* and *R. confervoides* additionally for 9 and 11 days, respectively. The harvested samples were oven-dried at 50 °C overnight, and then stored in sealed plastic bags under dry and dark conditions prior to MAA analysis.

MAA extraction and analysis

A 25 % aqueous methanol (v/v) extraction was made from 10 – 20 mg dry weight (DW) of the algal samples. After evaporating to dryness under vacuum (Speed Vac Concentrator SVC 100H) dried extracts were re-dissolved in 100% methanol. Samples were analysed with a Waters high-performance liquid chromatography (HPLC) system according to Hoyer *et al.* (2001). All total MAA concentrations are given as means of 3 replicates (±SD) randomly collected from the respective habitat. Means of the replicates from all experiments were taken and expressed as concentration per dry weight (mg g^{-1} DW). Differences in the MAA content under the distinct filter treatments in the induction experiments were statistically verified by using a one – way ANOVA test applying a multiple comparison post test (Tukey – Kramer HDS test) where significant differences occurred (probability at $p < 0.05$).

RESULTS

Radiation data

The ozone values measured over Antarctica (Neumayer Station) during the expedition period (austral summer 1997/98) ranged from 316 to 227 DU (Dobson Unit) with a mean of about 260 DU (Table 1) indicating an ozone depletion of almost 20 % compared to a typical ozone measurement of about 320 DU before 1980. Over the Arctic the ozone concentration is generally higher. Before 1980, an average value of about 450 DU was recorded, but it varied from 421 to 302 DU over Spitsbergen during the expedition time June and July 2000, representing a 23 %-loss of ozone (Table 2).

As a result of the ozone depletion in the stratosphere UVB increases. In December 1997, the average daily dose of UVB in air was higher (40.8 kJ m^{-2}) than in January 1998 (29.9 kJ m^{-2}) as measured on King George Island (Antarctic). Instantaneous UVB and PAR values reached 1.8 W m^{-2} and 1748 μmol photons m^{-2} s^{-1}. On Spitsbergen (Arctic), the average daily doses of UVB were lower in the months June and July 2000 compared to the same season in the Antarctica, 36.6 and 22.3 kJ m^{-2}, respectively, as well as instantaneous UVB and PAR values of 1.23 W m^{-2} and 1440 μmol photons m^{-2} s^{-1} (Table 2). This is also due to its location at a higher latitude than King George Island.

In Potter Cove (Antarctica), the light transmittance through the water was much lower in the inner fjord than in its outer part. A similar situation was found in Kongsfjord (Arctic), in both cases due to the input of turbid melt waters. The K_d values and the corresponding calculated depth for 1 % of remaining radiation for PAR and UVR (327 to 399 nm) are summarized in Table 2.

Table 2: Radiation measurements and ozone data for the expedition periods to Antarctica (1997/98) and Arctic (2000). Ozone data taken from Meterology Observatory of Neumayer Station, Antarctica (70°37´S, 8°22´W) and from TOMS satellite (Total Ozone Mapping Spectrometer), Arctic (78°55N, 11°56E). DU: Dobson Unit

	Antarctica		**Arctica**	
	Date	Units	Date	Units
Ozone				
Max. ozone	8 December 1997	316 DU	5 June 2000	421 DU
Min. ozone	16 November 1997	227 DU	20 July	302 DU
Ozone mean value	November 1997 – January 1998	260 DU	June – July 2000	346 DU
Atmosphere				
UVB (280 – 320 nm)				
Daily dose	average for December 1997	40.8 kJ m^{-2}	average for June 2000	36.6 kJ m^{-2}
Daily dose	average for January 1998	29.9 kJ m^{-2}	average for July 2000	22.3 kJ m^{-2}
example of irradiance at midday (UVB)	23 December 1997	1.80 W m^{-2}	13 June 2000	1.23 W m^{-2}
example of irradiance at midday (PAR)	20 January 1998	1748 μmol photons m^{-2} s^{-1}	21 June 2000	1440 μmol photons m^{-2} s^{-1}

Table 2: continued

	Antarctica		Arctica	
Attenuation coefficient				
$K_{d\ PAR}$	30 December 1998, outer fjord	0.17 m^{-1}; 1% depth: 26.9 m	22 June 2000, outer fjord	0.21 m^{-1}; 1% depth: 21.9 m
$K_{d\ UV\ (327\text{-}399\ nm)}$		0.19 m^{-1}; 1% depth: 24.2 m		0.39 m^{-1}; 1% depth: 11.8 m
$K_{d\ PAR}$	30 December 1998, inner fjord	0.45 m^{-1}; 1% depth: 10.2 m	21 June 2000, inner fjord	0.72 m^{-1}; 1% depth: 6.4 m
$K_{d\ UV\ (327\text{-}399\ nm)}$		1.1 m^{-1}; 1% depth: 4.2 m		0.94 m^{-1}; 1% depth: 4.9 m

MAA inventory

In the species examined, nine different MAAs were detected. Seven were identified as mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol and palythene, and were quantified by using the respective molar extinction coefficient (Karsten *et al.* 1998). Furthermore, two unknown UV-absorbing compounds with absorbance maxima of 324 and 357 nm, respectively, were also detected. The unknown substance-324 has previously been described in the Antarctic isolate of *P. crispa* (Hoyer *et al.* 2001), and has been detected in this study also as the main compound in *P. crispa* from the Arctic. MAA-357 is probably usujirene, the isomeric cis-form of palythene (Tsujino *et al.* 1979). Based on MAA composition the most abundant MAAs were palythine, porphyra-334 and shinorine, followed by asterina-330 in the Antarctic and Arctic field grown algal species presented here. In the case of *Rhodomela confervoides*, there was an almost equimolar concentration of palythinol and porphyra-334 of about 1 mg g^{-1} DW. However, in *Porphyra endiviifolium*, porphyra-334 was quantitatively the most abundant MAA (7.7 mg g^{-1} DW). In Fig. 1 all MAAs are summarized as total MAA concentration.

In Potter Cove (Antarctica), 19 algal species were examined of which one green alga was collected from the supralittoral, 6 red algae from the eulittoral, 7 from the upper, and 14 from the lower sublittoral. Out of them 6 species were found in more than one habitat. In Figure 1A, those algae containing MAAs are summarized, and collecting depths noted. A species not listed, *Georgiella confluens*, was found from the eulittoral

downwards. Only small traces of two different MAAs were detected in individuals of this species collected from the eulittoral, even less were found in those of the upper sublittoral, and nothing in those of the lower sublittoral. No MAAs were detected in four other species (*Hymenocladiopsis crustigena, Myriogramme smithii, Phycodrys austrogeorgica* and *Picconiella plumosa*) collected from the lower sublittoral (15, 20 m).

Ten macroalgal species from the Kongsfjord (Arctic) were analyzed. Red algae were not occurring in the eulittoral, but one green alga (*Prasiola crispa*) was collected from the supralittoral. Four red algal species derived from the upper, 8 from the lower sublittoral, and of those, 3 species were collected from both zones. These 3 species exhibited MAAs with distinct concentrations relating to growth depth (Figure 1B). *Coccotylus truncatus, Odonthalia dentata* and *Ptilota serrata* are deep-water plants, originating from 9 to 15 m depth and growing indeed deeper. These species contained no MAAs, even after exposure to enhanced UVR.

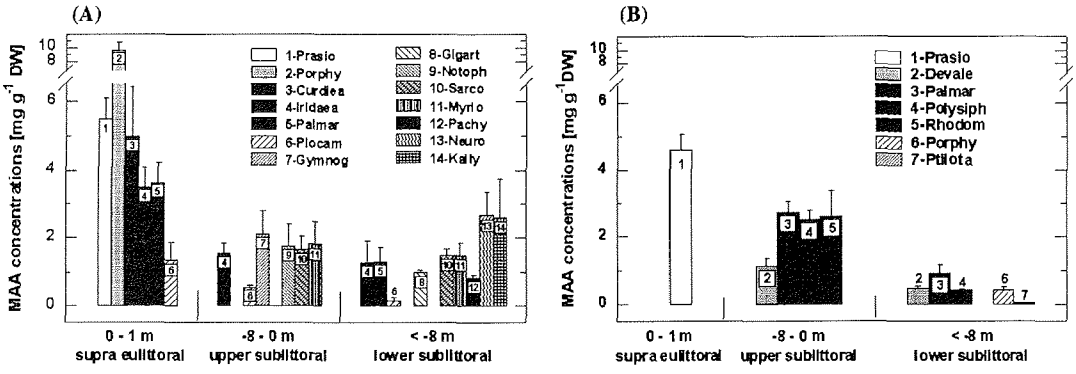


Figure 1: Total concentration of MAAs in macroalgae based on their vertical distribution (**A**) from Antarctica, (**B**) from the Arctic. Means ± SD (n = 3). Abbrevation, (**A**): Prasio – *Prasiola crispa* spp. *antarctica*, Porphy – *Porphyra endiviifolium*, Curdiea – *Curdiea racovitzae*, Iridaea – *Iridaea cordata*, Palmar – *Palmaria decipiens*, Plocam – *Plocamium cartilagineum*, Gymnog – *Gymnogongrus antarcticus*, Gigart – *Gigartina skottsbergii*, Notoph – *Notophycus fimbriatus*, Sarco – *Sarcothallia papillosa*, Myrio – *Myriogramme mangini*, Pachy – *Pachymenia orbicularis*, Neuro – *Neuroglossum ligulatum*, Kally – *Kallymenia antarctica*. (**B**): Prasio – *Prasiola crispa*, Devale – *Devaleraea ramentacea*, Palmar – *Palmaria palmata*, Polysiph – *Polysiphonia arctica*, Rhodom – *Rhodomela confervoides*, Porphy – *Porphyra* spec., Ptilota – *Ptilota gunneri*

In general, the supralittoral and the eulittoral species contained the highest concentrations of MAAs. Eulittoral species were collected from a broad intertidal platform influenced by a semidiurnal tide with an average amplitude of 135 cm in Potter Cove (Antarctica) (Schöne *et al.* 1998). These algae contained total MAA amounts of 9.7 mg g^{-1} DW (*Porphyra endiviifolium*), 4.9 mg g^{-1} DW (*Curdiea racovitzae*), and 3 mg g^{-1} DW (*Iridaea cordata*). As a comparable habitat is absent at Kongsfjord, most species were collected in the sublittoral and exhibited lower MAA concentrations, with increasing growth depth. In the lower sublittoral, all concentrations were found to be under 1 mg g^{-1} DW. In comparison, at Potter Cove, the lower sublittoral species contained higher MAA concentrations up to 2.6 mg g^{-1} DW and on an average about 1.5 mg g^{-1} DW.

Induction experiments

The induction of the MAA synthesis / accumulation after exposure to enhanced UVR was investigated in four red algal species from each polar region. An additional increase of total MAA concentration after incubation under PAR and PAR + UVR was observed in three Antarctic algae (*Kallymenia antarctica*, *Gymnogongrus turquetii*, *Palmaria decipiens*) and in *Devaleraea ramentacea* and *Palmaria palmata* from the Arctic (Fig. 2A, B). The increase in MAA concentration from the initial samples to those under the UVR treatment in *D. ramentacea*, *K. antarctica* and *P. decipiens* was statistically significant ($P < 0.03$, $P < 0.0002$, $P < 0.003$), as was from the initial sample to that under PAR-only in the latter species (Fig. 2A, B). In Antarctic *Neuroglossum ligulatum*, an increase in MAA concentration was observed between the initial sample and the PAR treated sample whilst the plants under the PAR + UVR condition exhibited almost the same values as the control. After 6 days of incubation, in *Polysiphonia arctica* and *Rhodomela confervoides*, no differences were visible between the initial and the treated samples (data not shown). After 9 days, a statistically higher concentration of MAAs was found under PAR-only and UVR conditions in *P. arctica* compared to the initial samples whereas in *R. confervoides* a decrease in MAA concentration was observed under both treatments after 11 days of incubation (Fig. 2B).

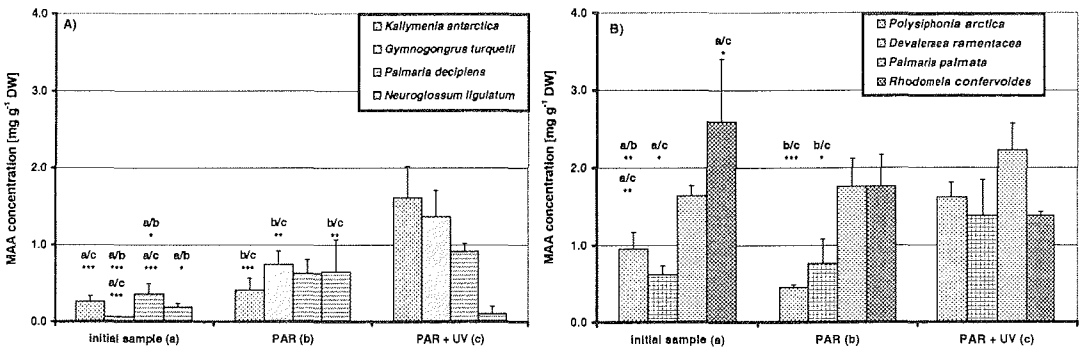


Figure 2: Total concentration of MAAs in (**A**) cultured Antarctic red algae (n = 4-5) after 12 days of exposure and (**B**) field-collected Arctic red algae after 6 (*D. ramentacea*, n = 4-5; *P. palmata*, n = 3-4), 9 (*P. arctica*, n = 4-5), and 11 (*R. confervoides*, n = 4, using the Kruskal-Wallis test) days of exposure under two different radiation conditions using specific cut-off filters. An asterisk indicates a significant difference in the concentrations of MAAs between treatments (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Means ± SD

In *K. antarctica*, shinorine and palythine were the quantitatively dominant MAAs, in *G. turquetii*, shinorine and porphyra-334 were also present in almost equimolar concentrations under the experimental radiation treatments, except in the initial sample, where porphyra-334 was absent. In *P. decipiens* porphyra-334 ocurred in highest concentrations, whereas in *N. ligulatum*, shinorine was the most abundant sunscreen compound in combination with traces of porphyra-334.

In *R. confervoides*, the initial samples contained porphyra-334 and palythinol in almost equimolar concentrations. After 11 days the concentrations increased proportionally. The same concentrations of porphyra-334 and palythine were found in the initial sample of *P. arctica* (ca. 0.3 mg g^{-1} DW). After 9 days of incubation, the concentration of porphyra-334 (0.575 mg g^{-1} DW) increased and was significantly ($P<0.02$) higher than of palythine (0.253 mg g^{-1} DW) under the treatment with UVR. In *P. palmata*, porpyhra-334 was the quantitatively most abundant MAA, whereas shinorine and palythine were detected in low concentrations (both were found to be about 10 % of the porphyra-334 concentration). Initially in *D. ramentacea*, the MAAs porphyra-334 and palythine did not show significant differences in their concentrations, however after the treatment with UVR, the content of porphyra-334 were significantly ($P<0.01$) higher with 1.1 mg g^{-1} DW than that of palythine (0.2 mg g^{-1} DW).

DISCUSSION

The effects of radiation on organisms depend on the intensity, spectral composition and the
duration of the incident radiation. The irradiance regime in the polar regions is one of the parameters that change markedly over the course of the year, as a result of fluctuating daylengths and atmospheric factors such as solar declination, cloud cover, aerosols and ozone concentrations (Lubin *et al.* 1998). Ozone depletion affects the irradiance by a very specific increase in the UVB radiation between the wavelengths 290 and 315 nm (Wängberg *et al.* 1996).

The trend of lower ozone values over the Antarctic compared with the Arctic (WMO 1998) has also been found in this study, correlating well with measurements of higher daily averages of atmospheric UVB in Potter Cove (Antarctica) than in the Kongsfjord (Arctic). However, it has to be considered that the former study site is located at lower latitudes with generally higher UVB values. Nevertheless, an increase in surface UVB also results in an augmentation of irradiance penetrating the water column.

The vertical attenuation coefficents (K_d values) measured in different parts of the Kongsfjord (Arctic) are in the same range as those of previous studies. $K_{d(UVB)}$ values between 0.3 and 1.34 m^{-1} depend on a stratified water column of different layers of turbidity, tidal level, and sun angle (Bischof *et al.* 1998, Hanelt *et al.* 2001). In Wängberg *et al.* (1996), the cited $K_{d(310)}$ values of 0.2 – 0.25 m^{-1} for Spitsbergen were lower than we have found, but can be explained by different measuring locations, i.e. inner versus outer fjord. The K_d values measured in Potter Cove (Antarctic) were slightly lower than those of the Kongsfjord indicating a deeper irradiance transmittance through the water column. In Antarctica, the 1 % depth of 26.9 m for remaining visible light, or even 40 m (Gómez *et al.* 1997), together with the 1 % depth of remaining radiation for UVR of 24 m indicate locally very high transparency and signify characteristic clear ocean waters (Smith and Baker 1981). Consequently, macroalgae might be affected by harmful UVB, particularly in the eulittoral and upper sublittoral, and at some Antarctic locations even much deeper (30 m depth, Karentz *et al.* 1989). The usually higher UVR conditions in Antarctica well explain the high MAA-concentrations of exposed supra- and eulittoral species as photoprotective defense against UV-induced damage. The relatively high production of MAAs in *Kallymenia antarctica* and *Neuroglossum ligulatum*, two Antarctic species collected in the outer

part of Potter Cove, growing at 20 and 15 m depth, respectively, can also be explained by high water transparency for UVR.

Algal species of the upper sublittoral of both polar regions exhibited similar MAA concentrations probably due to similiar radiation conditions at those depths. However, in the Antarctic, the macroalgae of the lower sublittoral usually exhibited only slightly lower MAA contents compared with the algae of the upper sublittoral. This was unexpected as in previous studies an obvious depth gradient in MAA concentrations was documented (Karsten *et al.* 1998, Franklin *et al.* 1999) and as confirmed in this study for the Arctic macroalgae. The relatively high MAA values even in Antarctic lower sublittoral species seems to be related with the higher PAR and UVR penetration depths, as biosynthesis of MAAs is a variable physiological process, controlled by radiation transmittance. In addition, other ecological parameters such as growth in shaded environments, e.g. in the understory, may influence the MAA content. Furthermore, MAA production is a very species-specific process, resulting in great differences in concentrations between species of the same habitat. Additionally, MAA synthesis may be determined on a genetic level, as suggested by those algae that do not have the capability to produce MAAs even after exposure to enhanced UVR as found for 4 Antarctic and 3 Arctic species.

In the Antarctic and Arctic, there are few bipolar species. Some species have a cosmopolitan distribution and some have disjunct bipolar distributions like *Acrosiphonia arcta* (Chlorophyta) (Bischoff & Wiencke 1995). The green alga *P. crispa* is a cosmopolitan species, but for the Antarctic it is described as a subspecies (*P. crispa* spp. *antarctica*). Nevertheless, the Arctic and Antarctic species have many similiarities. The *Prasiola* -samples investigated in this study were growing as nitrophilic species in comparable habitats: in penguin rockeries (*Phygoscelis adeliae*) near Potter Cove (Antarctica) and underneath a coastal bird-cliff (Kongsfjord, Arctic) colonized by breeding seagull *Rissa tridactyla*. Gross morphology of both isolates appears similiar, producing the same UV-absorbing compound (substance-324), which is unknown for red algae. The higher concentrations of MAAs in the Antarctic species was probably due to the higher atmospheric UVB experienced by the alga.

MAA concentrations are generally higher in macroalgae from Potter Cove (Antarctica) than in those from the Kongsfjord (Arctic). Further studies will be necessary to prove this as a general prediction as it might be simply an adaptation to the higher UVB found

in Antarctica. The collecting locations which are all in the inner fjord for the Arctic samples, exhibiting lower Kd values than the outer fjord may also play a role. Furthermore, there is no nutrient limitation at coastal Antarctic waters over the course of the year (Drew and Hastings 1992) which may effect the essentially important nitrogen uptake for the MAA molecules. In contrast, in Kongsfjord (Arctic) a strong depletion of phosphate and nitrate concentrations was found in the open-water period (Aguilera et al. 2002) which might be responsible for reducing the MAA synthesis / accumulation.

For the induction experiment, species of the upper and lower sublittoral were chosen, due to the flexible way they are able to react to environmental radiation changes in respect to MAA synthesis. The Antarctic species were taken from culture and were therefore low-light adapted, resulting in a significant lower initial MAA value compared to the Arctic algae collected from the field. Therefore, any comparison between these two set ups must be considered carefully. However, the experiments generally showed that MAA accumulation can be species – specifically induced by exposure to different light conditions with increasing MAA concentrations observed under high PAR-only treatment or under PAR + UVR as well as an additional increase under both conditions. Differences in MAA concentrations arising from exposure time are variable in the Arctic species. *P. arctica* and *R. confervoides* do not show any changes in MAA concentrations between the initial and harvested samples after 6 days, whereas distinct differences were seen in *P. palmata* and *D. ramentacea*. Furthermore, a MAA increase was found in *P. arctica* after 9 days, indicating species-specific differences in the enzymatic kinetics for the MAA biosynthesis / accumulation. In contrast, 11 days of exposure to UVR might be too high for *R. confervoides*, resulting in a MAA degradation or leakage into the medium, which is also observed in the Antarctic species *N. ligulatum* (Fig. 2A, B). No further induction of MAA formation or accumulation seems possible if species are "loaded-up" with MAAs (data not shown), indicating that each plant may have an maximum threshold of MAAs.

Generally, an induction of MAA synthesis by exposure to light and UVR was observed in species of both polar regions and indicates that more than one mechanism or photoreceptor might be involved in the MAA induction process. This is supported by the results of Franklin *et al.* (1999) who also postulated a signal transduction pathway or even interactions among various photoreceptors involved in the overall process leading to high MAA concentrations. At least, two different photoreceptors should be taken into

consideration referring to two types of induction one by MAA increment of PAR intensity and another by UVR.

The detection of high MAA concentrations in polar macroalgae is ecologically important for a better understanding of how benthic plants are able to cope with the increasing UVB in extreme environments. Furthermore, the investigations were based on algal vertical distribution in the shallow-water ecosystem. The physiological ability to adjust their MAA biosynthesis, depending on the environmental radiation was demonstrated.

Acknowledgements

This project was financially supported by the Deutsche Forschungsgemeinschaft (Ka 899/3-1/-2/-3). The authors like to thank M. Schoenwaelder for discussion and reading the manuscript, C. Gross and G. Koenig-Langlo for processing and providing UVB data and ozone data from the Neumayer Station, and the diving crews.

REFERENCES

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

Aguilera, J., Bischof, K., Karsten, U., Hanelt, D. & Wiencke, C. 2002. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defense systems against high light stress. Mar. Biol, in press

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

Bischof K., Hanelt D., Tüg H., Karsten U., Brouwer P.E.M. & Wiencke C. 1998. Acclimation of brown algal photosynthesis to ultraviolet radiation in Arctic coastal waters (Spitsbergen, Norway). Polar Biol. 20: 388-395

Bischoff, B. & Wiencke, C. 1995. Temperature ecotypes and biogeography of Acrosiphonales (Chlorophyta) with Arctic-Antarctic disjunct and Arctic/cold-temperate distributions. Eur. J. Phycol. 30: 19-27

Carroll, A.K. & Shick, J.M. 1996. Dietary accumulation of UV-absorbing mycosporine-like amino acids (MAAs) by the green sea urchin (*Strongylocentrotus droebachiensis*). Mar. Biol. 124: 561-569

Cockell, C.S. 2001. A photobiological history of Earth. In: Cockell, C.S. & Blaustein, A.R. (eds), Ecosystems, Evolution, and Ultraviolet Radiation, pp 1-35. Springer-Verlag, New York.

Cockell C.S. & Knowland J. 1999. Ultraviolet radiation screening compounds. Biol. Rev. 74: 311-345

Drew, E.A. & Hastings, R.M. 1992: A year-round ecophysiological study of *Himantothallus grandifolius* (Desmarestiales, Phaeophyta) at Signy Island, Antarctica. Phycologia 31: 262-277

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

Dunlap, W.C. & Yamamoto, Y. 1995. Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. Comp. Biochem. Physiol. 112B(1): 105-114

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

Gómez, I.,Weykam, G., Klöser, H. & Wiencke, C. 1997. Photosynthetic light requirements, metabolic carbon balance and zonation of sublittoral macroalgae from King George Island (Antarctia). Mar. Ecol. Prog. Ser. 148: 281-293

Groß, C., Tüg, H. & Schrems, O. 2001. Three years spectral resolved UV-measurements at Koldewey-Station 1997-1999. Mem. Nat. Inst. Polar Res. 54: 113-123

Hanelt, D., Tüg, H., Bischof, K., Groß, C., Lippert, H., Sawall, T. & Wiencke, C. 2001. Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. Mar. Biol. 138: 649-658

Hanelt, D., Wiencke, C. & Nultsch, W. 1997. Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. J.Photochem.Photobiol.B: Biol. 38: 40-47

Holm-Hansen, O., Lubin D. & Helbling E.W. 1993. Ultraviolet radiation and its effects on organisms in aquatic environments: In: Young, A.R., Björn, L.O.; Moan, J. & Nultsch W. (eds.), Environmental UV Photobiology, pp 379-425. Plenum press, New York.

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: 117-129

Karentz, D. 1989. Report on studies related to the ecological implications of ozone depletion on the Antarctic environment. Antarct. J. US. ,175-176

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

Kirk, J.T.O. 1994. Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge

Lubin, D., Jensen, E.H. & Gies, H.P. 1998. Global surface ultraviolet radiation climatology from TOMS and ERBE data. J. Geo. Res. 103(D20): 26061-26091

McClintock, J.B. & Karentz, D. 1997. Mycosporine-like amino acids in 38 species of subtidal marine organisms from McMurdo Sound, Antarctica. Ant. Sci. 9(4): 392-398

Schöne, T., Pohl, M., Zakrajsek, A.F., & Schenke, H.W. 1998. Tide Gauge Measurements – A contribution for the long term monitoring of the sea level. Ber. Polarforsch. 299: 12-14

Shick, J. M., 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 population of symbiotic dinoflagellates. Limnol. Oceanogr. 44(7): 1667-1682

Smith, R. C., Prézelin, B. B., Baker, K. S., Bidigare, R. R., Boucher, N. P., Coley, T., Karentz, D. ,

MacIntyre, S., Matlick, H. A. & Menzies, D. 1992. Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255: 952-959

Smith, R.C. & Baker, K.S. 1981. Optical properties of the clearest natural waters (200-800 nm). Applied Optics 20(2): 177-184

Tsujino, I., Yabe, K. & Sakurai, M. 1979. Presence of the near 358 nm UV-absorbing substances in red algae. Bull. Fac. Fish, Hokkaido University 30: 100-108

Vincent, W.F. & Neale, P.J. 2000. Mechanisms of UV damage to aquatic organisms. In: Cockell, C.S. & Blaustein, A.R. (eds), Ecosystems, Evolution, and Ultraviolet Radiation, pp 146-177. Springer-Verlag, New York.

Wängberg, S.A., Selmer, J.S., Eklund, N.G.A. & Gustavson, K. 1996. UV-B effects on Nordic Marine Ecosystem- A literature review. Tema Nord 1996. Nordic council of Ministers, Copenhagen.

Wiencke, C. 1990. Seasonality of red and green macroalgae from Antarctica- a long-term culture study under fluctuating Antarctic daylengths. Polar Biol. 10: 601-607

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

World Meteorological Organization. 1998. Scientifc Assessment of Ozone Depletion: 1998. World Meteorological Organization Global Ozone Research and Monitoring. Project 44

Publication 3

Hoyer, K., Karsten, U., Wiencke, C.

Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions

Marine Biology (2002) 141: 619-627

Copyright: Springer Verlag, reprinted with kind permission

Marine Biology (2002) 141: 619–627
DOI 10.1007/s00227-002-0871-0

K. Hoyer · U. Karsten · C. Wiencke

Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions

Received: 30 January 2002 / Accepted: 30 April 2002 / Published online: 4 July 2002
© Springer-Verlag 2002

Abstract The formation of UV-absorbing mycosporine-like amino acids (MAAs) as a photoprotective strategy against biologically harmful ultraviolet radiation was studied in Antarctic red macroalgae. After exposure to three different radiation treatments (PAR: 400–700 nm, PAR + UVA: 320–700 nm, PAR + UVA + UVB: 295–700 nm), using artificial irradiance sources under controlled conditions, the physiological capability to stimulate MAA synthesis was investigated. While 8 out of 18 species showed an induction of MAA formation and accumulation, the remaining ten, mainly deep water species, did not exhibit any traces of MAAs. The MAA-containing samples were divided into three physiological response types based on their MAA accumulation versus different radiation treatments. The first response type included *Kallymenia antarctica*, *Gymnogongrus antarcticus*, *Palmaria decipiens* and *Porphyra plocamiestris*, and exhibited additionally increasing MAA concentrations under the different radiation treatments, i.e. highest total MAA values were measured under the full radiation spectrum. The second response type included *Porphyra endiviifolium* and *Gymnogongrus turquetii*, showing highest MAA concentrations already under PAR + UVA. In contrast, *Neuroglossum ligulatum* and *Plocamium cartilagineum* exhibited a strong MAA decrease under PAR + UVR and were grouped in the third response type. No consistent MAA induction patterns could be found, even for individual MAAs, indicating that induction, formation and accumulation of individual MAAs is a very flexible and species-specific mechanism.

Communicated by O. Kinne, Oldendorf/Luhe

K. Hoyer (✉) · C. Wiencke
Foundation Alfred Wegener Institute,
Am Handelshafen 12,
27570 Bremerhaven, Germany

E-mail: khoyer@awi-bremerhaven.de
Fax: +49-471-48311425

U. Karsten
Institute of Aquatic Ecology,
University of Rostock, Albert-Einstein-Str. 3,
18057 Rostock, Germany

Introduction

Benthic macroalgae are sometimes exposed to detrimental ultraviolet-B radiation (UVBR; 280–315 nm), affecting biomolecules, such as proteins and nucleic acids, by so-called direct mechanisms (Vincent and Neale 2000). These molecules can be photochemically degraded or transformed, resulting in impairment or even complete loss of biological function. In this way, UVBR may also influence the genomic stability of plant populations (Ries et al. 2000). In addition, after ultraviolet radiation (UVR; 280–400 nm), indirect exposure mechanisms, such as the production of reactive oxygen species, may negatively affect cells. Macroalgae have developed a number of mechanisms to cope with the deleterious effects of UVBR, such as avoidance, i.e. growth in deep waters, DNA repair by photoreactivation processes and protection, i.e. quenching of reactive oxygen species through antioxidants (Bischof et al. 1998; Aguilera et al. 1999; Cockell and Knowland 1999; Poll et al. 2001). Another protection mechanism against enhanced UVBR is the synthesis and accumulation of sunscreen compounds, such as mycosporine-like amino acids (MAAs), that have been found in many marine primary producers (Bandaranayake 1998; Dunlap and Shick 1998; Jeffrey et al. 1999; Karsten et al. 1998a,b). Due to high extinction coefficients these compounds are very effective UVR absorbers in the wavelength range between 309 and 360 nm (Dunlap and Shick 1998; Cockell and Knowland 1999). To date, 19 distinct molecular structures of MAAs have been identified. It is assumed that these structures are synthesized via the shikimate pathway, which only occurs in plants and microorganisms (Herrmann and Weaver 1999; Shick et al. 1999).

The Southern Ocean, as a habitat for Antarctic macroalgae, features some peculiarities in comparison to other marine regions. Besides high nutrient concentra-

tions (Drew and Hastings 1992; Prézelin et al. 1994) and low water temperatures (Antarctic Surface Water, ranging from −1.7°C to 2.0°C) (Schodlok et al. 2002), ice cover and high amplitudes of irradiance fluctuations typically result in total darkness in winter and 24 h daylight in summer, hence producing large seasonal variability. Furthermore, the terrestrial impact caused by riverine influx is limited, and therefore the waters are generally very clear.

High water transparencies are evidenced by low diffuse vertical attenuation coefficients (K_d, Kirk 1994), resulting in a 1% depth of the photosynthetic active radiation (PAR; 400–700 nm) of up to 40 m in coastal waters near King George Island (Gómez et al. 1997). UVR also penetrates deeply into the water column, in some Antarctic marine areas even to depths of about 60–70 m (Smith et al. 1992). This becomes ecologically important in a scenario with increasing UVBR at the earth's surface caused by ozone depletion in the stratosphere, particularly over the Antarctic. In September 2000, the "ozone hole" extended to the southern tip of South America, with ozone values 50% below normal conditions at 75–80°S, which is a new record low (WMO 2001).

While the occurrence of MAAs in a few selected Antarctic macroalgae was documented for the first time by Karentz et al. (1991) and McClintock and Karentz (1997), a comprehensive inventory of the MAA contents of Antarctic species has recently been undertaken by Hoyer et al. (2001), investigating brown, green and red algae and showing that mostly red algae contain MAAs. The dependence of MAA accumulation on depth distribution, as well as differences in MAA concentrations within algal thalli, was demonstrated. Additionally, Post and Larkum (1993) investigated seasonal effects on UV-absorbing compounds in three Antarctic algae without identifying the chemical structure of the compounds. In Arctic macroalgae such as *Palmaria palmata* and *Devaleraea ramentacea* (Karsten and Wiencke 1999; Karsten et al. 1999), as well as in cold-temperate species such as *Chondrus crispus* (Karsten et al. 1998a; Franklin et al. 1999), MAA synthesis was investigated in field studies, resulting in species-specific MAA inductions under different radiation conditions, i.e. mainly under PAR, UVA and/or UVB. Therefore, in the present study, the three physiological algal groups based on MAA contents in the field (group I – no MAAs at all, group II – MAAs inducible in variable concentrations, and group III – always high MAA values) as described by Hoyer et al. (2001) were photobiologically investigated under controlled laboratory conditions by applying well-defined spectral ranges. The main aim was to better understand the species-specific physiological capability of Antarctic red macroalgae to form and accumulate MAAs as a photoprotective strategy. Emphasis was placed on deep-water species, which seem to produce no or lower MAA concentrations when collected in the field, probably due to the lack of UVBR in that particular environment (Dunlap et al. 1986).

Materials and methods

The 18 red macroalgal species studied (Table 1) were isolated on King George Island (Antarctica) in 1994, according to the method of Clayton and Wiencke (1986), and established as unialgal cultures in the laboratory of the Alfred Wegener Institute (Bremerhaven, Germany). Plants were grown under the following conditions: Provasoli-enriched North Sea water (Starr and Zeikus 1987) at a salinity of 30–32 PSU, aerated with membrane-filtered air (pore size 0.2 µm), at a temperature of 0°C, and an illumination of 2.2–4.4 W m^{-1} irradiance provided by daylight fluorescent lamps (Lumilux Deluxe, OsramL 36 W/12/950, Germany). The irradiance

Table 1. Investigated red macroalgal species with details on respective habitats in Antarctica, according to Wiencke and Clayton (2002), and on the physiological capability to synthesize and accumulate UV-absorbing mycosporine-like amino acids (*MAA*) after treatment with various radiation conditions (see "Materials and methods")

Species	Habitat	MAA induction
Antarcticothamnion polysporum Moe & Silva (Ceramiales)	Sublittoral (15–25 m), endemic	No
Audouinella purpurea (Lightfoot) Woelkerling (Acrochaetiales)	Eulittoral, sublittoral, cosmopolitan	No
Ballia callitricha Kützing (Ceramiales)	Tide pools, sublittoral (0–45 m), cold-temperate	No
Delesseria lancifolia (J.D. Hooker) J. Agardh (Ceramiales)	Sublittoral (2–30 m), cold-temperate	No
Gymnogongrus antarcticus Skottsberg (Gigartinales)	Tide pools, sublittoral (0–15 m), endemic	Yes
Gymnogongrus turquetii Hariot (Gigartinales)	Tide pools, sublittoral (0–30 m), sub-Antarctic/Antarctic endemic	Yes
Hymenocladiopsis crustigena Moe (Rhodymeniales)	Sublittoral (2–30 m), endemic	No
Kallymenia antarctica Hariot (Cryptonemiales)	Sublittoral (5–35 m), endemic	Yes
Myriogramme smithii (J.D. Hooker & Harvey) Kylin (Ceramiales)	Sublittoral (8–43 m), endemic	No
Neuroglossum ligulatum (Reinsch) Skottsberg (Ceramiales)	Sublittoral (1–10 m), endemic	Yes
Palmaria decipiens (Reinsch) Ricker (Palmariales)	Lower eulittoral, sublittoral (0–30 m), endemic	Yes
Pantoneura plocamioides Kylin (Ceramiales)	Sublittoral (2–45 m), cold-temperate	No
Phycodrys austrogeorgica Skottsberg (Ceramiales)	Sublittoral (2–45 m), endemic	No
Phycodrys quercifolia (Bory) Skottsberg (Ceramiales)	Sublittoral (0–25 m), cold-temperate	No
Phyllophora ahnfeltioides Skottsberg (Gigartinales)	Sublittoral (0–30 m), endemic	No
Plocamium cartilagineum (Linnaeus) Dixon (Plocamiales)	Sublittoral (2–40 m), cosmopolitan	Yes
Porphyra endiviifolium Chamberlain (Bangiales)	Upper eulittoral, endemic	Yes
Porphyra plocamiestris Ricker (Bangiales)	Sublittoral (1–20 m), endemic	Yes

intensity was related to the species-specific light requirements of photosynthesis (Weykam 1996). The daylength in the culture room was varied between 5 h (winter) and 20 h light (summer), thereby, simulating fluctuating Antarctic daylengths (Wiencke 1990). All samples were taken under spring conditions, when the daylength reached 18 h.

For the induction experiments, the low-light-acclimated cultures were transferred to the following radiation conditions: (1) only white light (PAR, photosynthetic active radiation: 400–700 nm), (2) PAR plus UVA radiation (PAR + UVA: 320–700 nm) and (3) PAR plus UVR (PAR + UVA + UVB: 295–700 nm). Daylight fluorescent lamps (Lumilux Deluxe, OsramL 36 W/12-950, Germany) in combination with Q-Panel UVA-340 fluorescent tubes (Cleveland, USA) emitting a spectrum similar to solar radiation in the UV range were used. The glass vessels were covered with specific filters to cut off UVBR (320 nm cut-off: Ultraphan URUV, Digefra, München, Germany) and UVB + UVA radiation (400 nm cut-off: Folex PR, Folex, Dreieich, Germany). The containers under the full spectrum were also covered with a filter with no transmission below 295 nm (Ultraphan UBT, Digefra, München, Germany).

Irradiance was measured with a Spectro 320D spectroradiometer (Instrument Systems, Germany) (see Fig. 1). PAR was 5.4 W m^{-2}, UVA and UVB 4.1 W m^{-2} and 0.5 W m^{-2}, respectively, at the vessel's surface. The UVA radiation corresponded to values measured in 7–8 m water depth during a sunny summer day on King George Island (Hoyer et al. 2001). In all cases a 16 h PAR + UVR:8 h dark photocycle was applied.

During the experiments algae were kept in glass beakers filled with filtered Provasoli-enriched seawater plus 2.1 mM sodium hydrogen carbonate as an inorganic carbon source. All experiments were performed in a constant temperature room at 0°C, except for those with *Porphyra plocamiestris*, which was kept at 5°C. After 12 days of exposure to the various radiation conditions the algae were harvested, oven-dried at 50°C overnight, and stored in Eppendorf tubes under dry and dark conditions prior to MAA analysis.

MAA extraction and analysis

Samples of about 10–20 mg dry weight (DW) were extracted for 2 h in Eppendorf tubes filled with 1 ml 25% aqueous methanol (v/v) and incubated in a waterbath at 45°C. This procedure was sufficient to obtain > 99.5% of MAAs into solution. After centrifugation at 5000 *g* for 5 min, 800 µl of the supernatants were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were re-dissolved in 800 µl 100% methanol and vortexed for 30 s. Samples were analyzed with a Waters high-performance liquid chromatography (HPLC) system according to the method of Karsten and Garcia-Pichel (1996), with the following modifications. The MAAs were separated on a Phenomenex SphereClone RP-C8 column (5 µm, 250×4 mm i.d.) protected with a RP-C8 guard cartridge (4×3 mm i.d.). The mobile phase was 5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at a flow rate of 0.7 ml min^{-1}. The MAAs were detected with a photodiode detector at 330 nm, and absorption spectra (290–400 nm) were recorded each second directly on the HPLC-separated peaks. Identification was done by spectra, retention time, and by co-chromatography with standards extracted from the marine red macroalgae *Chondrus crispus* and *Porphyra umbilicalis* from Helgoland, Germany, as well as from ocular lenses of the coral trout *Plectropomus leopardus* (kindly provided by Dr D. Bellwood, James Cook University, Townsville, Australia). Quantification was made using the molar extinction coefficients listed in Karsten et al. (1998c). All amounts are given as means (± SD) and expressed as concentration on a dry weight basis (mg g^{-1} DW).

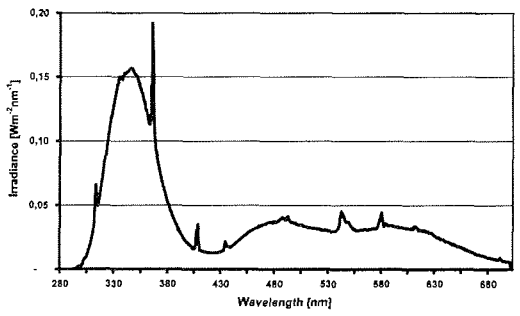


Fig. 1. Radiation spectrum in the range from 295 to 700 nm emitted by daylight fluorescent lamps in combination with Q-Panel UVA-340 fluorescent tubes

Statistical analyses

The influence of filter treatment on MAA content and significant differences within the different treatments were assessed by using one-way ANOVA followed by a multiple comparison test (Tukey–Kramer HSD-test). Differences were considered significant when probability was $P < 0.05$.

Results

Ten of 18 investigated red algae from Antarctica did not contain any MAAs, either in pre-culture or after exposure to various radiation conditions (Table 1). However, the other eight species exhibited seven different MAAs: shinorine, porphyra-334, palythine and asterina-330 as major components; palythinol in *Porphyra endiviifolium* in trace amounts as well as usujirene in *Palmaria decipiens*; and a minor content of an unknown substance (retention time: 4.6 min; absorption maxima: 332/3 nm) in *Neuroglossum ligulatum*. In this study, we refer mainly to the quantitatively dominant MAAs. Incubation under various radiation conditions resulted in species-specific differences in the quantity and quality of MAAs. Therefore, the macroalgae were grouped based on their response to the different irradiance treatments, resulting in distinct MAA induction/accumulation profiles (Table 2).

The first response type (a), including *Kallymenia antarctica*, *Gymnogongrus antarcticus*, *Palmaria decipiens* and *Porphyra plocamiestris*, exhibited an additional increase in total MAA concentration after incubation under PAR, PAR + UVA and PAR + UVA + UVB, with the highest concentrations under the latter treatment (Fig. 2A–D). The initial samples (before treatment) had a significantly lower MAA concentration compared to the exposed groups, except for *K. antarctica* and *P. plocamiestris*, in which no significant differences were found between the initial samples and the PAR treatment. The quantitative differences of MAAs between the PAR samples and those under the full radiation spectrum were statistically very significant ($P < 0.001$), except for *P. decipiens*. Additionally, significant differences were found in *G. antarcticus* between PAR and

Table 2. Classification of field-collected red algae in three physiological groups (*I–III*) based on MAA concentrations (Hoyer et al. 2001) and of cultured red algae belonging to the groups II and III, which were divided into three subgroups showing different responses (*response types a–c*) to MAA synthesizing and accumulation after treatment with distinct radiation conditions (PAR: 400–700 nm; PAR + UVA: 320–700 nm; PAR + UVA + UVB: 295–700 nm)

Field-collected algae	Group I	Group II		Group III
	Lacking MAAs	Inducibly variable MAA concentrations		Always high MAA concentrations
		Type a	Type b	Type c
Cultured algae after exposure to different radiation conditions	Lacking MAAs	Highest total MAA concentrations under PAR + UVA + UVB	Highest total MAA concentrations under PAR + UVA	MAA decrease under PAR + UVR

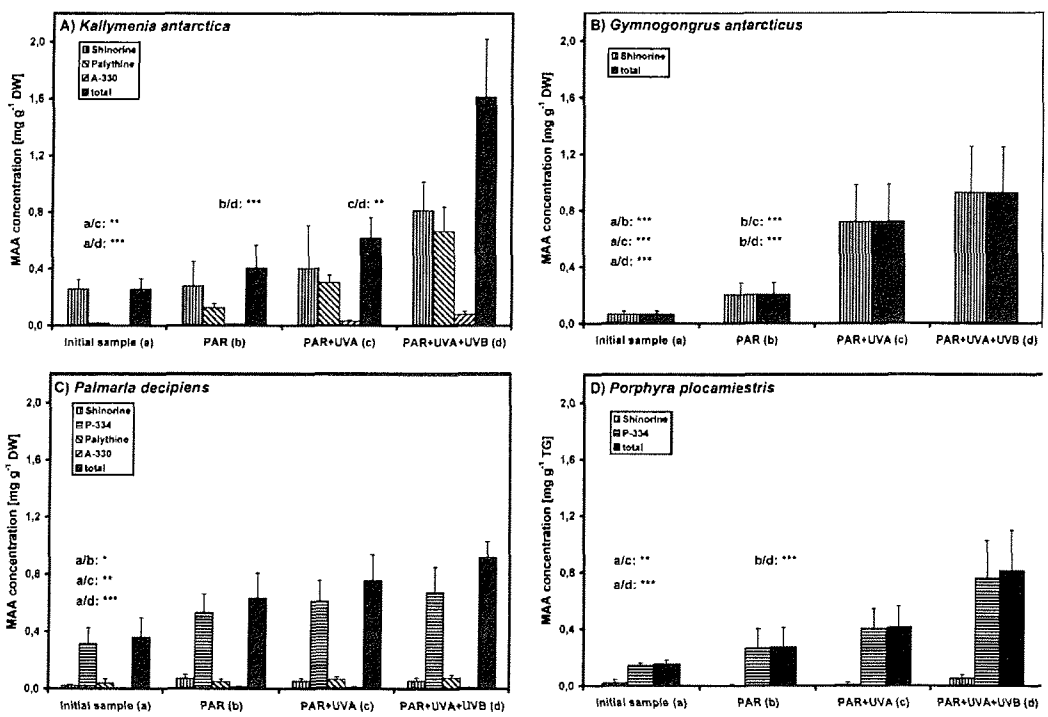


Fig. 2A–D. Shinorine, palythine, porphyra-334, asterina-330 and total concentration of MAAs in the first response type (a) of algal species showing a continuous increase of MAA concentration after 12 days of exposure to different radiation conditions. **A** *Kallymenia antarctica* ($n = 4–5$), **B** *Gymnogongrus antarcticus* ($n = 5–6$), **C** *Palmaria decipiens* ($n = 5–6$), **D** *Porphyra plocamiestris* ($n = 4–5$). *Lower case letters with asterisks* indicate significant differences in the concentrations of MAAs between respective treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Bars are means (± SD)

PAR + UVA conditions ($P < 0.001$). The increase in total MAAs from the initial samples to those with the full spectrum treatment was species dependent: 2.6-fold in *P. decipiens*, 5.3-fold in *P. plocamiestris*, 6.3-fold in *K. antarctica* and 14-fold in *G. antarcticus*. The relative changes in MAA concentration under the different radiation treatments are shown in Fig. 5A.

Within the first response type, the MAA compositions were different. *G. antarcticus* contained only shinorine as the sole MAA, while in *P. plocamiestris*, in addition to this compound, porphyra-334 also occurred in large quantities. This species also exhibited traces of palythine, asterina-330 and palythinol (data not shown) under PAR + UVA + UVB treatment. *K. antarctica* and *P. decipiens* exhibited three (shinorine, palythine, asterina-330) and four different UV-absorbing compounds (shinorine, porphyra-334, palythine, asterina-330), re-

spectively (Fig. 2A–D). In *K. antarctica*, shinorine and palythine were the quantitatively dominant MAAs, and in *P. decipiens* porphyra-334 occurred in highest concentrations.

A second response type (b), with *Porphyra endiviifolium* and *Gymnogongrus turquetii*, showed the highest concentration of total MAAs already under UVA treatment (Fig. 3A, B). In *P. endiviifolium*, statistically significant increases of MAAs from the high-value initial sample to plants kept under PAR + UVR conditions ($P < 0.0003$, $P < 0.03$) were detected, as well as between the PAR- and PAR + UVA-treated samples ($P < 0.02$, Fig. 3A). After additional exposure to UVB, a slightly lower concentration of MAAs compared to the PAR + UVA treatment was observed. In *G. turquetii*, the MAA concentration significantly increased from the low initial value in all radiation treatments ($P < 0.001$). Significant differences in MAA concentrations were also found between samples under PAR and both UVR treatments ($P < 0.005$, Fig. 3B). Although MAA levels were slightly lower under PAR + UVA + UVB compared to PAR + UVA, this difference was not statistically significant. The rise of total MAA values from the initial samples to the PAR + UVA treatment was 1.9-fold in *P. endiviifolium* and 25.2-fold in *G. turquetii*. While both species exhibited shinorine and porphyra-334 as the main MAAs, in *G. turquetii* these compounds occurred in almost equimolar concentrations under all experimental radiation treatments, except in the initial sample where porphyra-334 was absent. In contrast, in all samples of *P. endiviifolium* porphyra-334 was the quantitatively dominant MAA, whereas shinorine occurred in low amounts.

Neuroglossum ligulatum and *Plocamium cartilagineum* represent a third response type (c), as both species contained lower or alternatively no MAAs after exposure to PAR + UVA + UVB (Fig. 4A, B). In *N. ligulatum*, exposure to PAR and PAR + UVA was accompanied by a 3.6- and 8.7-fold increase in total

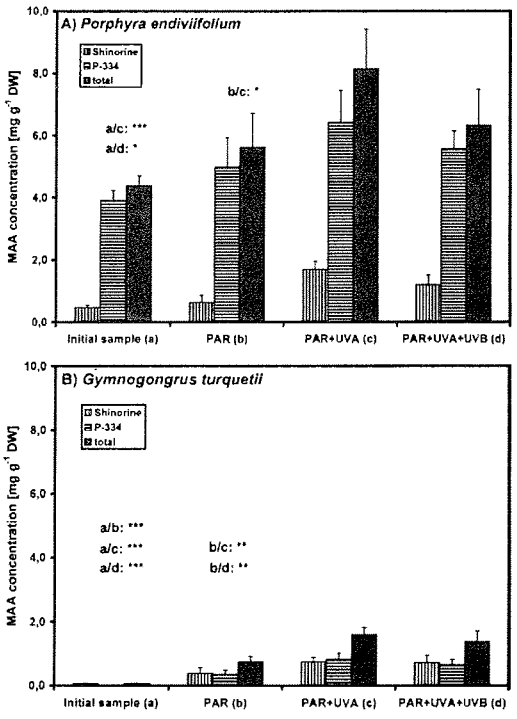


Fig. 3A, B. Shinorine, porphyra-334 and total concentration of MAAs in the second response type (b) of algal species showing the highest MAA concentration under PAR + UVA treatment after 12 days of exposure to different radiation conditions. **A** *Porphyra endiviifolium* ($n = 5$–7), **B** *Gymnogongrus turquetii* ($n = 4$–6). *Lower case letters with asterisks* indicate significant differences in the concentrations of MAAs between respective treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Bars are means (± SD)

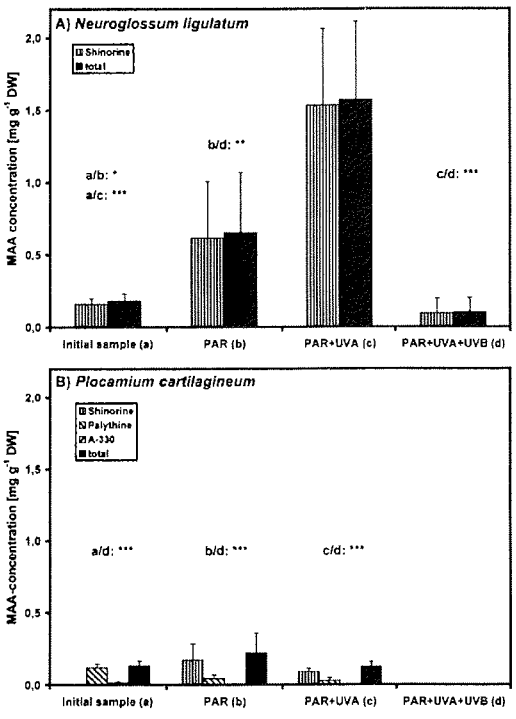


Fig. 4A, B. Shinorine, palythine, porphyra-334, asterina-330 and total concentration of MAAs in the third response type (c) of algal species showing a strong decrease of MAA concentration under PAR + UVR treatment after 12 days of exposure to different radiation conditions. **A** *Neuroglossum ligulatum* ($n = 3$–5), **B** *Plocamium cartilagineum* ($n = 3$–5). *Lower case letters with asterisks* indicate significant differences in the concentrations of MAAs between respective treatments (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). Bars are means (± SD)

MAAs, respectively, followed by a decrease similar to the initial value under PAR + UVA + UVB. In contrast, only a 1.7-fold increase from the initial concentration to the MAA content after the PAR treatment was evident in *P. cartilagineum*. Although exposure to PAR + UVA already led to a strong decrease in the MAA concentration, resulting in values similar to the initial ones after exposure to the full spectrum, only trace amounts of palythine (0.002 mg g^{-1} DW) were present. The relative changes in MAA concentration for the second and third response types are summarized in Fig. 5B.

Based on MAA composition, shinorine was the most abundant sunscreen compound in *N. ligulatum*, in addition to traces of porphyra-334 and an unknown UV-absorbing substance (data not shown). Whilst the initial sample of *P. cartilagineum* contained palythine as a major compound along with small amounts of asterina-330; shinorine was the quantitatively dominant MAA after exposure to higher PAR intensities.

Discussion

The most important result of the present study is that the algal response to the application of different radiation conditions can be clustered into three subgroups (a, b, c; Table 2), here called response types, based on MAA accumulation versus different radiation treatments.

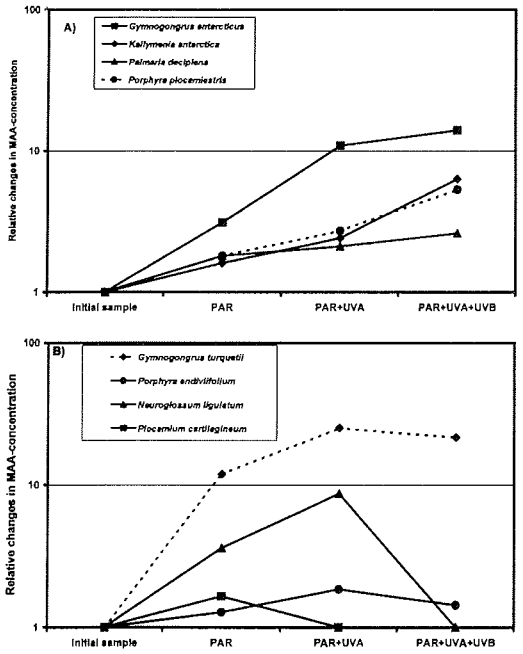


Fig. 5A, B. Relative changes in MAA concentrations under the different radiation conditions related to the normalized initial samples which were all set to 1. **A** First response type, **B** second and third response types of Antarctic algae after 12 days of exposure to different radiation conditions

The first response type (a) includes species that accumulated MAAs increasing under each treatment, i.e. strongest biosynthesis of total MAAs under the full artificial spectrum. The species of this response type (a: *Kallymenia antarctica*, *Gymnogongrus antarcticus*, *Palmaria decipiens*, *Porphyra plocamiestris*) are endemic to Antarctica and therefore generally well adapted to the harsh Southern Ocean environmental conditions, e.g. low temperatures (Wiencke and tom Dieck 1989; Bischoff-Bäsmann and Wiencke 1996; Eggert and Wiencke 2000). Furthermore, taxa of this subgroup are found over a wide depth range within the sublittoral, and *P. decipiens* occurs also in the eulittoral (Wiencke and Clayton 2002). This broad distribution pattern may result in adaptation to clear water conditions and consequently to UV radiation penetrating deeply into the water column. MAAs may thus be induced to assume the role of a sunscreen, as seen in *K. antarctica*, exhibiting high MAA concentrations even at a water depth of 20 m (Hoyer et al. 2001). The evolutionary development of endemism is coupled with environmental adaptation advantages with respect to temperature and irradiance. Such species flexibly allows the adjustment of their MAA concentrations to the prevailing radiation climate, as demonstrated for several field-collected Antarctic species from different depths (Hoyer et al. 2002).

Macroalgae of the second response type [b: *Gymnogongrus turquetii* (sub-Antarctic and Antarctic endemic), *Porphyra endiviifolium* (Antarctic endemic)] contain the highest MAA concentration under PAR + UVA, and additional UVB did not lead to any further MAA accumulation. The habitat of *P. endiviifolium* is the upper eulittoral, which is regularly exposed to full solar radiation. The perpetual high MAA concentrations found in this species have also been found in the closely related cold temperate and eulittorally growing *Porphyra umbilicalis* (Gröniger et al. 2000). High MAA concentrations are typically present in eulittoral species and, hence, may reflect a steady protective mechanism, thereby highlighting the photoprotective role of these UV-absorbing compounds (Karsten and West 2000). The generally lower MAA concentrations in *G. turquetii* can be explained by the broader depth range for growth (eulittoral to sublittoral down to 30 m, Wiencke and Clayton 2002), indicating that each algal species probably has an upper MAA concentration threshold that depends on the vertical distribution on the shore and the respective radiation conditions. However, one has to consider that the effect of dose, regardless of irradiance, was not explicitly tested. Therefore, we cannot ultimately conclude that the increase of MAAs is only due to the change in spectral composition.

Algae of the third response type (c) also exhibited higher MAA content after treatment with PAR or PAR + UVA. However, in contrast to type b, exposure to the full spectrum led to a strong decline in total

MAAs. Species of this type are the endemic *Neuroglossum ligulatum* and the cosmopolitan *Plocamium cartilagineum*. Under treatment with the full radiation spectrum, only traces of MAAs were found, which may either be explained by an almost complete degradation or decomposition of MAAs, or their possible leakage from the cells into the medium. However, the observation of thalli bleaching at the end of the UVR exposure experiment in these two species, indicates photodamage of the thallus. Additionally, this results in a decrease of MAA content per dry weight under the UVB treatment, whereas these plants show higher MAA concentrations under PAR/PAR+UVA conditions. Compared to *P. cartilagineum* from cold-temperate waters, the Antarctic isolate is extremely stenothermal, growing only at temperatures below 5°C and dying above 7°C (Bischoff-Bäsmann and Wiencke 1996), indicating the development of temperature ecotypes. The Antarctic *P. cartilagineum* seems to exhibit a high degree of physiological sensitivity to changing abiotic parameters, against which even the presence of MAAs does not provide full UV protection. Therefore, the presence of UV-absorbing compounds in *P. cartilagineum* and *N. ligulatum* is not sufficient to prevent bleaching of the tissue, suggesting that a MAA suite alone does not guarantee complete protection against UVR. However, even incomplete protection from UVR might reduce damage, so that any residual biological effects may be completely counteracted by other defenses, as suggested for phytoplankton species (Neale et al. 1998).

The MAA-containing species of this study belong to the two physiological groups II and III, classified by Hoyer et al. (2001). These algae also exhibited MAAs in the field (Karentz et al. 1991; McClintock and Karentz 1997). The generally lower MAA concentrations in this study may be due to the fact that cultured algae always show less MAAs due to the artificial and less extensive radiation climate (Carreto et al. 1990). The ten species which do not exhibit MAAs even after exposure to UVR belong to the first physiological group mentioned in Hoyer et al. (2001), including species such as *Hymenocladiopsis crustigena* and *Phycodrys austrogeorgica*, typically lacking MAAs in the field. These deep-water taxa are strongly shade adapted (Kirst and Wiencke 1995), and exhibit low photosynthetic light compensation and initial light-saturation points closely corresponding to the conditions of their habitat (Weykam et al. 1996). They are susceptible both to higher PAR and UVR and must live in a low radiation climate with little or no UVBR. This has also been demonstrated in the Arctic deep-water red algal species *Phycodrys rubens*. After transplantation from deeper to shallow water, strong photoinhibition and photobleaching occurred, suggesting that this plant is genetically adapted to a low-light environment and, hence, completely incapable of coping with higher ambient radiation (Karsten et al. 2001). In agreement with our data, these authors assumed that the lack of MAAs in *P. rubens* may be one reason for the observed response. Similarly, Bischof et al. (1998) found very strong sensitivity of photosynthesis to UVR, particularly in Antarctic deep-water species such as *Delesseria lancifolia* and *P. austrogeorgica*, both of which also lack MAAs (Table 1) shown by the present study. It may be possible that the presence or absence of MAA biosynthesis in Antarctic species may also be determined on a genetic level, and can be explained as adaptation to an almost UVB-free environment and to low light levels in general.

Six known MAA compounds (shinorine, porphyra-334, palythine, asterina-330, palythinol, usujirene) were detected in the Antarctic species studied here. The induction of individual MAAs is dependent on spectral radiation composition and intensity, resulting in species-specific induction patterns. The quantitatively dominant MAAs synthesized during the experiments were shinorine, porphyra-334 and palythine. The MAA composition differs across species, depending on the physiological and genetic characteristics of the individual algae. Cultured *P. endiviifolium* and *N. ligulatum* exhibited porphyra-334 and shinorine, whereas in field-collected samples palythine was also detected. The absence of some MAAs in cultured samples is a common observation, demonstrating that the artificial irradiance might not be efficient or natural enough to induce the whole MAA inventory. These data also indicate that red macroalgae must have highly specific trigger systems, which are capable of sensing solar radiation (dose, spectral composition, etc.) and which lead to a particular set of MAAs.

All data presented suggest that the induction, formation and accumulation of individual MAAs is a physiologically very flexible as well as species-specific process. The underlying mechanism seems to include several enzymatic steps for biosynthesis and interconversion depending on various environmental (radiation climate, water turbidity), ecological (growth habitat), physiological (enzymatic activity/regulation) and genetic factors (missing and/or silenced genes). Although experimental evidence for a particular trigger mechanism, as well as details concerning the biosynthetic pathway of individual MAAs, is still missing, we agree with the proposal of Franklin et al. (1999) that a signal transduction pathway or even interactions among various photoreceptors must be involved in the overall process leading to high MAA concentrations. At least two different photoreceptors should be taken into consideration, due to the two types of MAA induction patterns: one for the PAR range and another for UVR sensing.

As MAA induction occurs in *Chondrus crispus* under blue light and UVA, a cryptochrome photoreceptor should be involved in the triggering of MAA biosynthesis (Franklin et al. 2001). Furthermore, Portwich and Garcia-Pichel (2000) suggested reduced pterin as a UVB photoreceptor chromophore in the MAA metabolism of the cyanobacterium *Chlorogloeopsis* sp. PCC. Other mechanisms such as a redox reaction might be involved in blue-light-induced gene expression as well (Lin 2000).

As known from higher plant photobiology it seems that individual photoreceptors play unique roles in physiological regulation processes and that certain gene products are shared by light activation pathways initiated by different photoreceptors (Petridou et al. 1997 and references therein).

Although the trigger mechanism and the photoreceptor still have to be elucidated, it has been demonstrated that different radiation conditions lead to three different response types based on MAA formation. The MAA biosynthesis in mainly eu- and sublittoral red algae is a very flexible and species-specific biochemical pathway depending on irradiance, which may result in protection against harmful UVR as well as the production of other sunscreen substances important for life in the respective habitats.

Acknowledgements This project was financially supported by the Deutsche Forschungsgemeinschaft (Ka 899/3-1/-2/-3). The authors thank M. Schoenwaelder for discussion and reading the manuscript. The experiments we performed comply with the current laws of Germany.

References

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

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

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

Bischoff-Bäsmann B, Wiencke C (1996) Temperature requirements for growth and survival of Antarctic Rhodophyta. J Phycol 32:525–535

Caretto JI, Lutz VA, De Marco SG, Carignan MO (1990) Fluence and wavelength dependence of mycosporine-like amino acid synthesis in the dinoflagellate *Alexandrium excavatum*. In: Graneli E (ed) Toxic marine phytoplankton: proceedings of the 4th international conference on toxic marine phytoplankton. Elsevier, New York, pp 275–279

Clayton M, Wiencke C (1986) Techniques and equipment for culturing Antarctic benthic marine algae, and for preparing specimens for electron microscopy. Serie Científica, Instituto Antártico Chileno 34:93–97

Cockell CS, Knowland J (1999) Ultraviolet radiation screening compounds. Biol Rev Camb Philos Soc 74:311–345

Drew EA, Hastings RM (1992) A year-round ecophysiological study of *Himantothallus grandifolius* (Desmarestiales, Phaeophyta) at Signy Island, Antarctica. Phycologia 31:262–277

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, Chalker BE, Oliver JK (1986) Bathymetric adaptions of the reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J Exp Mar Biol Ecol 104:239–248

Eggert A, Wiencke C (2000) Adaptation and acclimation of growth and phototsynthesis of five Antarctic red algae to low temperatures. Polar Biol 23:609–618

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

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

Gröniger A, Sinha RP, Klisch M, Häder D-P (2000) Photoprotective compounds in cyanobacteria, phytoplankton and macroalgae – a database. J Photochem Photobiol B 58:115–122

Herrmann KM, Weaver LM (1999) The shikimate pathway. Annu Rev Plant Physiol Plant Mol Biol 50:473–503

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 211:117–129

Hoyer K, Karsten U, Wiencke C (2002) Inventory of UV-absorbing mycosporine-like amino acids in polar macroalgae and factors controlling their content. In: Huiskes A, Rozema J (eds) Antarctic biology in a global context: proceedings of the 8th SCAR international biology symposium. Buckhuys, Amsterdam (in press)

Jeffrey SW, MacTavish HS, Dunlap WC, Vesk M, Groenwoud 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, Mc Euen FS, Land MC, Dunlap WC (1991) Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108:157–166

Karsten U, Garcia-Pichel F (1996) Carotenoids and mycosporine-like amino acid compounds in members of the genus *Microcoleus* (Cyanobacteria): a chemosystematic study. Syst Appl Microbiol 19:285–294

Karsten U, West J (2000) Living in the intertidal zone – seasonal effects on heterosides and sun-screen compounds in the red alga *Bangia atropurpurea* (Bangiales). J Exp Mar Biol Ecol 254:221–234

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, 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, Sawall T, Wiencke C (1998c) A survey of the distribution of UV-absorbing substances in tropical macroalgae. Phycol Res 46:271–279

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

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

Kirk JTO (1994) Optics of UV-B radiation in natural waters. Schweizerbart'sche, Stuttgart

Kirst GO, Wiencke C (1995) Ecophysiology of polar algae. J Phycol 31:181–199

Lin C (2000) Plant blue-light receptors. Trends Plant Sci 5:337–342

McClintock JB, Karentz D (1997) Mycosporine-like amino acids in 38 species of subtidal marine organisms from McMurdo Sound, Antarctica. Antarct Sci 9:392–398

Neale PJ, Banaszak AT, Jarriel CR (1998) Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like

amino acids protect against inhibtion of photosynthesis. J Phycol 34:928–938
Petridou S, Foster K, Kindle K (1997) Light induces accumulation of isocitrate lyase mRNA in a carotenoid-deficient mutant of *Chlamydomonas reinhardtii*. Plant Mol Biol 33:381–392
Poll van de WH, Eggert A, Buma AGJ, Breeman AM (2001) Effects of UV-B induced DNA damage and photoinhibition on growth of six temperate marine red macrophytes: distinct habitat related differences in ultraviolet-B tolerance. J Phycol 37:30–37
Portwich A, Garcia-Pichel F (2000) A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912. J Photochem Photobiol 74:493–498
Post A, Larkum AWD (1993) UV-absorbing pigments, photosynthesis and UV exposure in Antarctica: comparison of terrestrial and marine algae. Aquat Bot 45:231–243
Prézelin BB, Boucher NP, Schoefield O (1994) Evaluation of field studies of UVB radiation effects on Antarctic marine primary productivity. In: Biggs RH, Joyner MEB (eds) Stratospheric ozone depletion. UV-B reduction in the biosphere. NATO ASI Ser Ser I Global Environ Change 18:181–194
Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK, Hohn B (2000) Elevated UV-B radiation reduces genome stabiltiy in plants. Nature 406:98–101
Schodlok M, Hellmer H, Beckmann A (2002) On the transport, variability and origin of dense water masses crossing the South Scotia Ridge. Deep-Sea Res (in press)
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 population of symbiotic dinoflagellates. Limnol Oceanogr 44:1667–1682
Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z, Waters KJ (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959
Starr RC, Zeikus JA (1987) UTEX – The culture collection of algae at the University of Texas at Austin. J Phycol 23[Suppl]:1–47
Vincent WF, Neale PJ (2000) Mechanisms of UV damage to aquatic organisms. In: Cockell CS, Blaustein AR (eds) Ecosystems, evolution, and ultraviolet radiation. Springer, New York Heidelberg Berlin, pp 146–177
Weykam G (1996) Photosynthetic characteristics and life-strategies of Antarctic macroalgae. Rep Polar Res 192:1–132
Weykam G, Gómez I, Wiencke C, Iken K, Klöser H (1996) Photosynthetic characteristics and C:N ratios of macroalgae from King George Island (Antarctica). J Exp Mar Biol Ecol 204:1–22
Wiencke C (1990) Seasonality of red and green macroalgae from Antarctica – a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601–607
Wiencke C, Clayton M (2002) Antarctic seaweeds. In: Wägele JW (ed) Synopses of the Antarctic benthos. Koeltz Scientific Books, Königstein
Wiencke C, tom Dieck I (1989) Temperature requirements for growth and temperature tolerance of macroalgae endemic to the Antarctic region. Mar Ecol Prog Ser 54:189–197
WMO (World Meterological Organization) (2001) World climate news. WMO 18:1–16. http://www.wmo.ch/indexflash.html. Cited 11 June 2002

Publication 4

Hoyer, K., Schäfer, S., Karsten, U., Wiencke, C.

Interactive effects of temperature and radiation on polar macroalgae

Journal of Phycology (in preparation)

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

Kirsten Hoyer[1], Sabine Schäfer[1], Ulf Karsten[2], Christian Wiencke[1]

[1]Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

[2]Institute of Aquatic Ecology, University of Rostock, Albert-Einstein-Str. 3, D-18057 Rostock, Germany

Author for correspondence:

Kirsten Hoyer

Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12

D- 27570 Bremerhaven, Germany

E-mail: khoyer@awi-bremerhaven.de

ABSTRACT

INTRODUCTION

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

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

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

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

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

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

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

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

MATERIALS AND METHODS

Algal material

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

Experimental set up

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

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

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

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

Determination of optimal quantum yield of PSII

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

MAA extraction and analysis

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

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

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

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

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

RESULTS

MAA inventory

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

Temperature and radiation effects on total MAA concentrations

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

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

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

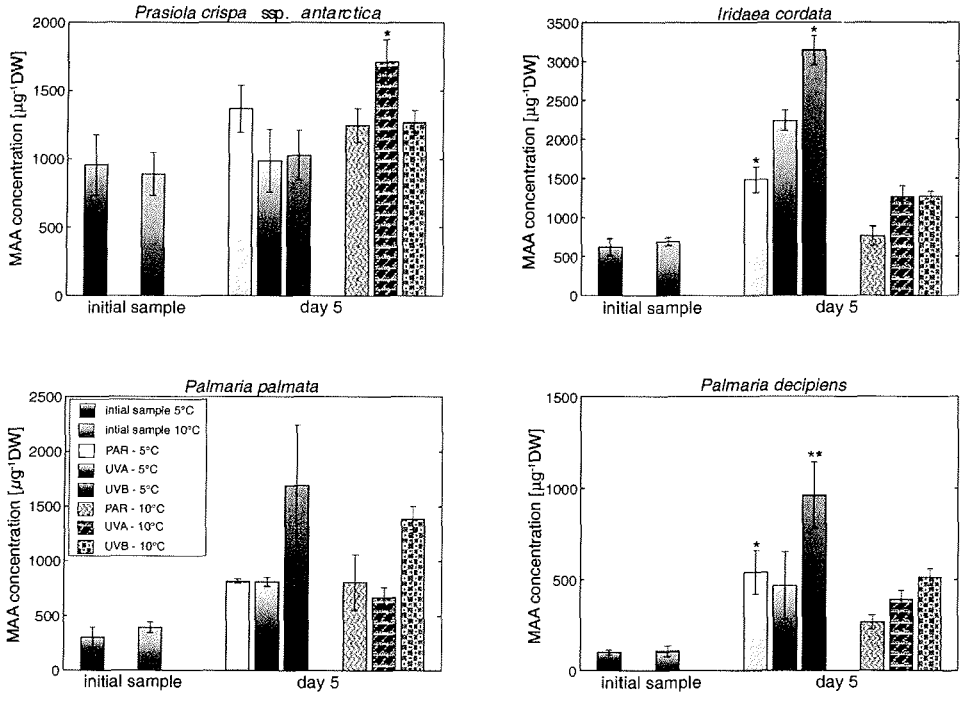


Figure 1: Total concentration of MAAs of the initial samples and after five days of exposure to different radiation conditions (PAR, PAR+UVA, PAR+UVA+UVB) at 5 and 10°C. *Prasiola crispa* ssp. *antarctica* from King George Island (Antarctica). *Iridaea cordata* (at 5 °C the PAR+UVA sample and at 10 °C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

Temperature and radiation effects on individual MAAs

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

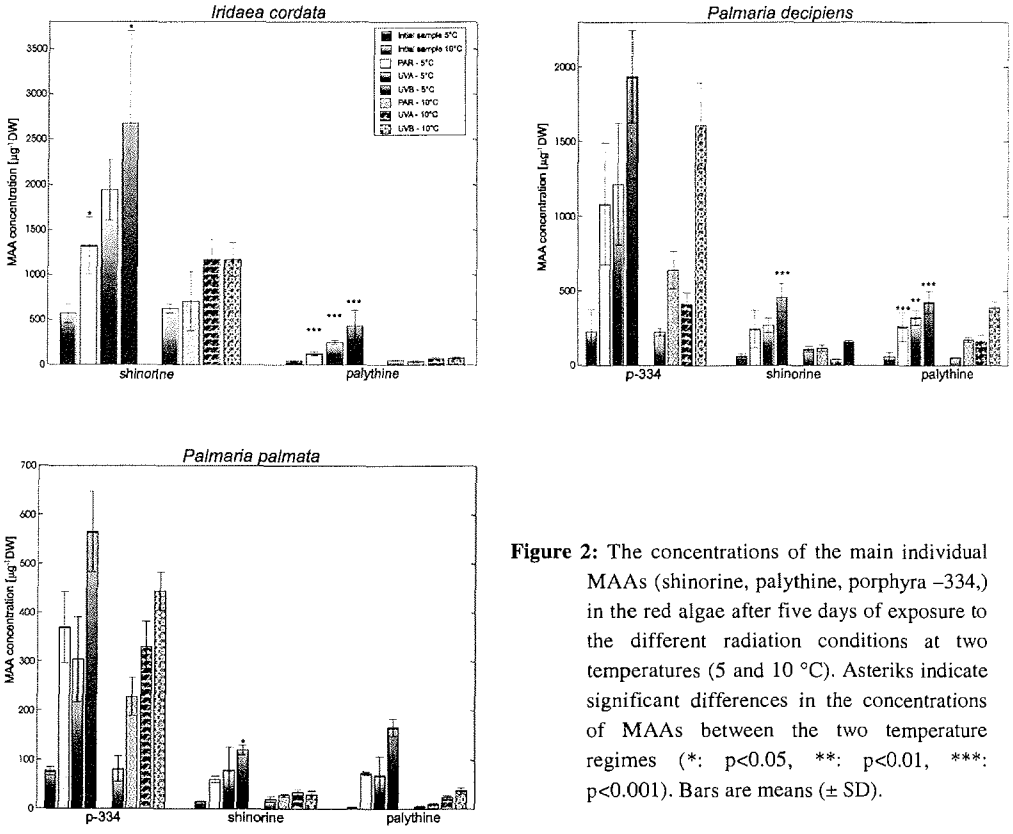


Figure 2: The concentrations of the main individual MAAs (shinorine, palythine, porphyra –334,) in the red algae after five days of exposure to the different radiation conditions at two temperatures (5 and 10 °C). Asteriks indicate significant differences in the concentrations of MAAs between the two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

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

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

Spectral radiation effects

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

Effects on photosynthesis (optimal quantum yield)

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

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

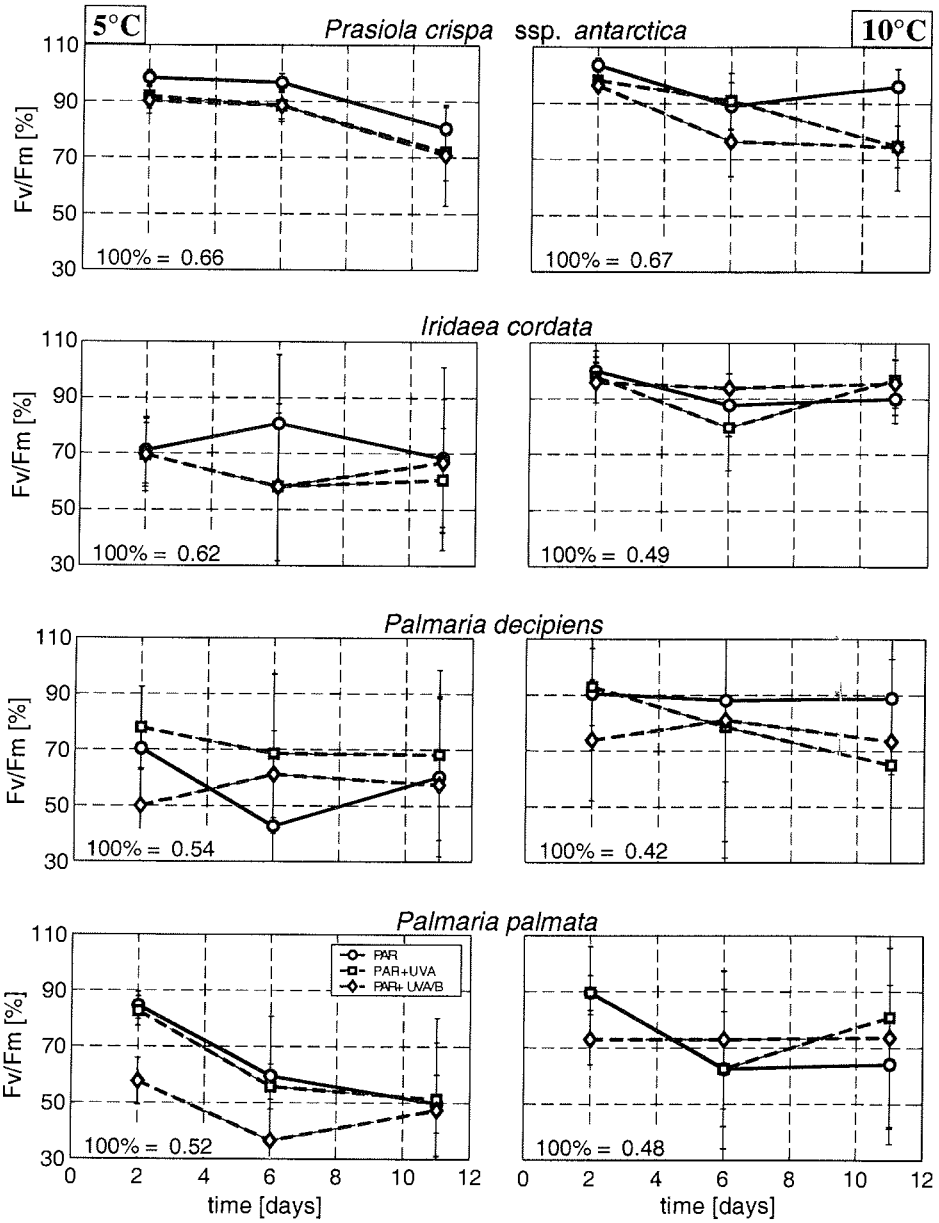


Figure 3: Relative differences in optimal quantum yield of PS II (Fv/Fm) of algae exposed to different radiation conditions PAR; PAR+UVA; PAR+UVA+UVB) at two temperatures (5 and 10 °C). Initial samples were standardized to 100 %. Fv/Fm was measured after a recovery period of 6-8 h darkness at day 2,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

DISCUSSION

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

Temperature effects

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

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

Radiation effects

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

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

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

Interactive effects

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

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

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

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

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

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

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

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

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

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

Acknowledgements

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

REFERENCES

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: 109-19.

Barry, J.P., Baxte, C.H., Sagarin, R.D. & Gilman. S.E. 1995. Climate-related, long-term faunal changes in a California rocky intertidal communitiy. Science 267:672-5.

Beardall, J., Beer, S. & Raven, J.A. 1998. Biodiversity of marine plants in an era of climate change: some predictions based on physiological performance. Botanica Marina 41:113-23.

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

Bischoff, B. & Wiencke, C. 1995. Temperature ecotypes and biogeography of Acrosiphonales (Chlorophyta) with Arctic-Antarctic disjunct and Arctic/cold-temperate distributions. Eur. J. Phycol. 30: 19-27.

Breemann, A. M. 1988. Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: Experimental phenological evidence. Helgol. Meeresunters. 42: 199-241.

Davison, I.R. 1991. Environmental effects on algal photosynthesis: temperature. J. Phycol. 27:2-8.

Dring, M.J., Makarov, V., Schoschina, E., Lorenz, M. & Lüning, K. 1996. Influence of ultraviolet-radiation on chlorophyll fluorescence and growth in different life-history stages of three species of Laminaria (Phaeophyta). Mar. Biol. 126:183-91.

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

Eggert, A. & Wiencke, C. 2000. Adaptation and acclimation of growth and phototsynthesis of five Antarctic red algae to low temperatures. Polar Biol. 23:609-18.

Franklin, L.A., Kräbs, G. & Kuhlenkamp, R. 2001. Blue light and UVA radiation control the synthesis of mycosporine-like amino acids in Chondrus crispus (Florideophyceae). J. Phycol. 37:257-70.

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.

Gómez, I., Figueroa, F.L., Sousa-Pinto, I., Viñegla, B., Pérez-Rodríguez, E., Maestre, C., Coelho, S., Felga, A. & Pereira, R. 2001. Effects of UV radiation and temperature on photosynthesis as measured by PAM flourescence in the red alga Gelidium pulchellum (Turner) Kützing. Botanica Marina 44:9-16.

Gröniger, A. & Häder, D.-P. 2002. Induction of the synthesis of an UV-absorbing substance in the green alga Prasiola stipitata . J. Photochem. Photobiol. 66:54-9.

Gröniger, A., Hallier, C. & Häder, D.-P.. 1999. Influence of UV radiation and visible light on Porphyra umbilicalis: Photoinhibition and MAA concentration. J. App. Phycol. 11:437-45.

Groß, C., Tüg, H. & Schrems, O. 2001. Three years spectral resolved UV-measurements at Koldewey-Station 1997-1999. Mem. Nat. Inst. Polar Res. 54: 113-23.

Hanelt, D., Tüg, H., Bischof, K., Groß, C., Lippert, H., Sawall, T. & Wiencke, C. 2001. Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. Mar. Biol. 138: 649-658

Hanelt, D., Wiencke, C. & Nultsch, W. 1997. Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. J.Photochem.Photobiol.B: Biol. 38: 40-47

Holm-Hansen, O., Lubin, D. & Helbling, E.W. 1993. Ultraviolet radiation and its effects on organisms in aquatic environments: In: Young, A.R., Björn, L.O.; Moan, J. & Nultsch W. [Eds.] Environmental UV Photobiology. Plenum press, New York, pp. 379-425.

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.

Hoyer, K., Karsten, U. & Wiencke, C. 2002. Induction of sunscreen compounds in Antarctic macroalgae by different radiation conditions. Mar. Biol. 141:619-627

Jackson, A.E. & Seppelt, R.D. 1995. The accumulation of proline in Prasiola crispa during winter in Antarctica. Physiol. Plant. 94(1):25-30.

Jacob, A, Kirst, G.O., Wiencke, C. & Lehmann, H. 1991. Physiological responses of the Antarctic green alga Prasiola crispa ssp. antartica to salinity stress. J. Plant Physiol. 139:57-62.

Karentz, D. 1989. Report on studies related to the ecological implications of ozone depletion on the Antarctic environment. Antarct. J. US. 175-76.

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., 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., Sawall, T.& Wiencke, C. 1998c. A survey of the distribution of UV-absorbing substances in tropical macroalgae. Phycol. Res. 46:271-79.

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.

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.

Kirk, J.T.O. 1994. Light and photosynthesis in aquatic ecosystems. 2nd ed. Cambridge University Press, Cambridge, 509 pp.

Klöser, H., Mercuri, G., Laturnus, F., Quartino, M.L. & Wiencke, C. 1994. On the competitive balance of macroalgae at Potter Cove (King George Island, South Shetlands). Polar Biol. 14:11-6.

Pakker, H., Martins, R.S.T., Boelen, P., Buma, A.G.J., Nikaido, O. & Breeman, A.M. 2000. Effects of temperature on the photoreactivation of ultraviolet-B-induced DNA damage in Palmaria palmata (Rhodophyta). J. Phycol. 36:334-41.

Poll, van de W.H., Eggert, A., Buma, A.G.J. & Breeman, A.M. 2002. Temperature dependence Of UV radiation effects in Arctic and temperate isolates of three red macrophytes. Eur. J. Phycol. 37:59-68.

Ross, J.C. & Vincent, W.F. 1998. Temperature depedence of UV radiation effects on Antarctic Cyanobacteria. J. Phycol. 34:118-25.

Schodlok, M., Hellmer, H. & Beckmann, A. 2002. On the transport, variability and origin of dense water masses crossing the South Scotia Ridge. Deep-Sea Research II. 49:4807-4825

Shick, J. M., 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 population of symbiotic dinoflagellates. Limnol. Oceanogr. 44(7): 1667-82.

Starr, R.C. & Zeikus, J.A. 1987. UTEX-The culture collection of algae at the University of Texas at Austin. Suppl. J. Phycol. 23:1-47.

Wiencke, C. & tom Dieck, I. 1990. Temperature requirements for growth and survival of macroalgae from Antarctica and southern Chile. Marine Ecology Progress Series 59:157-70.

Wiencke, C. & tom Dieck, I. 1989. Temperature requirements for growth and termperature tolerance of macroalgae endemic to the Antarctic region. Mar. Ecol. Prog. Ser. 54:189-97.

Wiencke, C., Rahmel, J., Karsten, U., Weykam, G. & Kirst, G.O. 1993 Photosynthesis of marine macroalgae from Antarctica: Light and temperature requirements. Bot. Acta. 106:78-87.

Wiencke, C., Bartsch, I., Bischoff, B., Peters, A.F. & Breeman, A.M. 1994. Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. Botanica Ma. 37:247-59.

Wiencke, C., Gómez, I., Pakker, H., Flores-Moya, A., Altamirano, M., Hanelt, D, Bischof, K. & Figueroa, F. 2000. Impact of UV radiation on viability, phototsynthetic characteristics and DNA of brown algal zoospores: Implications for depth zonation. Mar. Ecol. Prog. Ser. 197: 217-29.

Publication 5

Karsten, U., Dummermuth, A., Hoyer, K., Wiencke, C.

Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters

Polar Biology (in press)

Copyright: Springer Verlag, reprinted with kind permission

Interactive effects of ultraviolet radiation and salinity on the ecophysiology of two Arctic red algae from shallow waters

Ulf Karsten[1], Angelika Dummermuth[2], Kirsten Hoyer[2] and Christian Wiencke[2]

[1]University of Rostock, Institute of Aquatic Ecology, Albert-Einstein-Strasse 3, D-18059 Rostock, Germany
[2]Alfred-Wegener-Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Author for correspondence:
Prof. Dr. Ulf Karsten
University of Rostock, Institute of Aquatic Ecology
Albert-Einstein-Strasse 3
D-18059 Rostock, Germany
Fax: ++49-381-4986072; Email: ulf.karsten@biologie.uni-rostock.de

Abstract

In a comparative ecophysiological study the abundant red macroalgae *Devaleraea ramentacea* (L.) Guiry and *Palmaria palmata* (L.) O. Kuntze from shallow waters of the Arctic Kongsfjord (Spitsbergen) were exposed to hyposaline and hypersaline media in combination with and without UV-radition to evaluate the interactive effects of both environmental parameters on optimum quantum yield of photosynthesis, as well as on the physiological capability to synthesise and accumulate photoprotective mycosporine-like amino acids (MAAs). While *D. ramentacea* exhibited euryhaline features and acclimated well to the UV radiation applied, *P. palmata* can be characterised as stenohaline plant because of its high mortality already under mild hyposaline conditions (15 PSU). In addition, the latter species showed a limited ability to acclimate to changing PAR/UV radiation pointing to a relatively low physiological plasticity. Both species synthesised and accumulated MAAs after UV treatment. However, only in *D. ramentacea* a correlation between increasing MAA concentration and decreasing photosynthetic sensitivity under UV was observed. All ecophysiological data well correlate with field observations where both red algal species co-exist in the same shallow water habitat of the Kongsfjord. However, while *P. palmata* becomes more often greenish, sometimes slightly bleached over the summer months, *D. ramentacea* appears much more healthy and hence unstressed under the prevailing environmental factors.

Key words

Arctic, *Devaleraea ramentacea, Palmaria palmata*, MAAs, mycosporine-like amino acids, photoinhibition, photosynthesis

Introduction

The Arctic Kongsfjord on Spitsbergen is a marine coastal ecosystem which has intensively been studied over the last years as model for global change (Hanelt *et al.*, 2001 and references therein). A typical feature of the fjord is a well structured phytobenthic community down to depth of almost 40 m (Hop *et al.*, 2002) that plays an important role in primary production being a food source for herbivores and detrivores, as well as nursery area and habitat for fish and invertebrates (Lippert *et al.*, 2001). Marine macroalgae of such high latitudes are exposed to seasonally fluctuating environmental factors such as solar radiation and temperature, as well as to long periods of ice cover (Hanelt *et al.*, 2001; Hop *et al.*, 2002).

Compared to the „ozone hole" over Antarctica which is known since the 70ies (Smith *et al.*, 1992), the increase in ozone depletion over the Arctic represents a more recent phenomenon (Wängberg *et al.*, 1996; Rex *et al.*, 2000; Hanelt *et al.*, 2001). As a consequence of ozone springtime reduction in the polar regions UV-radiation particularly of the UVB-waveband (280-320 nm) markedly rises. Although the biological consequences of changes towards higher doses of UV-radiation in marine ecosystems are not fully understood, many phototrophic organisms living in the intertidal as well as in the upper subtidal zone of the coasts are strongly affected (Franklin & Forster, 1997).

The macroalgal species *Devaleraea ramentacea* (L.) Guiry and *Palmaria palmata* (L.) O. Kuntze are the most abundant Rhodophyta in the upper sublittoral of the Kongsfjord. While the first species represents one of the few endemics of the Arctic region, the latter one occurs from temperate to cold waters of the Atlantic ocean, and exhibits on Spitsbergen its northern distribution limit. In spring/summer both organisms are often exposed to high solar radiation, and hence their photophysiology and protecting strategies avoiding or counteracting UV-induced damage has been studied in great detail (Hanelt *et al.*, 1997; Aguilera *et al.*, 1999, 2002; Karsten & Wiencke, 1999; Karsten *et al.*, 1999, 2001). From these studies it could be concluded that *D. ramentacea* and *P. palmata* are capable to physiologically acclimate to diurnally changing solar radiation due to dynamic photoinhibition, i.e. the up- and down-regulation of photosynthesis in response to the respective prevailing low and high visible light, as well as UV conditions (Hanelt, 1998). In addition, to prevent UV-photodamage these macroalgal species are biochemically capable to synthesise and accumulate UV-absorbing substances, the so-called mycosporine-like amino acids

(MAAs) (Dunlap & Shick, 1998; Karsten & Wiencke, 1999; Karsten *et al.*, 1999). As passive sunscreens MAAs preferentially absorb UV photons in the spectral range of 310-360 nm followed by dissipating the absorbed radiation energy in form of harmless heat and fluorescence without generating photochemical reactions (Bandaranayake, 1998; Cockell & Knowland, 1999), and thereby protecting, at least partially, photosynthesis and growth of phototrophic organisms (Garcia-Pichel *et al.*, 1993; Neale *et al.*, 1998).

The motivation for the present study was the field observation that in shallow waters of the Kongsfjord during the summer season thalli of *P. palmata* often looked rather greenish, sometimes slightly bleached compared to the mainly, although not always red-coloured *D. ramentacea* from similar locations. Although intuitively radiation stress seemed to be the responsible factor, earlier results indicated a relative high photosynthetic tolerance of *P. palmata* under increasing natural PAR and UV-doses (Hanelt *et al.*, 1997; Karsten *et al.*, 2001). Since the large discharge of melting water into the fjord can locally and temporary decrease the seawater salinity down to 23 PSU (Hanelt *et al.*, 2001) and because of the fact that subtidal red algae are generally stenohaline (Kain & Norton, 1990), we assumed that this abiotic factor may act as additional stressor on the macroalgal physiology. Therefore, in a comparative study we exposed *D. ramentacea* and *P. palmata* under controlled conditions on Spitsbergen to hyposaline and hypersaline media in combination with and without UV-radition to evaluate the interactive effects of both environmental parameters on photosynthetic performance, as well as on the ability to synthesise and accumulate MAAs.

Materials and methods

Algal material and study site

The red macroalgae *Devaleraea ramentacea* (L.) Guiry and *Palmaria palmata* (L.) O.Kuntze preferentially grow in shallow waters at the study site in the Kongsfjord (Ny-Ålesund, Spitsbergen, 78°55.5´ N; 11°56.0´ E). Both species are typically attached to coarse gravel and single rocks on sandy sediments in the fjord or occur as epiphytes on rhizoids of kelps such as *Laminaria saccharina* (L.) Lamour.. In the Kongsfjord *D. ramentacea* typically grows in depths from 1 m down to 8 m, while *P. palmata* is found slightly deeper from 2 m to 10 m. All algal samples were collected from healthy

looking, dark red plants at 3-5 m by SCUBA diving and kept in black bags to avoid exposure to higher solar irradiances prior laboratory experiments.

Radiation and salinity experiments

Thalli of both species were cut at 3-4 cm from the apical part using a razor blade to get almost homogeneous pieces of the same age class for the exposure experiments. All plantlets were kept 24-36 h in running seawater at 3-5°C and dim light conditions (< 5 μmol PAR $m^{-2}s^{-1}$) to minimize potential wound healing responses. Afterwards algae were treated with hypo- and hypersaline media in combination with PAR and PAR+UV exposure over a period of 4 days. Hypersaline media of 50 PSU were prepared by freezing-out fresh water from fully marine fjord water. The dilution of fjord water with MilliQ water resulted in a hyposaline solution of 15 PSU. Salinity was checked using a refractometer. All salinity treatments were carried out in 300 ml glass containers. These vessels were irradiated from the top with 30 μmol PAR $m^{-2}s^{-1}$, 6.7 W m^{-2} UV-A (320-400 nm) and 0.25 W m^{-2} UV-B (280-320 nm). As radiation source a combination of Philips daylight fluorescence tubes and Q-Panel UV-A-340 fluorescence tubes (Q-Panel Company, Cleveland, Ohio, USA) was used. Radiation measurements were carried out with a Li-Cor LI-190-SB cosine corrected sensor connected to a Li-Cor LI-1000 datalogger (Lambda Instruments, Lincoln, Neb., USA) for PAR, and with a RM-21 broad-band UV radiometer (Dr. Gröbel, Ettlingen, Germany). While half of the containers (15, 34 and 50 PSU) were exposed to the full radiation spectrum, the other half was kept under a specific filter foil to cut-off UV-A+B (PAR treatment) (400 nm cut-off; Folex PR, Folex, Dreieich, Germany). All thalli were exposed to 24 h PAR per day, while supplemented UV-radiation was applied for only 10 h per day resulting in a 10 h UV treatment interval followed by a 14 h recovery period. Temperature was kept constant at approximately 5°C. As physiological fitness parameter photosynthetic performance was measured always 8 h after on-set, as well as 7 h after off-set UV-radiation. After 1, 2 and 4 days treatment with the different salinity and radiation combinations samples for MAA analysis were taken.

Photosynthesis

After sampling algal thalli were kept for 5-10 minutes inside a light-tight box. Afterwards photosynthetic activity was determined in this container by measuring

variable chlorophyll-fluorescence of photosystem II using a portable pulse amplitude modulated fluorometer (Diving-PAM, Walz, Effeltrich, Germany). The main application of the Diving-PAM is the determination of effective PS II quantum yield by the saturation pulse method ($\Delta F/F_m'$ = effective quantum yield of an irradiated sample, $\Delta F = F_m' - F_t$, Genty *et al.*, 1989). However, if determined in the dark, as undertaken in the present study, the effective quantum yield equals the optimum quantum yield which was calculated as the ratio of variable to maximum fluorescence F_v/F_m. Minimal fluorescence (Fo) was measured with a pulsed measuring beam (approximately 0.3 μmol photons m^{-2} s^{-1}, 650 nm), followed by short pulses of saturating white light (0.4-0.8 s, 1000-5000 μmol photons m^{-2} s^{-1}) to record Fm ($F_v = F_m - F_o$) (Hanelt, 1998). F_v/F_m values of both red algal species acclimated for 24-36 h to the dim light conditions in the laboratory were characteristic for photosynthetically non-inhibited plants and consequently set to 100% (=control). While *D. ramentacea* exhibited a maximum F_v/F_m value of 0.65±0.02 (n=6), *P. palmata* showed F_v/F_m value of 0.59±0.03 (n=9). All data recorded are expressed in relation to the respective value.

MAA extraction and analysis

After sampling, plants were oven-dried at 50°C, and then stored in sealed plastic bags under cool, dry and dark conditions until analysis. Samples (4-5 replicates) of about 10-20 mg dry weight (DW) were extracted for 1.5-2 h in screw-capped centrifuge vials filled with 1 mL 25% aqueous methanol (v/v) and incubated in a waterbath at 45°C. After centrifugation at 5000 g for 5 min, 700 μL of the supernatants were evaporated to dryness under vacuum (Speed Vac Concentrator SVC 100H). Dried extracts were re-dissolved in 700 μL 100% methanol and vortexed for 30 s. After passing through a 0.2 μm membrane filter, samples were analysed with a Waters HPLC system according to the method of Karsten *et al.* (1998a), modified as follows. MAAs were separated on a stainless-steel Phenomenex Sphereclone RP-8 column (5 μm, 250 x 4 mm I.D.) protected with a RP-8 guard cartridge (20 x 4 mm I.D.). The mobile phase was 5% aqueous methanol (v/v) plus 0.1% acetic acid (v/v) in water, run isocratically at a flow rate of 0.7 ml min^{-1}. MAAs were detected online with a Waters photodiode array detector at 330 nm, and absorption spectra (290-400 nm) were recorded each second directly on the HPLC-separated peaks. Identification was done by spectra, retention time and by co-chromatography with standards extracted from the marine red

macroalgae *Chondrus crispus* Stackhouse (Karsten *et al.*, 1998) and *Porphyra umbilicalis* (L.) Kützing, as well as from ocular lenses of the coral trout *Plectropomus leopardus* (Lacepède, 1802), kindly sent by Dr. David Bellwood, James Cook University, Townsville, Australia. Quantification was made using the molar extinction coefficients given in Karsten *et al.* (1998b).

Statistics

Mean values and standard deviation per treatment were calculated. Statistical significance of differences in photoinhibitory response in plants kept under different salinities and radiation scenarios was tested by one-way analysis of variance (ANOVA) followed by a multi-range test using Fisher's protected least significant difference (LSD) according to Sokal & Rohlf (1981). Calculations were done using the program InStat (GraphPad, San Diego, USA).

Results

During the course of the experiment the optimum quantum yield (F_v/F_m) of the control thalli of *Devaleraea ramentacea* (34 PSU, PAR) remained always high exhibiting values between 86 and 98% of the maximum, i.e. of non-inhibited plants (Fig. 1). Algae treated with 15 PSU showed a slight, but significant decrease in F_v/F_m ($p<0.01$) down to 76% of the control over the first 39 h, followed by some recovery resulting in 87% of non-treated samples at the end of the experiment. In contrast, in plants kept at 50 PSU F_v/F_m much stronger and continuously declined to 58% of the optimum after 39 h ($p>0.01$) (Fig. 1). Afterwards optimum quantum yield gradually increased up to 76% of the control.

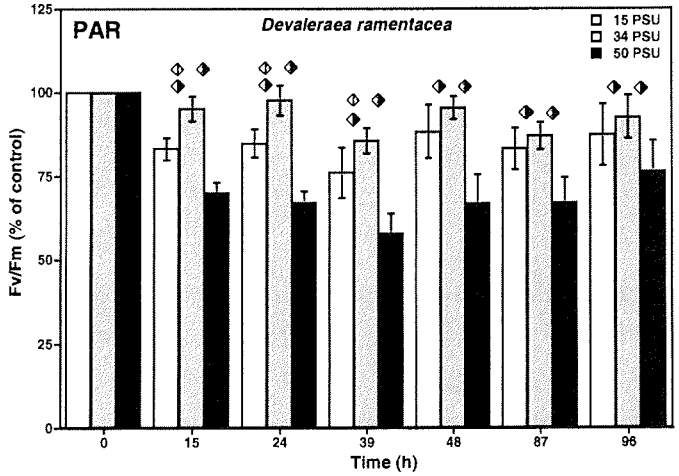


Figure 1: Changes in photosynthetic optimum quantum yield (F_v/F_m) of *Devaleraea ramentacea* under various salinity conditions (15, 34, 50 PSU) and visible light (PAR) over the course of 96 h. F_v/F_m of non-inhibited plants was determined as 0.65±0.02 and standardized to 100%. Given are the mean values±SD (n=10). Significant differences ($P < 0.01$) among samples under various salinity treatments are marked with squares: 15 PSU versus 34 PSU (white–pointed triangle), 15 PSU versus 50 PSU (white-black triangle), 34 PSU versus 50 PSU (pointed-black triangle).

F_v/F_m of *D. ramentacea* treated with salinity plus UV radiation was generally much more affected compared to the salinity only experiment (Fig. 2). Thalli kept at 15 and 34 PSU showed at the end of the first two UV exposure intervals a decline in optimum quantum yield down to 50-60% of the control. However, during each recovery period F_v/F_m increased to 75-80% of the maximum. In contrast, under hypersaline conditions photosynthesis was stronger inhibited under UV (45-50% of control; $p<0.01$) and did not show marked recovery under PAR conditions within 48 h ($p<0.01$). However, after the last interval of the UV treatment at 50 PSU F_v/F_m in *D. ramentacea* was much less affected resulting in 67% of the maximum. The final measurement of recovery at the end of the experiment clearly indicated for all salinities identical optimum quantum yields >81% of the control (Fig. 2).

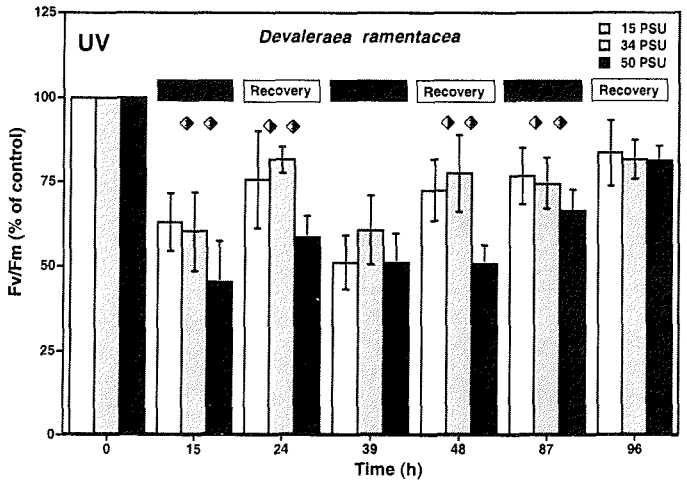


Figure 2: Changes in photosynthetic optimum quantum yield (F_v/F_m) of *Devaleraea ramentacea* under various salinity conditions (15, 34, 50 PSU) and ultraviolet radiation (UV) over the course of 96 h. F_v/F_m of non-inhibited plants was determined as 0.65±0.02 and standardized to 100%. Given are the mean values±SD (n=10). Significant differences ($P < 0.01$) among samples under various salinity treatments are marked with squares: 15 PSU versus 34 PSU (white–pointed triangle), 15 PSU versus 50 PSU (white-black triangle), 34 PSU versus 50 PSU (pointed-black triangle). The black bars indicate the 10 h UV treatment interval followed by the 14 h recovery period.

The proportional degree of photoinhibition (F_v/F_m) in *D. ramentacea* due to salinity and UV treatment is shown in Fig. 3. Under hyposaline conditions over the first 48 h 15 PSU and UV led to nearly equal photoinhibitory responses (Fig. 3A). However, after 87 and 96 h exposure, the UV effect strongly decreased resulting in only 23-28% of the total decline in optimum quantum yield. In contrast, under hypersaline conditions the UV effect on F_v/F_m in *D. ramentacea* was generally much less pronounced and continuously decreased over the course of the experiment (Fig. 3B). After 87 and 96 h treatment only the salinity factor was responsible for photoinhibition.

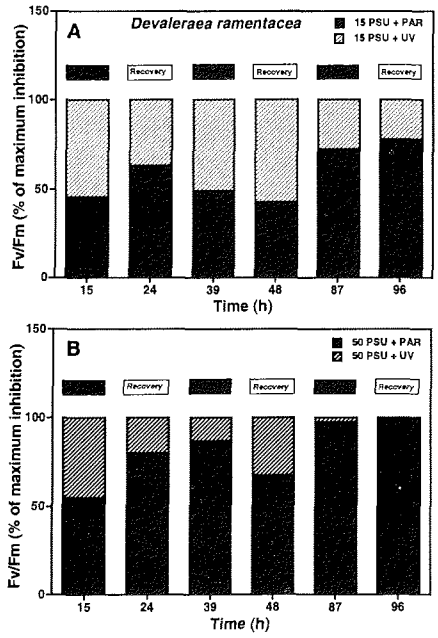


Figure 3: The effect of salinity and UV treatment on the maximum decrease in the photosynthetic optimum quantum yield (F_v/F_m) of *Devaleraea ramentacea* over the course of 96 h. From the mean value data presented in figures 1 and 2 the proportional degree of photoinhibition due to both stress factors was calculated and expressed as percentage of maximum photoinhibition. A: 15 PSU ± UV treatment; B: 50 PSU ± UV treatment.

Seven different mycosporine-like amino acids (MAAs) were detected in *D. ramentacea*, namely mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol and palythene (data not shown). Plants at the beginning of the experiment contained total MAAs of 1.2 mg g^{-1} dry weight (DW) (Fig. 4). After 1 day treatment with salinity and UV both 15 PSU samples showed a decrease in total MAAs (0.8 mg g^{-1} DW) and both 50 PSU samples an increase in total MAAs (1.5-1.7 mg g^{-1} DW). MAAs in plants kept at 34 PSU with and without UV were unaffected. While after 2 days exposure thalli at all 15 PSU and 34 PSU conditions showed unchanged total MAA concentrations, algae at both 50 PSU conditions exhibited a decrease in total MAAs (1.0-1.2 mg g^{-1} DW). A strong UV-induced increase in total MAAs was observed at the end of the experiment. Under all salinities UV-exposure led to almost doubling of the MAA contents. However, while at 15 PSU and 50 PSU 1.5 mg and 1.7 mg MAAs g^{-1} DW, respectively, were measured in *D. ramentacea*, at 34 PSU the highest total MAA concentration of 2.2 mg g^{-1} DW was determined (Fig. 4).

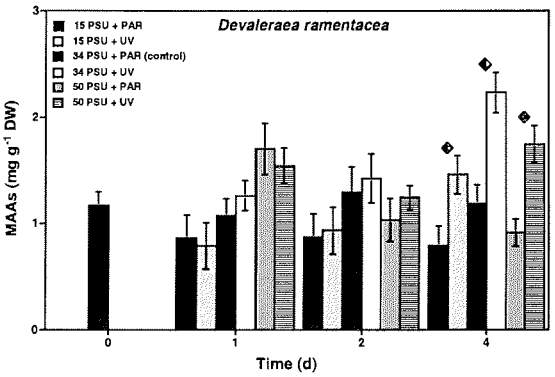


Figure 4: The interactive effects of salinity and UV treatment on the total intracellular mycosporine-like amino acid contents (MAAs) in *Devaleraea ramentacea* over the course of 96 h. Given are the mean values ± SD (n=4-5).

While in *D. ramentacea* F_v/F_m of the control conditions (34 PSU, PAR) remained unchanged over the course of the experiment, in *P. palmata* a small, but continuous decline of this parameter was observed resulting in 75-80% of the maximum (Fig. 5). Compared to *D. ramentacea*, the optimum quantum yield of *P. palmata* was much stronger affected by salinity, particularly at 50 PSU over the first 48 h ($p<0.01$) (Fig. 5). After that period F_v/F_m in algae kept at 15 PSU also strongly declined resulting in fully bleached and hence dead thalli at the end of the experiment. While after 96 h the 34 PSU samples exhibited 78%, the 50 PSU plants showed only 48% of the optimum photosynthesis (Fig. 5).

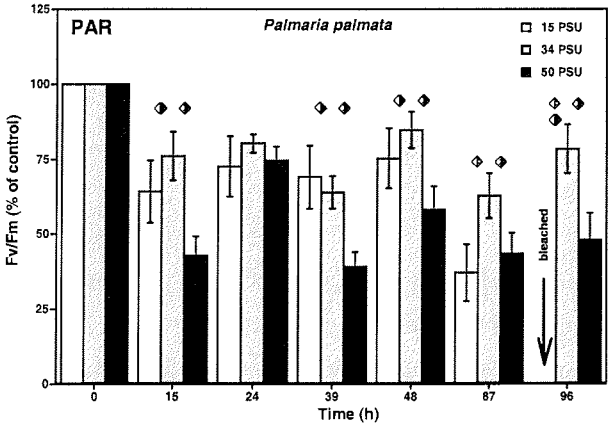


Figure 5: Changes in photosynthetic optimum quantum yield (F_v/F_m) of *Palmaria palmata* under various salinity conditions (15, 34, 50 PSU) and visible light (PAR) over the course of 96 h. F_v/F_m of non-inhibited plants was determined as 0.59±0.03 and standardized to 100%. Given are the mean values±SD (n=10). Significant differences ($P < 0.01$) among samples under various salinity treatments are marked with squares: 15 PSU versus 34 PSU (white-pointed triangle), 15 PSU versus 50 PSU (white-black triangle), 34 PSU versus 50 PSU (pointed-black triangle). Arrow indicates completely bleached (dead) thalli at 15 PSU.

Under the salinity plus UV treatments, F_v/F_m in *P. palmata* decreased even more indicating strong interactive effects of both abiotic factors (Fig. 6). While the 34 PSU samples showed during on-set of UV radiation always declining optimum quantum yields (41-54% of the control), after off-set the UV source marked recovery occured (67-84% of the control). Algae incubated at 15 PSU plus UV also died after 87 h treatment as indicated by completely bleached tissue (Fig. 6). Thalli of *P. palmata* at 50 PSU plus UV exhibited over the course of the experiment a similar photosynthetic response compared with plants at 50 PSU without UV.

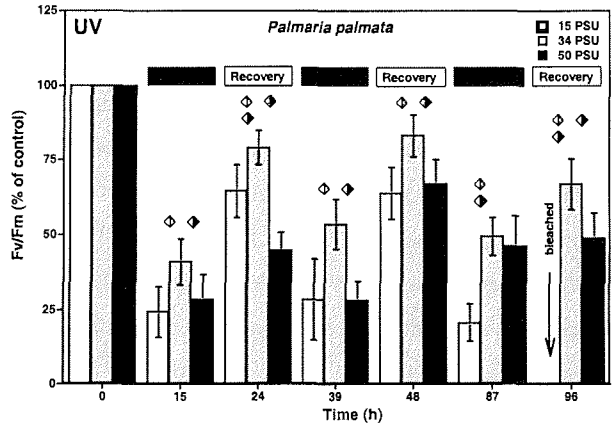


Figure 6: Changes in photosynthetic optimum quantum yield (F_v/F_m) of *Palmaria palmata* under various salinity conditions (15, 34, 50 PSU) and ultraviolet radiation (UV) over the course of 96 h. F_v/F_m of non-inhibited plants was determined as 0.59±0.03 and standardized to 100%. Given are the mean values±SD (n=10). Significant differences ($P < 0.01$) among samples under various salinity treatments are marked with squares: 15 PSU versus 34 PSU (white–pointed triangle), 15 PSU versus 50 PSU (white-black triangle), 34 PSU versus 50 PSU (pointed-black triangle). Arrow indicates completely bleached (dead) thalli at 15 PSU. The black bars indicate the 10 h UV treatment interval followed by the 14 h recovery period.

As in *D. ramentacea*, the proportional degree of photoinhibition (F_v/F_m) in *P. palmata* due to both abiotic factors was in average mainly because of salinity treatment (Fig. 7). Although at the beginning of the experiments UV radiation also led to some decrease in optimum quantum yield, this effect got weaker after 48 h.

In *P. palmata* six different MAAs were detected, namely shinorine, porphyra-334, palythine, asterina-330, palythinol and palythene (data not shown). Plants at 34 PSU plus UV showed already after 24 h a small, but significant increase in total MAA concentration ($p<0.01$) (Fig. 8). These samples continuously accumulated MAAs 2-fold over the course of the experiment. Although under hyposaline conditions total MAAs decreased at the beginning, after 48 h a significant UV-induced formation could be observed ($p<0.01$) followed by bleaching of the tissue. Under hypersaline treatment thalli of *P. palmata* exhibited after 48 h a strong accumulation of MAAs due to UV ($p<0.01$) and after 96 h a decline from 2.4 to 1.9 mg MAAs g^{-1} DW (Fig. 8).

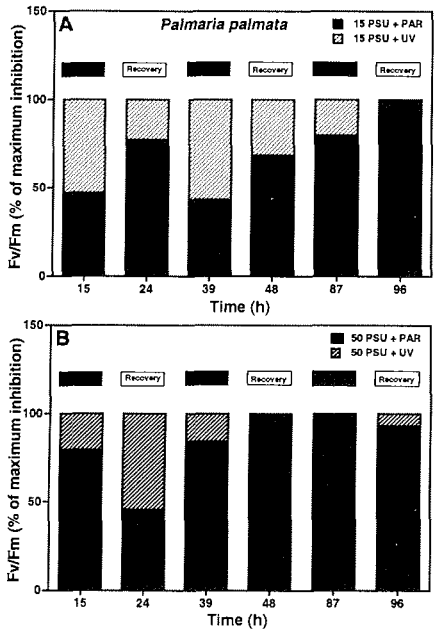


Figure 7: The effect of salinity and UV treatment on the maximum decrease in the photosynthetic optimum quantum yield (F_v/F_m) of *Palmaria palmata* over the course of 96 h. From the mean value data presented in figures 1 and 2 the proportional degree of photoinhibition due to both stress factors was calculated and expressed as percentage of maximum photoinhibition. A: 15 PSU ± UV treatment; B: 50 PSU ± UV treatment.

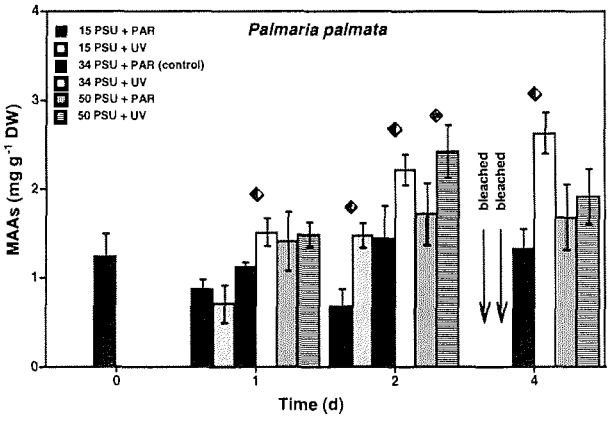


Figure 8: The interactive effects of salinity and UV treatment on the total intracellular mycosporine-like amino acid contents (MAAs) in *Palmaria palmata* over the course of 96 h. Given are the mean values ± SD (n=4-5). Arrows indicate completely bleached (dead) thalli at 15 PSU.

Discussion

On Spitsbergen solar radiation as primary environmental factor for photosynthesis and productivity of macroalgae is not only seasonally fluctuating, but also diurnally extremely variable at the earth's surface due to rapidly changing weather conditions (Hanelt *et al.,* 2001). In addition, during summer the underwater light climate of the Kongsfjord is further affected by calving glaciers and strong melting water influx resulting in increasing turbidity due to suspended particles and hence in a strong decrease of the water column transmittance (Bischof *et al.,* 1998). The irradiance *Devaleraea ramentacea* and *Palmaria palmata* were exposed to in the laboratory was much lower compared to nature. While in Arctic summer typical insolation at the earth's surface may reach 1300 μmol m^{-2} s^{-1} PAR, 19 W m^{-2} UV-A and 1.1 W m^{-2} UV-B (Bischof *et al.,* 1998), we used only 30 μmol m^{-2} s^{-1} PAR, 6.7 W m^{-2} UV-A and 0.25 W m^{-2} UV-B to simulate realistic underwater values. In the water column maximum transmittance for PAR and UV-B as expressed by the 1% depth ranges from 6.2 to 24.2 m and 3.4 to 9 m, respectively (Bischof *et al.,* 1998). Consequently, both red algal species may experience in their habitat the irradiances applied.

Macroalgae living under such fluctuating conditions need a broad physiological plasticity to acclimate to the wide range of incident solar radiation to receive on one hand side always sufficient energy for photosynthesis and on the other hand to avoid

photodamage. Shallow water and intertidal macroalgae are known to undergo dynamic photoinhibition when exposed to excessive sun light that typically occurs at midday (Häder & Figueroa, 1997; Hanelt, 1998). Dynamic photoinhibition is considered as photoprotective mechanism, which dissipates excessively absorbed energy as physiologically harmless thermal radiation (Osmond, 1994). Previous studies have shown that for the evaluation of PAR- and UV-induced inhibition of photosynthesis in macroalgae the *in vivo* chlorophyll fluorescence of photosystem II, as used in the present investigation, is a suitable method (Häder & Figueroa, 1997; Hanelt *et al.*, 1997; Hanelt, 1998; Bischof *et al.*, 1998). The optimum quantum yield (F_v/F_m) was demonstrated to be a sensitive parameter to evaluate the physiological status of the photosynthetic apparatus (Cordi *et al.*, 1997), and hence represents a measure for fitness.

The experimental set-up was designed to test the photosynthetic performance of the shallow-water species *D. ramentacea* and *P. palmata* in response to UV radiation and salinity. While in the first species the strongest photoinhibitory effect was measured under hypersaline conditions without UV (25% inhibition after 4 days), at 15 PSU only a small decrease in optimum quantum yield was observed. In strong contrast, *P. palmata* did not survive hyposaline treatment over the course of the experiment, and showed also at 50 PSU 50% inhibition in F_v/F_m. Consequently, while *D. ramentacea* can be characterised as euryhaline species, *P. palmata* exhibits rather stenohaline features. From an ecological standpoint stenohalinity with respect to growth is typical for sublittoral red algae compared to the broad salinity tolerance of intertidal species (Kain & Norton, 1990). In agreement with these authors it has to be mentioned that *P. palmata* has the main distribution in temperate/cold-temperate waters of the Northern Atlantic where it sublittorally grows in depths down to 20 m or protected as typical understorey plant of kelp forests (Irvine, 1983; Lüning, 1990). These habitats are characterised by rather stable salinity conditions which support the development of stenohaline organisms. Consequently, the strong inhibition of photosynthesis and high mortality of the Arctic isolate of *P. palmata* at 15 PSU can be explained by a limited physiological capacity to acclimate to external salinity fluctuations. In addition, the occurrence of this species at the Northern distribution limit on Spitsbergen which is characterised by extremely low water temperatures may contribute to the reduced photosynthetic tolerance. The primary metabolism of temperate/cold-temperate organisms growing under Arctic conditions is most probably slowed down according

the Q10-rule and therefore it is reasonable to assume that acclimation responses are affected as well. This hypothesis is supported by the fact that temperature optima for photosynthesis and growth are only significantly lower in endemic Antarctic macroalgae compared to Arctic and cold-temperate species that typically exhibit strong decline of both processes with decreasing temperatures (Healey, 1972; Wiencke *et al.*, 1993, 1994; Kirst & Wiencke, 1995). Consequently, while many Antarctic seaweeds seem to be relatively strongly adapted, Arctic and cold-temperate counterparts show a much weaker adaptation to low temperatures, and hence the general fitness may be species-specifically more or less affected. Although *P. palmata* is abundant in the Kongsfjord, the data presented in combination with the observation of rather greenish, sometimes slightly bleached thalli during summer in the field indicate stressed plants. However, interactive effects of salinity and temperature must still be experimentally evaluated.

Although salinities in the upper layers of the water column does generally not decline to values lower than 23 PSU (Hanelt *et al.*, 2001), it should be mentioned that 15 PSU as tested in the present study represents a mild hyposaline stress for marine organisms. In contrast to *P. palmata* many other red macroalgae from intertidal as well as sublittoral habitats well or even preferentially grow and photosynthesise at this salinity (Bird *et al.*, 1979; Kirst, 1990; Mostaert *et al.*, 1995).

When UV-radiation was applied on top of the salinity treatment both species studied exhibited at the beginning similar photosynthetic responses, i.e. a decline of the optimum quantum yield after UV on-set followed by some degree of recovery after UV off-set. While UV-induced photoinhibition compared to the control was in *D. ramentacea* relatively small, *P. palmata* exhibited a much stronger response (Figs. 2, 6). In addition, the first species showed an increasing UV tolerance of photosynthesis over the course of the experiment, while the latter species seemed unable to photoacclimate. This confirms data of Hanelt (1998) who showed that photosynthesis of *P. palmata* collected along a depth profile in the Kongsfjord did not acclimate to the prevailing radiation gradient. Within other macroalgal species from the Arctic and Antarctica, the degree of photoinhibition is normally a function of the collecting depth, i.e. shallow-water isolates are more PAR/UV resistent than plants from deeper waters (Bischof *et al.*, 1998a, b). The difference in the acclimation potential of the photosynthetic performance between *D. ramentacea* and *P. palmata* under UV is reflected by the vertical distribution in the Kongsfjord, since the first species grows in shallower waters.

In addition, at more temperate locations *P. palmata* preferentially inhabits deeper waters than in the Arctic and hence the shallow water growth habit in the Kongsfjord appears unusual. Due to incomplete osmotic adjustment both algae may be more able to tolerate increase in UV radiation as compared to salinity change.

In recent studies, the photobiological function of MAAs as a cellular defense system against the harmful effects of UV-radiation on growth, photosynthesis and other processes has been reported for various marine phototrophic organisms (Garcia-Pichel *et al.,* 1993; Dunlap & Shick, 1998; Neale *et al.,* 1998). In a convincing bio-assay, Adams and Shick (1996) experimentally documented that UV-treated and subsequently fertilized sea urchin eggs typically show a UV-dose-dependent delay in the first cell division compared to unirradiated eggs from the same batch. The determination of the cleavage delay in eggs having different MAA contents, produced by feeding adults different macroalgal diets (with high and low MAA levels) in the absence of UV proved to be a perfect indicator for the sunscreen function of these compounds. The authors documented that the greater the MAA concentration in the eggs, the less they were affected by UV radiation. In the present study both *D. ramentacea* and *P. palmata* synthesised and accumulated MAAs over the course of the experiment in response to the UV treatment, except for those sample treated with 15 PSU. The highest MAA concentrations were usually measured at 34 PSU (Fig. 4, 8). The UV-induction data for MAAs are well supported by earlier experiments in the field, where both species were transplanted in the Kongsfjord from deeper waters to the surface, followed by exposure to natural full, as well as filtered solar radiation (Karsten & Wiencke, 1999; Karsten *et al.,* 1999). However, the most striking point is the fact that although both *D. ramentacea* and *P. palmata* form MAAs in a similar manner and concentration, this increase well correlates with the rising photosynthetic tolerance under UV in the first species only. In *P. palmata*, the optimum quantum yield under UV does not seem to benefit from higher MAA contents. These contradictory results on the potential sunscreen function of MAAs in both red algae clearly indicate species-specific physiological advantages are not solely due to the synthesis and accumulation of UV-absorbing compounds.

In conclusion, while *D. ramentacea* is able to resist different environmental stress factors in the upper sublittoral of the Arctic Kongsfjord indicating a relatively high degree of physiological plasticity, *P. palmata* exhibits a marked sensitivity against salinity and a limited capabilty to acclimate to changing PAR/UV radiation pointing to a rather inflexible metabolism.

Acknowledgements

The authors like to thank Heike Lippert, Eva Philipp and Stefan Kremb for providing field samples and the Ny-Ålesund International Research and Monitoring Facility for their support. This project was financially supported by the Deutsche Forschungsgemeinschaft (Ka 899/3-1/2) and the German Research Minister (BMBF) (project MONA-03F0229).

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.

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. Progr. Ser.*, **191**: 109-119.AGUILERA, J., DUMMERMUTH, A., KARSTEN, U., SCHRIEK, R. & WIENCKE, C. (2002) Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. *Pol. Biol.* (in press).

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

BIRD, N.L., CHEN, L.C.M. & MCLACHLAN, M. (1979) Effects of temperature, light and salinity on growth in culture of *Chondrus crispus*, *Furcellaria lumbricalis*, *Gracilaria tikvahiae* (Gigartinales, Rhodophyta), and *Fucus serratus* (Fucales, Phaeophyta). *Bot. Mar.*, **22**: 521-527.

BISCHOF, K., HANELT, D., KARSTEN, U., BROUWER, P., TÜG, H. & WIENCKE, C. (1998a) Acclimation of brown algal photosynthesis to penetration of light and ultraviolet radiation in Arctic coastal waters (Kongsfjord, Spitsbergen). *Pol. Biol.*, **20**: 388-395.

BISCHOF, K., HANELT, D. & WIENCKE, C. (1998b) UV-radiation can affect depth-zonation of Antarctic macroalgae. *Mar. Biol.*, **131**: 597-605.

COCKELL, C.S. & KNOWLAND, J. (1999) Ultraviolet radiation screening compounds. *Biol.Rev.*, **74**: 311-345.

CORDI, B., DEPLEDGE, M.H., PRICE, D.N., SALTER, L.F. & DONKIN, M.E. (1997) Evaluation of chlorophyll fluorescence, in vivo spectrophotometric pigment absorption and ion leakage as biomarkers of UV-B exposure in marine macroalgae. *Mar. Biol.*, **130**: 41-49.

DUNLAP, W.C. & SHICK, M.J. (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. & FORSTER, R.M. (1997) The changing irradiance environment: consequences for marine macrophyte physiology, productivity and ecology. *Eur. J. Phycol.*, **32**: 207-232.

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**: 170-176.

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. & FIGUEROA, F.L. (1997) Photophysiology of marine macroalgae. P*hotochem. Photobiol.*, **66**: 1-14.

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

HANELT, D., WIENCKE, C. & NULTSCH, W. (1997) Influence of UV radiation on the photosynthesis of Arctic macroalgae in the field. *J. Photochem. Photobiol. B: Biology*, **30**: 179-187.

HANELT, D., TÜG, H., BISCHOF, K., GROß, C., LIPPERT, H., SAWALL, T. & WIENCKE, C. (2001) Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. *Mar. Biol.*, **138**: 649-658.

HEALEY, F.P. (1972) Photosynthesis and respiration of some Arctic seaweeds. *Phycologia*, **11**: 267-271.

HOP, H., PEARSON, T., HEGSETH, E.N., KOVACS, K.M., WIENCKE, C., KWASNIEWSKI, S., EIANE, K., MEHLUM, F., GULLIKSEN, B., WLODARSKA-KOWALCZUK, M., LYDERSEN, C., WESLAWSKI, J.M., COCHRANE, S., GABRIELSEN, G.W., LEAKEY, R., LÖNNE, O.J., ZAJACZKOWSKI, M., FALK-PETERSEN, S., KENDALL, M., WÄNGBERG, S.A., BISCHOF, K., VORONKOV, A.Y., KOVALTCHOUK, N.A., WIKTOR, J., POLTERMANN, M., DI PRISCO, G. PAPUCCI, C. & GERLAND, S. (2002) The marine ecosystem of Kongsfjorden, Svalbard. *Pol. Res.* ,21: 167-208

IRVINE, L.M. (1983) Seaweeds of the British Isles, Vol. 1 Rhodophyta. British Museum, London.

KAIN, J.M. & NORTON, T.A. (1990) Marine ecology. In *Biology of the Red Algae* (Cole, K.M. & Sheath, R.G., eds), pp. 377-422. Cambridge University Press, Cambridge.

KARSTEN, U., FRANKLIN, L.A., LÜNING, K. & WIENCKE, C. (1998a) Natural ultraviolet and photosynthetic active radiation induce formation of mycosporine-like amino

acids in the marine macroalga *Chondrus crispus* (Rhodophyta). *Planta*, **205**: 257-262.

KARSTEN, U., SAWALL, T. & WIENCKE, C. (1998b) A survey of the distribution of UV-absorbing substances in tropical macroalgae. *Phycol. Res.*, **46:** 271-279.

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 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., BISCHOF, K. & WIENCKE, C. (2001) Photosynthetic performance of Arctic macroalgae after transplantation from deep to shallow waters followed by exposure to natural solar radiation. *Oecologia*, **127**: 11-20.

KIRST, G.O. (1990) Salinity tolerance of eukaryotic marine algae. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **41**: 21-53.

KIRST, G.O. & WIENCKE, C. (1995) Ecophysiology of polar algae. *J. Phycol.*, **31**: 181-199.

LIPPERT, H., IKEN, K., RACHOR, E. & WIENCKE, C. (2001) Epifauna associated with macroalgae in the Kongsfjord. *Pol. Biol.*, **24**: 512-522.

LÜNING, K. (1990) *Seaweeds: Their Environment, Biogeography, and Ecophysiology*. Wiley, New York.

MOSTAERT, A.S., KARSTEN, U. & KING, R.J. (1995) Physiological responses of *Caloglossa leprieurii* (Ceramiales, Rhodophyta) to salinity stress. *Phycol. Res.*, **43**: 215-222.

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**: 928-938.

OSMOND, C.B. (1994) What is photoinhibition? Some insights from comparisons of shade and sun plants. In *Photoinhibition of Photosynthesis. From the Molecular Mechanisms to the Field* (Baker, N.R. & Bowyer, J.R., eds), pp. 1-24. BIOS Scientific Publications, Oxford.

REX, M., DETHLOFF, K., HANDORF, D., HERBER, A., LEHMANN, R., NEUBER, R., NOTHOLT, J.,RINKE, A., GATHEN, P. VON DER, WEISHEIMER, A., GERNANDT, H. (2000). Arctic and Antarctic ozone layer observations - chemical and dynamical aspects of variability and long-term changes in the polar stratosphere. *Pol. Res.*, **19**: 193-204.

SMITH, R.C., PREZELIN, B.B., BAKER, K.S., BIDIGARE, R.R., BOUCHER, N.P., COLEY, T., KARENTZ, D., MACINTYRE, S., MATLICK, H.A., MENZIES, D., ONDRUSEK, M., WAN,

Z. & WATERS, K.J. (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. *Science*, **255**: 952-959.

SOKAL, R.R. & ROHLF, F.J. (1995) *Biometry*. The principle and practice of statistics in biological research. Freeman WH, New York.

WÄNGBERG, S.A., SELMER, J.S., EKELUND, N.G.A. & GUSTAVSON, K. (1996) UV-B effects on nordic marine ecosystem. *Tema Nord* 1996, Nordic Council of Ministers, Copenhagen.

WIENCKE, C., RAHMEL, J., KARSTEN, U., WEYKAM, G. & KIRST, G.O. (1993) Photosynthesis of marine macroalgae from Antarctica: light and temperature requirements. *Bot.Acta*, **106**: 78-87.

WIENCKE, C., BARTSCH, I., BISCHOFF, B., PETERS, A.F. & BREEMAN, A.M. (1994) Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. *Bot. Mar.*, **37**: 247-259.

4 SUMMARY OF RESULTS

4.1 Environmental radiation factors

For ecological studies on the importance of MAAs as sunscreen compounds, it was essential to characterise the radiation climate in the environment of the investigated algae. Therefore, atmospheric and underwater radiation measurements were taken at both study sites, Potter Cove, Antarctica and Kongsfjord, Arctic. Generally, the surface irradiance at Potter Cove was higher than at Kongsfjord. Hence, for example , the average daily dose of UVB in air in austral summer (December/January 1997/98) was 10 and 34 % higher as those comparable with the summer months June and July 2000 at Kongsfjord (for details see Table 2, Publ.1; Table 2, Publ.2).

The radiation transmittance in the water column was measured at two different stations in the inner and outer fjord of Potter Cove (Antarctic) and Kongsfjord (Arctic). From measurements at different depths, the vertical attenuation coefficient of downward irradiance (K_d) for PAR and UVR (327 – 400 nm) and additionally, the corresponding depth for 1 % of remaining radiation were calculated (Kirk 1994). A high K_d indicates a strong absorption of the radiation in the water column. The K_d values from the inner fjords of both polar areas were much higher compared to the outer fjords due to the input of turbid melt waters and terrestrial input (see Table 2, Publ.1; Table 2, Publ.2). For Kongsfjord, underwater radiation spectra of different depths are presented in figure 7, in which the irradiance typically shows a strong absorption of the UV range but also of the higher PAR range (600 – 700 nm) in the water column.

Radiation measurements differed greatly because of their dependence on several variable atmospheric and water characteristics (see 1.2 and 1.3 of the Introduction). Another parameter affecting the UV flux is the ozone concentration, which was roughly 25 % lower over Antarctica than over the Arctic during austral summer 1997/98 compared with the summer months June and July 2000 of the northern hemisphere (Table 2, Publ. 2).

4.2 Individual MAAs

In all publications, the MAAs palythine, porphyra-334, and shinorine were found most frequently and at least one of them was also the primary MAA both in field and laboratory cultured red algae. Other identified MAAs were namely mycosporine-

glycine, asterina-330, palythinol and the trans-cis molecular configuration palythene/usujirene, but these compounds were present mostly in low concentrations. One exception was in *Rhodomela confervoides* (Hudson) P.C. Silva from the Arctic, which had an almost equimolar concentration of palythinol and porphyra-334 of about 1 mg g^{-1} DW. In both *Prasiola* species from the Antarctic and Arctic, an unknown UV-absorbing substance was detected with an absorption maximum at 324 nm, which was firstly described by HPLC characteristics in Publication 1. In general, the field algae exhibited higher MAA levels and more variation in MAA type compared with the cultured algae.

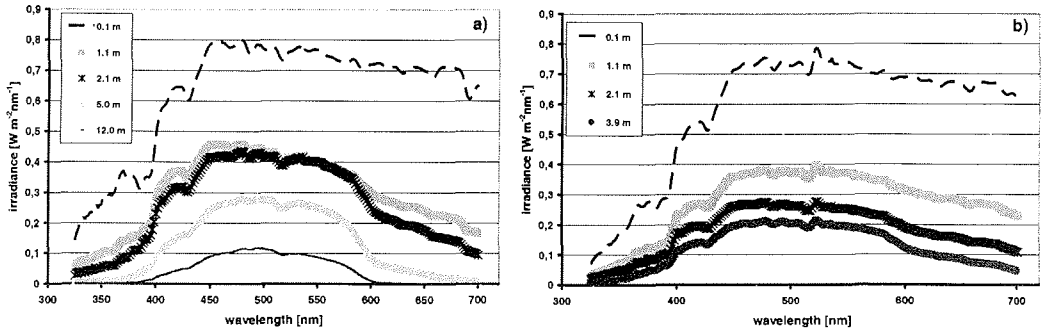


Figure 7: Underwater spectrum of solar irradiance measured at different depths. a) outer Kongsfjord at 22.06.01 midday. b) inner Kongsfjord at 21.06.01 midday.

4.3 MAA occurrence and induction

For a broad MAA survey of the Antarctic, 28 macroalgal species were analysed, 18 Rhodophyceae, five Phaeophyceae and three Chlorophyceae (Publ. 1). In addition, the MAA determination of 10 Antarctic red algae from cultures of the Alfred Wegener Institute, Bremerhaven, Germany, was performed to complete the number of investigated Antarctic species. In total, 28 red algae were analysed, of which 16 species contained MAAs. Traces of MAAs were found in one Antarctic field-collected Phaeophyceae, and in all three tested Chlorophyceae. The green alga, *Prasiola crispa* ssp. *antarctica*, contained a high concentration of the unkown substance-324. Algal species were also sampled at different depths. The MAA concentration decreased with increasing depth within most samples. Supra- and eulittoral algae, which were exposed

to mostly unattenuated solar radiation contained the highest MAA levels (< 9 mg g^{-1} DW) (Fig. 8).

Furthermore, variation in MAA concentrations was detected in different tissues of vegetative thalli of three red algal species (*Iridaea cordata*, *Palmaria decipiens*, *Curdiea racovitzae*). The margins of these leathery species contained 1.5 to 3.6 fold higher MAA concentrations as the basal tissue. No significant differences in MAA contents were found neither in tetrasporophytes and gametophytes of the red algae *Gigartina skottsbergii* and *Iridaea cordata*, nor after transplantation of the two species *Kallymenia antarctica* and *Gigartina skottsbergii* from deep (20 m) to shallow (0.3 – 10 m) water depths (Publ. 1). In figure 9, total MAA concentrations in several algae in relation to their depth distribution in the sublittoral are presented.

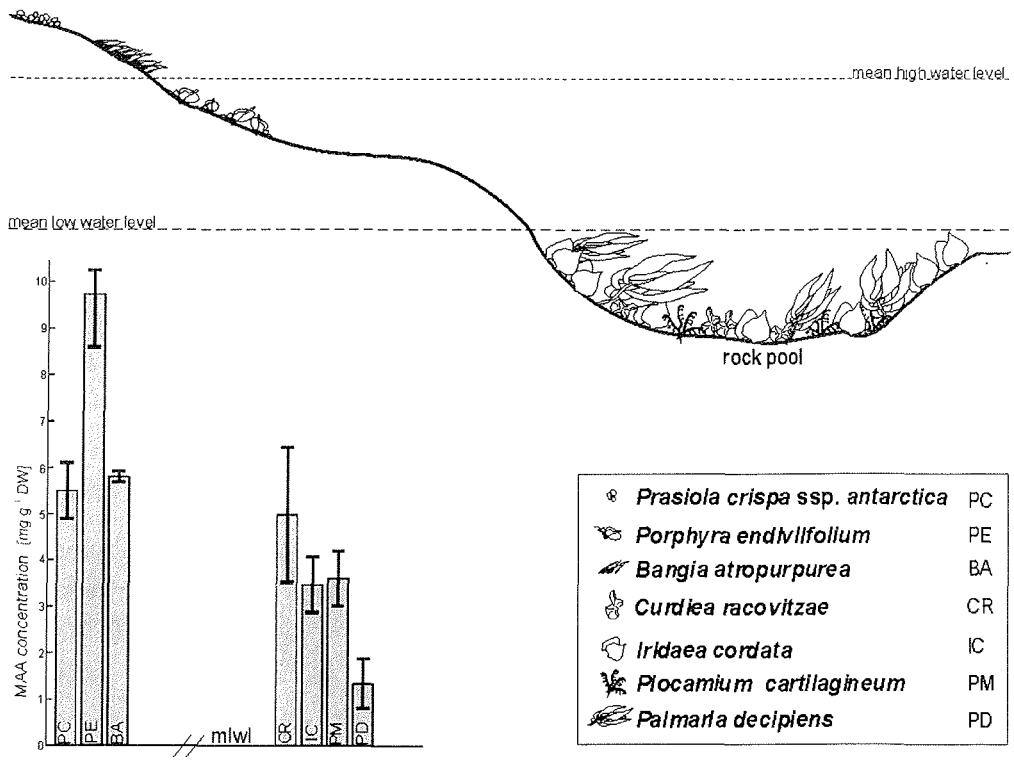


Figure 8: Supra- and eulittoral zone with a tidal rock pool from Potter Cove, Antarctica, and vertical distribution of six red and one green algal species investigated in this study, including their respective total MAA concentrations. Means ± SD (n=3). mlwl: mean low water level

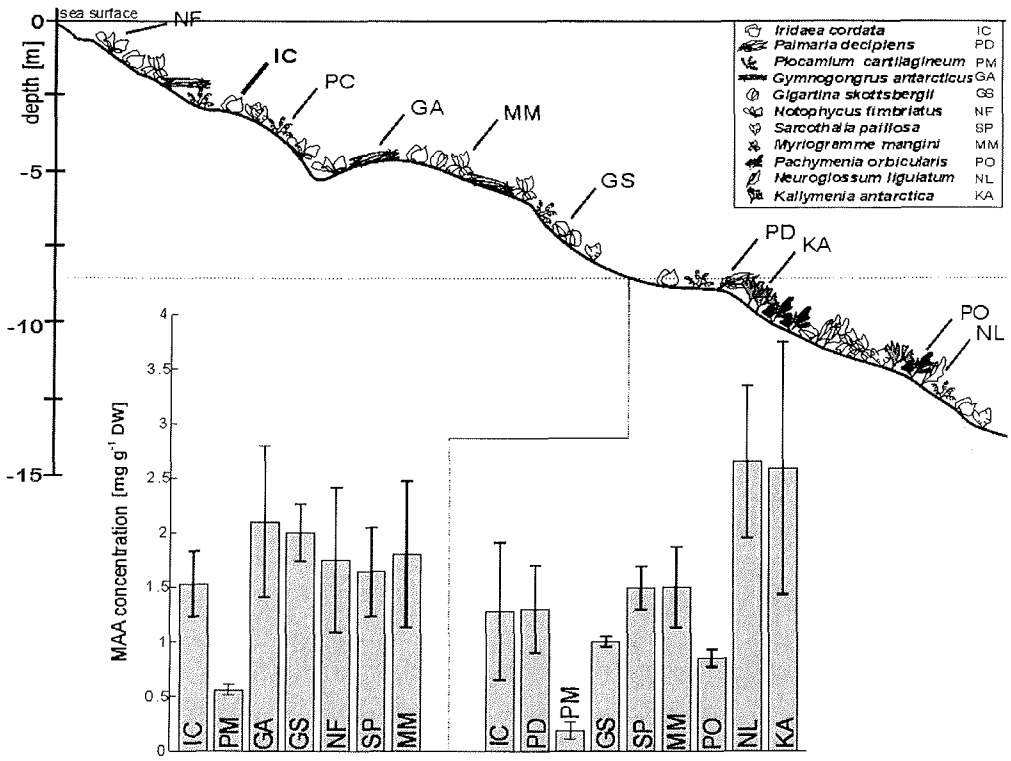


Figure 9: Total MAA concentration in several sublitoral Antarctic red macroalgae from Potter Cove related to their vertical distribution collected in the sublittoral of Potter Cove, Antarctica. Means ± SD (n=3).

In comparison with the Antarctic, fewer species were analysed from the Arctic, among them were nine red and one green algae (Publ. 2). The latter species contains the same unknown UV-absorbing substance-324 as *P. crispa* ssp. *antarctica* from Antarctica. Intertidal rocky platforms are absent in Kongsfjord, hence most species were collected in the sublittoral. The resulting MAA concentrations from lower sublittoral algae were usually under 1 mg g^{-1} DW. In comparison, at Potter Cove, the lower sublittoral species contained higher MAA concentrations up to 2.6 mg g^{-1} DW and on an average about 1.5 mg g^{-1} DW. Figure 10 shows total MAA concentrations of algae growing in different depths in the Kongsfjord, Arctic.

Interactive effects of irradiance and other abiotic factors such as temperature (Publ. 4) and salinity (Publ. 5) on MAA accumulation and photosynthetic performance were also investigated. In the temperature study, the cultured Antarctic and Arctic algae (*Iridaea cordata*, *Palmaria decipiens*, *P. palmata*, *Prasiola crispa* and *P. crispa* ssp. *antarctica*)

normally grown at 0 °C were acclimated to experimental temperatures of 5 and 10 °C for

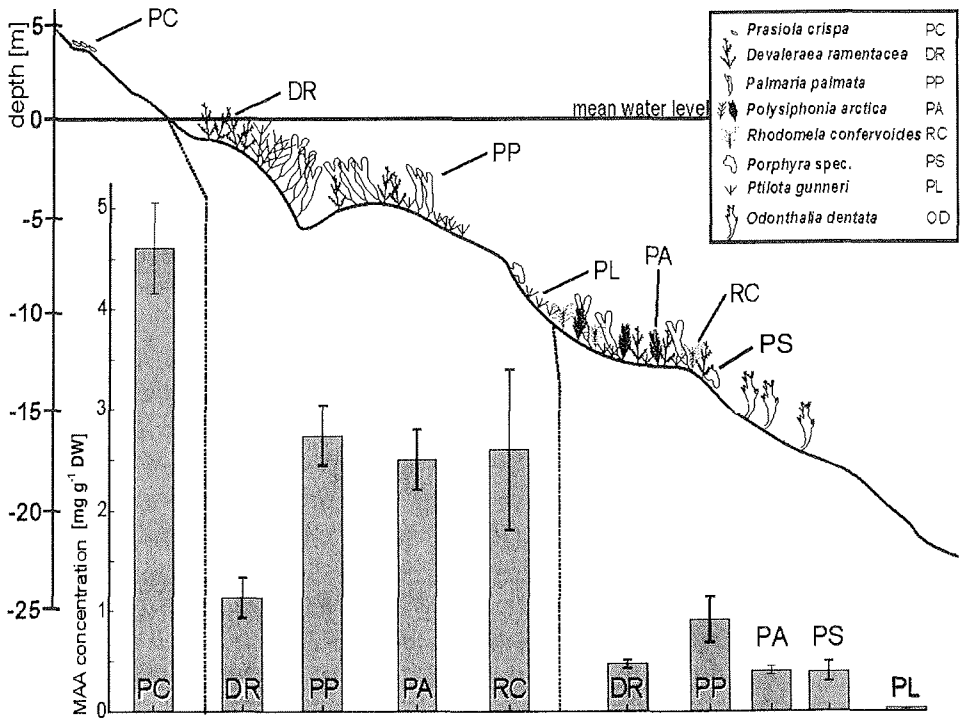


Figure 10: Total MAA concentration in several Arctic red macroalgae related to their vertical distribution collected in the sublittoral of Kongsfjord, Arctic. One green algal species is originated from a supralittoral/terrestrial location. Means ± SD (n=3).

23 days under low-light conditions. MAA concentrations were all very similar. In contrast, the optimum quantum yield of photosynthesis (F_v/F_m) was higher in all 5 °C samples than at 10 °C.

However, after exposure to the distinct radiation conditions, the Antarctic red algae exhibited significantly higher MAA concentrations under nearly all radiations treatments at 5 °C compared with those at 10 °C. Whereas in *P. palmata* from the Arctic and in the Antarctic green alga the MAA concentrations were similar under all experimental conditions. The degree of photoinhibition was less pronounced at 10 °C in all red algae after recovery for 8 hours in the darkness measured after 2, 6 and 11 days. In the green alga, those differences were not so marked and became relatively equalized after day 11.

The interactive effects of salinity and irradiance on MAA accumulation and photosynthetic performance were investigated in two Arctic red algae growing in a similar habitat. The endemic species *Devaleraea ramentacea* is a more euryhaline plant than *Palmaria palmata* (Publ. 5). At the end of the experiment, all *D. ramentacea* individuals under the UV treatment exhibited higher MAA concentrations (1.5 - 2.2 mg g^{-1} DW) compared with the PAR-only treated samples, containing 1.2 mg g^{-1} DW at 34 PSU and even lower for the other salinity values. The photosynthetic performance (F_v/F_m) showed a relatively slight decrease during the course of the experiment at the three salinity conditions (15, 34, 50 psu) without UVR, most pronounced in the 50 psu-treated samples. Samples treated with an addition of UV showed similar results, and photosynthesis recovered in all treatments to almost identical F_v/F_m values of over 80 % of the control by the end of the experiment. The photoinhibition effect was appeared to be caused by the hypersalinity, and to a lesser extent by the UV-stress.

Palmaria palmata has the greater physiological capability to survive in hypersalinity rather than hyposalinity, as indicated by totally bleached thalli at 15 PSU with and without UVR at the end of the experiment. The highest increase in MAA concentration was detected at 34 PSU plus UVR (2.7 mg g^{-1} DW) after a continuous MAA accumulation over the course of the experiment. Without UVR, MAAs remained more or less equal with concentrations similar to the control (1.2 mg g^{-1} DW), and even less in the samples at 15 PSU. Optimum quantum yields of *P. palmata* generally showed lower values compared with those of *D. ramentacea,* in the UV-treated samples, and the photoinhibition was mainly caused by the salinity in both samples at 15 and 50 PSU.

4.4 MAAs as sunscreen

The induction of MAA accumulation with PAR alone, led to a 1.4 fold higher MAA concentration in *Palmaria decipiens,* and to a 4.2 fold higher concentration in *Palmaria palmata* (Table 3), containing the same MAA suite (p-334, palythine, shinorine, and asterina-330, in this order). The initial values of the photosynthetic activity (F_v/F_m) of all samples are listed in Table 3.

Table 3: MAA concentrations and F_v/F_m initial values of each algal set-up

	Palmaria decipiens		*Palmaria palmata*	
	without induction	exposed to PAR (40 μmol m^{-2} s^{-1}) for 5 days	without induction	exposed to PAR (40 μmol m^{-2} s^{-1}) for 4 days
MAA concentration	231 ± 39 μg g^{-1} DW	323 ± 82 μg g^{-1} DW	114 ± 31 μg g^{-1} DW	479 ± 64 μg g^{-1} DW
initial values of Fv/Fm (control)	0.58 ± 0.04	0.36 ± 0.06	0.52 ± 0.037	0.42 ± 0.03

In *P. decipiens* from the Antarctic, the photosynthesis is markedly less affected in the samples containing higher MAA concentrations after one hour of UV exposure (Fig. 11). Consequently, the recovery is also better, given that within the first hour, the values rose up to 100 % of the initial values, whereas the samples containing less MAAs only reached 80 % of the initial F_v/F_m. Within five hours of UV exposure, F_v/F_m dropped to under 50 % in both set-ups, but in samples with a higher MAA concentration, the F_v/F_m values rose again to over 100 % after five hours of UV stress and recovered after cessation of the UV treatment, reaching F_v/F_m values of over 120 %. In contrast, the MAA-low samples recovered only moderately reaching F_v/F_m values of 80 % (Fig. 11).

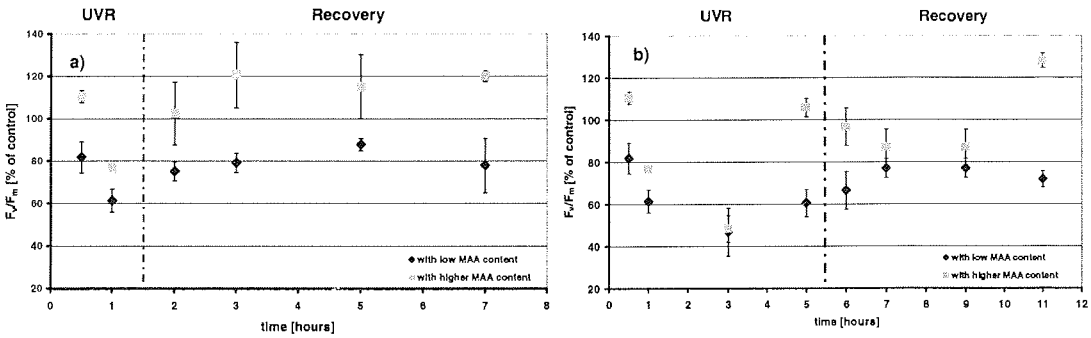


Figure 11: Optimum quantum yield of photosynthesis in samples of *Palmaria decipiens* with low and higher MAA contents. a) during 1 h exposure to UV and 6 h of recovery. b) during 5 h exposure to UV and 6 h of recovery. Means ± SD (n=3).

In *P. palmata* from the Arctic, the photosynthesis inhibition was similar in all samples experiencing one hour UV exposure. Recovery started slightly better in those exhibiting a higher MAA content, but after six hours of recovery, both values became relatively

similar, reaching 100 % of the initial values (Fig. 12). After three hours of UV-stress, the F_v/F_m values decreased to 20 % in the indiviuals containing a lower MAA concentration, whereas the values of the other samples did not drop any further. Within four hours after the cessation of UV-stress, the recovery of photosynthesis was up to 100 % in the MAA-high samples. In contrast, in the MAA-low samples, the photosynthesis only recovered up to about 60 % of the initial values (Fig. 12).

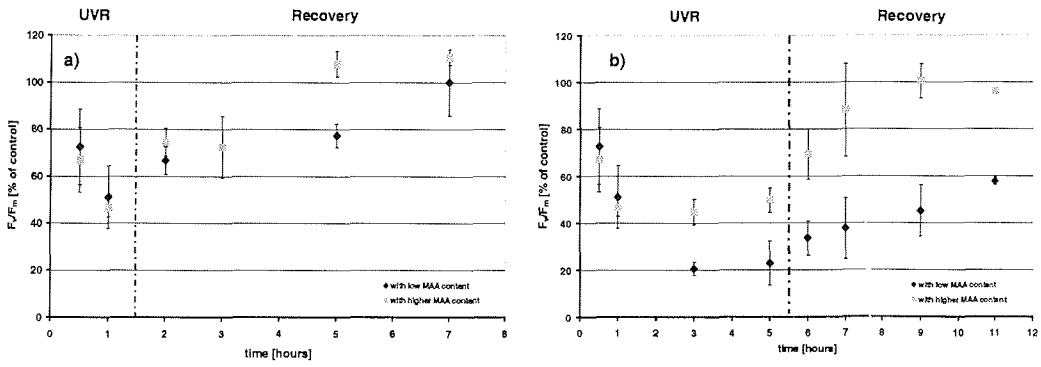


Figure 12: Optimum quantum yield of photosynthesis in samples of *Palmaria palmata* with low and higher MAA contents. a) during 1 h exposure to UV and 6 h of recovery. b) during 5 h exposure to UV and 6 h of recovery. Means ± SD (n=3).

The Arctic red algae, *Odonthalia dentata* and *Coccotylus truncatus* were exposed to different radiation conditions, and covered with the shinorine- and porphyra-334-biofilters, in order to investigate the photosynthetic performance under UV stress. In *O. dentata*, the F_v/F_m values decreased under the PAR-only treatment. In all the other samples, the photoinhibition was shown to be very strong, significantly lower F_v/F_m values were found to occur as compared to the PAR-samples. The PAR+UVA and biofilter treated samples were shown to recover slightly after the first UV treatments, reaching values of up to 50 and almost 60%, respectively, of the initial F_v/F_m values. Interestingly, after day 5 of UV exposure the UV effect was most attenuated in the samples under the biofilter compared with those under the PAR+UVA and PAR+UVA+UVB. But in the recovery their F_v/F_m values corresponded again with those of the PAR+UVA treated samples. These results suggest that the biofilters in this experiment also had a similar function to that of the 320 nm cut-off filter. The results

from day 3 were similar to that of day 2, therefore only data from day 1, 2 and 5 are shown in the graph below (Fig. 13).

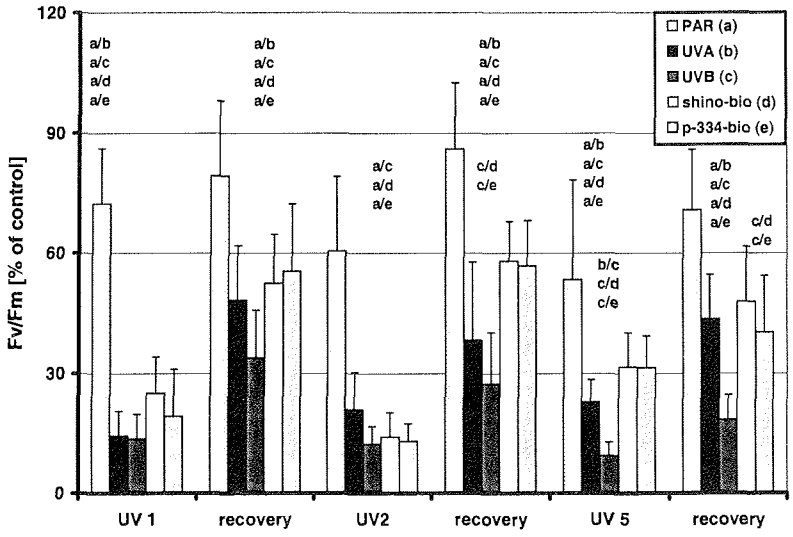


Figure 13: Optimum quantum yield of photosynthesis (F_v/F_m) after UV exposure (9 h) and recovery (12 h) of *Odonthalia dentata* at day 1, 2 and 5. Means ± SD (n=5-10).

During the experiment with *C. truncatus* the F_v/F_m values of the PAR-treated samples remained stable at about 90 % (Fig. 14). The optimum quantum yield of all other samples responded in the same way; F_v/F_m decreased when exposed to UV followed by a recovery. The samples under PAR+UVA+UVB had the lowest F_v/F_m values. However, the individuals seemed to acclimate to the radiation conditions, as indicated by the good recovery of the F_v/F_m values of up to 80 % in the PAR+UVA-treated samples and up to 70 % in the biofilter treated samples at the end of the experiment. Generally, these two latter samples showed the same response pattern exhibiting relatively similar values of photosynthesis activity. In this case however, photosynthetic recovery as determined in the significantly higher F_v/F_m values of the biofilter treated samples compared to those of the PAR+UVA+UVB treatment was seen. Data are shown for day 1, 2 and 9 in figure 15, day 5 and 8 depicted the same pattern.

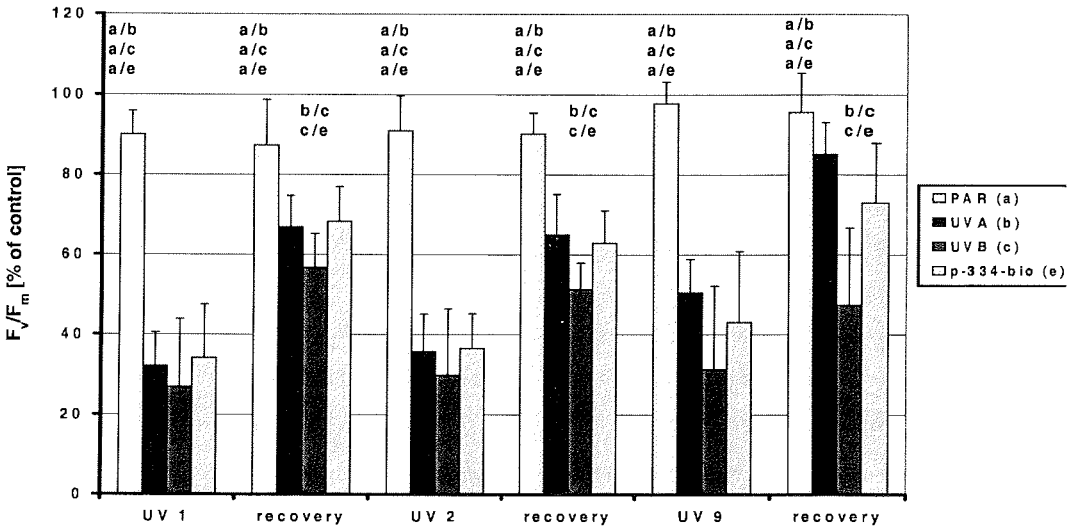


Figure 14: Optimum quantum yield of photosynthesis (F_v/F_m) after UV exposure (9 h) and recovery (12 h) in *Coccotylus truncatus* at day 1, 2 and 9. Means ± SD (n=5-10).

5 DISCUSSION

5.1 MAA occurrence in field and cultured algae

In inventories of MAA occurrence in Antarctic (Publ. 1) and Arctic macroalgae (Karsten et al. 1998b, Publ. 2), MAAs have been found most commonly in red algae. Methanolic extracts from green and brown algal species typically showed no strong absorption in the UV range. These results agree well with other survey studies (Karentz et al. 1991, 2001, Karsten et al. 1998b,c, 2000). In green macroalgal species, MAAs and UV-absorbing compounds have been detected in relatively low concentrations, although high contents of an unknown UV-absorbing substance-324 have been detected in species of the genus *Prasiola* (Fig. 8 and 9; Publ. 1, 2, Post and Larkum 1993, Gröniger and Häder 2002). Another exception has been found by Banaszak et al. (1998), who detected high amounts of mycosporine-glycine in the calcerous green algal species *Halimeda opuntia*. However, only one plant was tested in that study. Given the great variation in MAA concentrations under field conditions, this level of replication is not sufficient. Furthermore, the ethanol extract of the phenolic compound coumarin, from species of the green algal family Dasycladaceae also absorbs in the UV range with absorption maxima at 210, 265 and 345 nm (Menzel et al. 1983), and one could speculate that it could also be used as a photoprotective mechanism, in addition to its function as antifeedant compound, antimicrobial defence and epiphytic growth regulator. Additionally, Pérez-Rodríguez et al. (1998) and Gómez et al. (1998) have reported the existence of two chemically unknown UV-absorbing substances in the thallus and in water surrounding *Dasycladus vermicularis*. It is suggested that coumarin is one of the underlying structures and an effective photoprotectant (Pérez-Rodríguez, personal communication). The study by Xiong et al. (1999) also demonstrated a common occurrence of MAAs in freshwater and terrestrial microalgae (Chlorophyta), resulting that green algae generally contain UV-absorbing compounds.

Brown algae exhibited only very low concentrations of MAAs or none at all (Publ. 1, Karsten et al. 1998b, c). These might not come from the brown algae themselves, but from epiphytes such as diatoms, if samples are not carefully cleaned (Publ. 1). Although Banaszak et al. (1998) and Banaszak and Lesser (1995) reported that they rinsed the brown algal samples to remove epibionts. In some brown algal species they found quite high levels of MAAs, however, once again only one individual was tested. Thus, it is

assumed that brown algae have other photoprotective mechanisms apart from MAAs. Phlorotannins, which are polyphenolic compounds, are widely distributed amongst brown algae. They absorb UV radiation in the range from 280 to 320 nm, suggesting that they may play a role in photoprotection, as these compounds are inducible after exposure to UV radiation (Pavia et al. 1997, reviewed in Schoenwaelder 2002). These data on green and brown algae led to the investigation of MAAs in mainly red algae and one green algal genus in the present study.

The algae growing in different habitats can be classified into three distinct physiological groups, related to their MAA characteristics: I) species with high concentrations of MAAs, II) species with MAAs in variable concentrations, III) species containing no MAAs (Publ. 1, 2).

I) species with high concentrations of MAAs

In the first group, supra- and eulittoral species such as *Bangia atropurpurea, Porphyra endiviifolium,* and *Prasiola* sp., originating from a habitat in which the solar radiation is barely attenuated, generally contained the highest MAA concentrations (Fig. 8, 10). This has been also observed in red algae from the (upper) eulittoral zone from temperate to warm temperate regions (Karsten et al. 1998b,c, Karsten and West 2000, Karsten et al. 2001). Supra- to upper eulittoral species have probably developed an effective protection mechanism under high PAR and UVR, and a significant protection from UV damage might be achieved by the presence of MAAs. *Porphyra* and *Bangia* both belong to the order Bangiales, which is considered to be primitive and ancestral, due to its simple vegetative and reproductive organisation (Kraft and Wölkerling 1990). In the past, these ancestral genera would probably have had to cope with high UVR typical for palaeozoic times, and thus they may have benefitted from MAAs as protection mechanism, enhancing their chances of survival. Consequently, this may allow them to grow and successfully reproduce in the intertidal zone of exposed rocky shores, where extreme environmental conditions prevail today.

II) species with MAAs in variable concentrations

Sublittoral species with a broad depth distribution are able to adjust their MAA content to the respective depth or radiation climate, and were integrated in the second group (Fig. 9, 10). This agrees well with the finding that MAA concentrations decrease with increasing depth (Dunlap et al. 1986, Karsten et al. 1998a,b, Karsten and Wiencke 1999,

Karsten et al. 1999, Franklin et al. 1999). This observation is also connected with the underwater radiation climate, as radiation is attenuated as it penetrates through the water column. Moreover, the attenuation is very variable due to inherent optical properties associated particularly with particle-rich coastal waters (Kirk 1994). It was for this reason that Jerlov (in Mobley 1994) defined nine coastal water and three ocean types based on different transmission characteristics. The relatively low vertical attenuation coefficient, K_{dUVR} (0.19 m^{-1}; ocean water type 3 [Mobley 1994]), in the outer fjord of Potter Cove in the Antarctic, results in deep penetration of UVR through the water column (1% depth = 24 m). This may explain the high MAA levels in *Kallymenia antarctica*, collected in the lower sublittoral (Publ. 1, 2). Typically, the MAA concentrations are higher in lower sublittoral algae from Potter Cove compared with algae collected from the same position in Kongsfjord where the calculated K_d-values are higher (Table 2, Publ. 2). The relatively low MAA concentrations found in Arctic *Devaleraea ramentacea* from the upper sublittoral (2m), may have been due to highly turbid water conditions in the time just prior sampling.

III) species containing no MAAs

Strongly shade-adapted (mostly understorey species) and deep-water algae lack the capacity to produce MAAs, and form the third group (Publ. 1 and 2). Among the field-collected algae, the Antarctic species *Hymenocladiopsis crustigena, Myriogramme smithii, Phycodrys austrogeorgica,* and *Picconiella plumosa* from the deeper sublittoral are characterised by low initial photosynthetic light saturation values ($I_k < 30$ μmol m^{-2} s^{-1}, Publ. 1, Weykam et al. 1996). They typically inhabit deep-waters, grow as understorey species, and hence are well protected from harmful UVBR. Consequently, there is no physiological need to synthesise and accumulate UV-absorbing substances, therefore saving energy, carbon and nitrogen. Similarly, the Arctic deep-water algae *Coccotylus truncatus*, *Odonthalia dentata* and *Ptilota serrata* do not contain MAAs. Only *Ptilota gunneri* exhibited very low MAA concentrations, possibly indicating that the algal thalli were contaminated with epiphytes (Karsten, personal communication). Furthermore, these species are usually very susceptible to UVR and high PAR, as indicated by strong photoinhibition. However, this might be due to a combination of the lack of MAAs and their adaptation to the deep water low radiation climate (Karsten et al. 2001). Moreover, it is generally observed that the light saturation point (I_k) of algal

photosynthesis, positively correlates with MAA concentrations (r = 0.831); the higher the I_k, the higher the MAA content and vice versa (Publ. 1).

Overall, it may be concluded that MAAs play a role in photoprotection of the algae, and that the MAA accumulation may be connected with adaptation to their respective habitats. Hence, the increase in MAA concentration tends to be coupled with a decrease in incident radiation attenuation.

One major aim of this thesis was a comparison of MAA occurrence from macroalgae of the two polar study areas, keeping in mind the following differences between the study sites:

1. both fjords were located at different latitudes, and as a result the solar elevation angles were different, resulting in different daylengths (Kongsfjord: 24 h, Potter Cove: 20 h in polar summer) and varying radiation intensities (radiation conditions in Table 2, Publ. 2).
2. the generally thicker ozone layer over the Arctic result in a higher attenuation of UVB compared with the Antarctic.
3. macronutrient availability is not limited in the Antarctic, in contrast to the seasonal decrease in macronutrient concentrations in the Kongsfjord (Drew and Hastings 1992, Strutton et al. 2000, Aguilera et al. 2002b). As Litchman et al. (2002) demonstrated, the availability of nitrogen is important for the synthesis/accumulation of MAA molecules in the algae.
4. different hydrodynamic conditions (Kongsfjord being roughly 20 times larger than Potter Cove), and higher terrestrial input, resulting in higher K_d values in Kongsfjord, affect the macroalgae.
5. macroalgal habitats differ, tidal rocky platforms, including rock pools do not occur at the Arctic study site.

These differences might show that it is reasonable to expect that the MAA concentrations on average are higher in algae from Potter Cove (Antarctica) than from Kongsfjord (Arctic). The higher the natural solar UV radiation in the respective habitat, the more MAAs are formed and accumulated in marine red macroalgae.

Nevertheless, it should be noted that the great variability found in MAA concentrations are also dependent on other factors. Significant tissue-specific differences have been found in MAA levels between the margins and basal parts of thalli in some Antarctic species (Publ. 1). Different MAA values have also been seen within a tissue gradient in

Arctic species (Karsten and Wiencke 1999, Karsten et al. 1999), which might be due to self-shading effects. Additionally, differences in the absorption of UV-absorbing compounds and MAA concentrations have been found in different developmental stages of *Gracilaria chilensis* and *Caloglossa apomeiotica* (Molina and Montecino 1996, Karsten et al. 2000), but not in the few tested Antarctic species (Pub. 1). This suggests that MAA accumulation might be species-specific, however further studies are necessary to confirm this. Furthermore there are seasonal differenes in MAA and UV-absorbing compound concentrations (Post and Larkum 1993, Karsten et al. 1999).

5.2 MAA induction - laboratory studies

In laboratory experiments the capacity for the induction of MAA synthesis/accumulation under exposure to different artificial radiation treatments (PAR; PAR+UVA; PAR+UVA+UVB) was investigated from the field and from cultures in several Arctic and Antarctic red algal species, belonging to the three physiological groups (Publ. 2 and 3). The results were largely MAA- and species-specific. Species originating in the same habitat responded differently to radiation exposure and enhanced UVR, as did the individual MAA. Generally, it can be concluded that the algae can be grouped into three different physiological response types based on MAA levels; (a) highest MAA concentration under PAR+UVA+UVB, (b) highest MAA concentration under PAR+UVA, (c) MAA decrease under PAR+UVR. No differentiation between the UVR treatments were made for the Arctic algae (field-collected) and additionally their radiation histories were not known; this may have had an influence on the response to the radiation treatments and the accumulation of MAAs (Publ. 2).

(a) Algal species with highest concentrations under PAR+UVA+UVB formed the first response type (Fig. 2, Publ. 2, Fig. 2, Publ. 3). The MAA concentrations increased either under each additional radiation treatment or only under the latter radiation treatment. Algae belonging to this group are found over a wide depth range within the sublittoral, and even occur in the eulittoral as does *Palmaria decipiens* (Wiencke and Clayton 2002). These plants are able to adjust their MAA concentrations to suit the prevailing radiation climate, as demonstrated for several field–collected Antarctic and Arctic species from different depths (Publ. 1, Publ. 2, Karsten et al. 1998 b). Arctic *Devaleraea ramentacea* and *Palmaria palmata* exhibit the highest MAA concentrations under PAR+UVA+UVB after transplantation from deep to shallow water (Karsten and

Wiencke 1999, Karsten et al. 1999). Based on these studies both species have been classified into this group. Karsten and Wiencke (1999) demonstrated that *P. palmata* showed an increase in MAAs under all radiation treatments after transplantation, which was also found for Antarctic species such as *Gymnogongrus turquetii* and *Kallymenia antarctica* in the present study (Fig. 2, Publ 3). In contrast, in the endemic *D. ramentacea,* an increase in MAA concentration was only found under the full solar radiation spectrum (Karsten et al. 1999). From those experiments, however, it cannot be confirmed whether the accumulation was due to UVB alone, or an interactive effect between UVB and PAR occurred. However, in the laboratory experiment, interactive effects can probably be excluded due to the low PAR intensity, suggesting that the accumulation may be stimulated by UVB alone. Nevertheless, the effect of dose, regardless of irradiance was not specifically tested. Therefore, it cannot be unequivocally concluded that the increase in MAAs is only due to the change in spectral composition.

(b) The second response type contains species with maximal MAA values under PAR+UVA, which means that there was no further stimulation of MAA accumulation under the UVB spectral range. *Porphyra endiviifolium,* inhabiting the upper eulittoral, and thus regularly exposed to full solar radiation and *Gymnogongrus turquetii,* originating from a broad depth range from the eulittoral to sublittoral down to 30 m (Wiencke and Clayton 2002) belong to this group. The resulting MAA levels must be high enough to protect against the full radiation spectrum. *Porphyra endiviifolium* contains much higher MAA concentrations than the other species, most probably due to their habitat. A similar result has also been demonstrated in the closely related cold temperate *P. umbilicalis*, but this species contained constantly high MAA concentrations under the different radiation conditions without any further stimulation of MAA production (Gröniger et al. 1999). However, the exposure time was only for 3 days (12:12 light:dark cycle), which may have been too short to induce any MAA production. In general, high MAA concentrations are commonly present in eulittoral species, and may reflect a steady protective mechanism. In the present study, PAR+UVA seems to be already strong enough to act as stressor to produce the highest MAA levels in those species.

(c) Within the third response type, are algae that show a decrease of MAAs under enhanced UVR. These species seem to be susceptible to UVR stress, as photodamage

was observed, as indicated by bleaching of the thalli after exposure to UVR. This suggests that decomposition of photosynthetic pigments occurs under enhanced UVR. Photodestruction has also been shown for cyanobacteria and the authors concluded that it was due to direct damage caused by UVR (Lao and Glazer 1996). That fact may also explain the almost total MAA decrease under PAR+UVA+UVB and PAR+UVA in *Neuroglossum ligulatum* and *Plocamium cartilagineum*, respectively (Fig. 4, 5b, Publ. 3). Both species probably exhibit a high degree of physiological sensitivity against changing environmental factors, as shown for the Antarctic isolate *P. cartilagineum;* an extremely stenothermal species, growing only at temperatures below 5°C and dying above 7°C (Bischoff-Bäsmann and Wiencke 1996).

A decrease in MAAs was also found in Arctic *Rhodomela confervoides* after 11 days of UV-exposure. This was probably a stress response to PAR and UVR, as samples were collected at a depth of 12 m and thus were low-light adapted. Interestingly, *R. confervoides* seemed to have been 'loaded-up' with MAAs at the beginning of the experiment, because it contained a high concentration of 2.6 mg MAAs g^{-1} DW. Therefore, it may be concluded that MAAs were capable in this species to protect the tissue against UVR, at least during the first 6 days of exposure where no degradation or leakage was detected. However, due to the decease of MAAs after 11 days, it can be suggested that a MAA suite at steady state conditions does not guarantee complete protection against UVR. On the other hand, even an incomplete protection might reduce UV-damage at least for a short time. Decrease of MAAs may occur passively by photodestruction or by leakage, when the cell is affected by UV-damage, which can result in an alteration of the membranous permeability. On the other hand, it also may occur MAA degradation due to an active enzymatic process by the plant itself or even due to bacterial impact.

In deep-water and shade-adapted algae, no stimulation of MAA biosynthesis even under enhanced irradiance occurred. These deep-water plants are strongly shade-adapted (Kirst and Wiencke 1995), and exhibit low photosynthetic light compensation and initial light-saturation points, closely corresponding to their habitat conditions (Weykam et al. 1996). They are susceptible both to enhanced PAR and UVR, and consequently live in a habitat with a low radiation climate and little or no UVBR. This agrees well with other studies, in which the authors found that photosynthesis was very

sensitive to UVR in polar deep-water algae, which do not contain MAAs (Karsten et al. 2001). This may indicate that the MAA formation is determined on a genetic level, and missing and/or silenced genes may be the cause of the incapacity to synthesise MAAs. It may be explained as an adaptation to an almost UVR-free environment and to low-light levels in general. Consequently, there is no need to synthesise and accumulate UV-absorbing substances with the concomitant expense of energy, carbon and nitrogen.

Furthermore, the results of these induction experiments are related to enhanced UVR conditions and can only be partly used to determine the influence of PAR-only, under which normally also a moderate enhancement of MAAs occurred. Hence the threshold of fluence rate of effective induction was not tested, but it is assumed that it varies with species, as it does in the production of scytonemin in cyanobacteria (García-Pichel and Castenholz 1991). Additionally, Carreto et al. (1989) demonstrated that an augmentation of the PAR-intensity from 20 to 250 μmol $m^{-2}s^{-1}$ resulted in an increase in the content of UV-absorbing compounds in dinoflagellates, occurring rapidly and reversibly within hours. They concluded that MAA synthesis was dependent on light intensity and the spectral radiation composition, as exposure to sunlight resulted in a much more efficient synthesis than exposure to artificial radiation (Carreto et al. 1990a). This confirms the findings of the present study, as cultured algae exposed to artificial radiation never reached the high MAA concentrations that the same species exhibited in the field. Also, the absence of some individual MAAs in cultured samples was commonly observed, once again suggesting that the artificial irradiance might not be efficient or natural enough to induce the whole MAA suite. The natural PAR:UVA:UVB ratio at 60° solar elevation is 179:21:1 (Thiel et al. 1996). Under artificial radiation, using typically the Q-Panel UVA-340 nm tube and the daylight fluorescence lamps together, the ratio is around 20:12:1. Hence, in the present study the PAR intensity is much too low to compare with natural conditions and should be defined as a background radiation, whereas the UVA:UVB ratio is relatively similar to solar radiation but still lower by a factor of 1.8.

Nevertheless, the PAR intensities used in the present experiments are close to those that algae experience in their natural environment. Therefore, in this case, the low PAR intensity is not a limiting factor for photosynthesis, and hence should not influence their energetic requirements nor mask the effects of UVR. The accumulation of UV-absorbing compounds due to UVB also depends on PAR intensity (Molina and

Montecino 1996). Nevertheless, one should remain aware of the methodological differences between laboratory and field experiments when placing the results into an ecological and physiological context. As the underwater light climate is very complex and several other biotic and abiotic factors also influence field populations, laboratory experiments under controlled conditions are helpful to elucidate specific mechanisms involved in the physiological responses to UVR stress. Hence, by combining field and laboratory experiments we might understand the importance of the individual components of the underwater light climate (Sagert and Schubert 2000). This is emphasised by the results of the transplantation experiment in the Antarctic with *Kallymenia antarctica* (Publ. 1). In this species no radiation effects were detectable in the field experiment, whereas in the laboratory experiments low-light adapted cultured isolates of the same species showed MAA accumulation under all radiation treatments (Publ. 2, 3). Hence, from both experiments it may be suggested that the transplanted field plants were already 'loaded up' with MAAs, so that during the exposure to enhanced radiation no further MAA accumulation was possible.

However, all data from MAA surveys, field and laboratory experiments show that irradiance has a large influence on MAA accumulation and that MAA synthesis/accumulation is inducible by radiation, which is one prerequisite for confirming their photoprotective role in the algae. A summary of those results is shown in Figure 16.

The distinct physiological responses of MAA accumulation to different radiation treatments are not only found in macroalgae but also in microalgae and cyanobacteria (e.g. Carreto 1989, 1990a,b, Villafañe et al. 1995, Banaszak and Trench 1995b, Hernando et al. 2002). Helbling et al. (1996) showed that two species of centric diatoms cultured under high PAR, increased their MAA concentration in response to exposure to solar radiation. In contrast, two species of pennate diatoms only increased MAA accumulation in the presence of UVR. The prymnesiophytes *Phaeocystis antarctica* and *P. pouchetii* produce significant amounts of UV-absorbing compounds in response to UVB exposure, whereas in several other diatoms, induction was most effective at wavelengths between 370 and 460 nm (Marchant et al. 1991, Riegger and Robinson 1997). In the dinoflagellate *Heterocapsa triquetra,* synthesis of MAAs increased under exposure to UVB (Wängberg et al. 1997), whereas *Amphidinium carterae* did not accumulate high amounts of MAAs even under UVR exposure (Hannach and Sigleo

1998). García-Pichel and Castenholz (1993) have shown that MAA concentrations increased with increased UVR fluence rates for several cyanobacteria. The data indicate that the distinct physiological responses of all organisms to the different radation treatment are species-specific rather than due to a common physiological and/or biochemical mechanism.

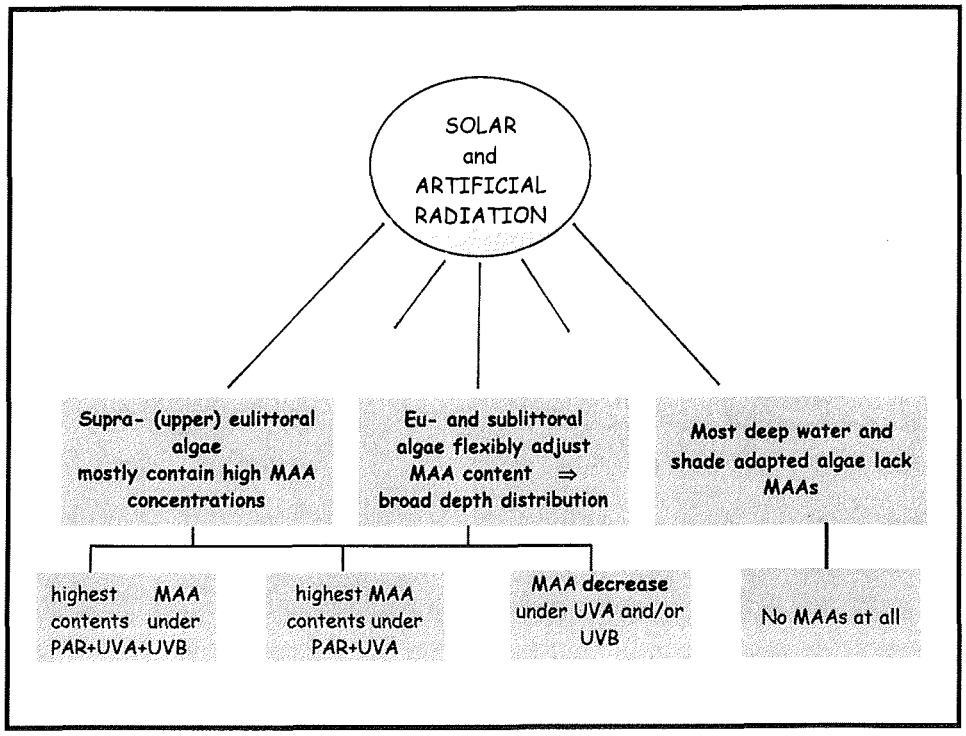


Figure 16: Summarised result of field investigations and laboratory experiments referring to MAAs according to their habitat and their responses to different irradiance conditions

The complement of identified MAAs in the present study (mycosporine-glycine, shinorine, porphyra-334, palythine, asterina-330, palythinol, palythene, usujirene) is characteristic for red macroalgae of different biogeographic regions. Shinorine, palythine and p-334 are the most abundant MAAs. However, the distribution patterns do not indicate any obvious trend within or across red algal taxa. On the other hand,

preliminary studies have shown that the unknown UV-absorbing substance-324 in the green algal genus *Prasiola* is also found in several other species belonging to the order Prasiolales (data not shown), indicating a chemotaxonomic marker function for this group. Usually shinorine and palythine are the predominant MAAs found in subtidal Antarctic and Arctic red algae, whereas in eulittoral taxa p-334 predominates (Publ. 1, 2).

In calculated response spectra for the induction of MAAs in *Chondrus crispus* from Helgoland, Germany, the short wavelength UVA exhibted the highest quantum efficiency in the synthesis of shinorine. In contrast, synthesis of asterina-330, palythinol and palythine was mainly induced by UVB (Kräbs et al. 2002). In *Caloglossa* species p-334 was mainly stimulated by PAR, while UVA and UVB predominately led to the accumulation of shinorine and palythine (Karsten et al. 2000). In *Porphyra endiviifolium* the highest p-334 concentration was found under PAR+UVA and in *Porphyra plocamiestris* under PAR+UVA+UVB (Fig. 2, 3,5 Publ. 3). Discrepancies in MAA induction patterns may be explained by species-specific responses of the plants to spectral radiation composition and intensity. Furthermore, differences in MAA composition across species probably depends on physiological and genetic characteristics of the individual algae.

Nevertheless, it is still an open question how the trigger mechanism of the induction of MAA accumulation/synthesis functions, and what type of photoreceptor(s) is (are) involved. The shikimate pathway, where MAAs are probably synthesised (Shick et al. 1999), is assumed to be light-regulated (Weaver and Herrmann 1997). Interestingly enough, the transcription of genes coding for the first enzyme in the shikimate pathway (DAHP synthase) increases under UVR exposure (Shick et al. 2000). Thus both the irradiance, which may act through cell surface receptors (Shick et al. 2000), and the direct effect of UVR on the shikimate pathway may indicate that probably more than one mechanism or photoreceptor might be involved in the MAA induction and formation process. This is supported by the results of Franklin et al. (1999) who postulated a signal transduction pathway or even interactions among various photoreceptors involved in the overall process leading to high MAA concentrations. Studies in higher plant photobiology have also shown that individual photoreceptors play unique roles in physiological regulation processes, and that certain gene products are shared by light activation pathways initiated by different photoreceptors (Petridou et

al. 1997 and references therein). Portwich and Garcia-Pichel (2000) have suggested a reduced pterin as a UVB photoreceptor chromophore acting in the MAA metabolism of the cyanobacterium *Chlorogloeopsis* sp. for two reasons; (1) the action spectrum of MAA synthesis was congruent with that of a reduced pterin (with a maximum at 310 nm), (2) both an inhibitor of the biosynthetic pathway and an antagonist of excited states of pterins depressed the photosensory efficiency of induction. Furthermore, Franklin et al. (2001) suggested a cryptochrome photoreceptor was active in the triggering process for MAA biosynthesis, as MAA induction occurred in *Chondrus crispus* under blue light and UVA. In contrast, Shick et al. (2000) raised the question whether the MAA accumulation may be stimulated by an indirect effect via reactive oxygen species (ROS).

The question of whether MAA formation is constitutive or only produced when induced by radiation remains unanswered (Karentz 2001). From the present study it may be assumed that MAA occurrence is constitutive because even in many low-light cultured algae, MAAs were always found albeit only in trace amounts, which then increased under enhanced radiation conditions. But in those species that did not contain any MAAs, there was no induction of MAA production. In contrast, in some cyanobacterial species, MAA-synthesis could be induced after enhanced radiation exposure, even when the cells had no traces of MAAs (Portwich and Garcia-Pichel 1999). Hence the constitution of MAAs might also be species-specific.

5.3 Do other abiotic factors control the MAA occurrence/induction ?

Radiation may not be the only trigger for MAA production. Investigation of the effect of enhanced temperature on MAA accumulation in polar algae (Pub. 4), showed that at 5 and 10 °C the MAA concentration in dim-light acclimated species remained unchanged (Publ. 4). When the Antarctic red algae (*Iridaea cordata*, *Palmaria decipiens*) were exposed to enhanced PAR and UVR, MAA concentrations were higher at 5 °C than at 10 °C, and remained almost unchanged in the Antarctic green and Arctic red algae (*Prasiola crispa* ssp. *antarctica*, *Palmaria palmata*). Nevertheless, temperature has a strong influence on almost all types of biochemical reactions according the Q_{10} rule. However, exceptions to this rule have been found in endemic

Antarctic macroalgae, in which enzymatic processes of MAA accumulation and synthesis seem to be well adapted to the low temperature habitats of Antarctic species. Indeed, other cellular processes are also well adapted to these cold temperatures, for example growth, light saturated photosynthesis, dark respiration (e.g. Wiencke and tom Dieck 1989, 1990, Wiencke 1990, Thomas and Wiencke 1991, Bischoff-Bäsmann and Wiencke 1996, Eggert and Wiencke 2000).

In the Arctic *P. palmata,* slight or even no interactive effects between temperature and UVR have been detected (Fig. 1, Publ. 4). This agrees well with a recent investigation by Poll et al. (2002), about temperature dependence of UV effects on growth, photosynthesis and DNA damage. The authors concluded that the contribution of temperature to UV effects in *P. palmata* was small within the tested temperature range from 6 to 18 °C. Arctic cold temperate red algal species are generally less stenothermal than Antarctic ones, and exhibit a broader temperature range for growth (up to maximal 25 °C) and survival at higher maximum temperatures between 17 and 25 °C (Wiencke et al. 1994, Bischoff-Bäsmann and Wiencke 1996).

There was no obvious interactive effect between UVR and temperature on the synthesis/accumulation of the UV-absorbing substance-324 in the *P. crispa* ssp. *antarctica* (Fig. 1). A characterisation by Wiencke and tom Dieck (1989) indicate this species as a mainly eurythermal alga due to its wide growth temperature range from 0 to 20 °C, with a growth optimum at 5 °C. In nature, *P. crispa* ssp. *antarctica* has to cope with very extreme environmental conditions, and needs to be able to combat desiccation, freezing, UV- and excessive PAR-stress as well as fluctuating salinity and temperature stress (Jakob et al. 1991). A broad environmental tolerance (*sic*) has also been found in other green algal species explaining their ecological success in extreme habitats (Taylor et al. 2001).

In summary, it may be concluded that temperature alone has no effect on MAA synthesis/accumulation in polar algae under the tested temperature range under low-light conditions, but togehter with enhanced irradiance, interactive stimulating effects are species-specific.

Recovery of photosynthesis after UVR offset was generally lower at 5 than at 10 °C in the red algae studied. Ross and Vincent (1998) have suggested that UVR is more detrimental at lower temperatures, possibly indicating that the enzymatic repair

mechanisms are too slow to compensate for the damaging UVR effect. Hanelt et al. (1997a) also reported that in laboratory experiments the temperature showed a pronounced effect on the reaction kinetics of photosynthesis, and suggested that higher temperatures may be beneficial for the photoprotective process in macroalgae. However, in the present study the recovery of the photoinhibition was retarded at the lower temperature, although in the two Antarctic red algal species, the MAA concentrations were higher at 5 °C compared to 10 °C. In Arctic *P. palmata* MAA levels were more or less equal at both temperature treatments. Hence, it might be an interactive effect that in samples exposed to UVR and enhanced temperature, photosynthesis is not noticeably protected by the presence of MAAs.

Hypersaline stress had no influence on the MAA synthesis/accumulation in two Arctic macroalgae (*Devaleraea ramentacea* and *Palmaria palmata*). In contrast, hyposalinity led to a decrease in the MAA concentration, particularly in *P. palmata,* which did not survive this treatment. Over the course of the experiment, thalli of this species became bleached at 15 psu independent of the presence of UVR. Therefore, *P. palmata* is more stenohaline than *D. ramentacea*. However, in response to UVR treatment, coupled with different salinities (50, 34 psu), MAAs were accumulated in both species over the course of the experiment. The highest MAA concentrations were usually measured at 34 psu and UVR (Fig. 4, 8, Publ. 5). In contrast to both macroalgae, a slight stimulation of MAA and scytonemin accumulation under enhanced salinity and low-light conditions have been demonstrated in some cyanobacteria (Portwich and García-Pichel 1999, Karsten 2002, Dillon et al. 2002). Stenohalinity with respect to growth is particularly typical for sublittoral red algae, compared to the broad salinity tolerance of intertidal species (Kain and Norton 1990, Reed 1990). *Palmaria palmata* is mainly found in temperate/cold–temperate waters of the North Atlantic, where it grows sublittorally at depths down to 20 m, or protected as typical understorey plant of kelp forests (Irvine 1983, Lüning 1990). These habitats are characterised by stable salinity conditions, which support the development of stenohaline organisms. Consequently, the strong inhibition of photosynthesis, the decrease of MAAs and high mortality of the Arctic isolate of *P. palmata* at 15 psu can be explained by a limited physiological capacity to acclimate to external salinity fluctuations.

Oren (1997) and Portwich and García-Pichel (1999) also reported a decrease in levels of MAAs in cyanobacteria when their growth medium was diluted. MAAs in the medium increased within minutes, with a strong preferential leakage of mycosporine-glycine over shinorine. MAAs were not reabsorbed by the organisms (Portwich and García-Pichel 1999). However, leakage into the medium under hyposaline conditions was not tested in the present study. It has been suggested that MAAs may also serve as organic osmolytes, exhibiting many typical characteristics necessary for this role; i.e. their high concentration in the cells, their chemical structures, being polar, uncharged or zwitterionic amino acid derivates, high water solubility, their cytoplasmatic location (Karsten 2002, references therein). However, the concentrations of the osmolytes trehalose and sucrose are typically much higher in cyanobacteria than those of the MAAs, leading to the conclusion that MAAs are not playing an important role in osmotic acclimation (Portwich and Garíca-Pichel 1999).

In addition to the MAA accumulation under irradiation and osmotic stress, Jokiel et al. (1997) have also observed a mechanical stimulation mechanism. Corals exhibited elevated MAA levels after exposure to high water velocities. However, this has not been reported for macroalgae.

5.4 Evidence for MAAs acting as a sunscreen

The efficiency of MAAs in protecting photosynthesis after exposure to UV-stress in two polar red algal species (*Palmaria palmata* and *Palmaria decipiens*) was investigated. After exposure to UVR, species-specific responses of photosynthesis was measured in these two plants. The results may indicate that in *P. palmate,* photosynthesis is protected not only by MAAs, but probably also by other UV-defence mechanisms, playing an ancillary role during short time stress. Futhermore, the efficiency of MAAs does not only seem to be concentration-dependent, but also species-specific because the photosynthesis of *P. palmata* was more affected by UVR, although containing a higher MAA concentration than that of *P. decipiens.*

Generally, the results of both *Palmaria* species provide evidence that a higher MAA concentration in the same taxa, under identical UV-conditions, reduces the degree of photoinhibition, and consequently allows the acceleration of the recovery of photosynthesis. Karsten et al. (1999) who tested the relative effectiveness of the

presence of MAAs to reduce UV-induced inhibition of photosynthesis in *Devaleraea ramentacea* showed very similar data. Isolates collected from different depths, and therefore containing different MAA concentrations were exposed to distinct radiation conditions with and without UVR. Karsten et al. (1999) reported that increasing MAA contents were positively correlated with a higher photosynthetic resistance against UVR. However, it is known that photoinhibition is a regulative mechanism of photosynthesis in algae to protect against excessive PAR and UV-stress in the field (Hanelt et al. 1994, 1997a, Hanelt 1998).

Neale et al. (1998) also showed in a photosynthesis experiment with a low-light (MAA-low) and high-light (MAA-rich, 14-fold higher MAA concentration) adapted dinoflagellate (*Gymnodinium sanguineum*) that in the MAA-rich samples the resistance to UVR increased. MAAs appeared to act as spectrally-specific UV-screens, and hence photoprotectants. This view is also supported by Adams and Shick (1996, 2001), demonstrating the photoprotective role of MAAs due to the more successful development (less abnormalities, less delay in development) of green sea urchin embryos (up to the pluteus stage) in MAA-rich eggs compared with MAA-low eggs under UV-stress.

Samples of two Arctic, deep-water red algal species (*Odonthalia dentata*, *Coccotylus truncatus*), lacking MAAs, were exposed to enhanced PAR and UVR in order to investigate their photosynthetic response covered with different filters (400, 320 and 295 nm cut off filters, and shinorine and/or p-334 biofilters). The MAA biofilter attenuated UVR (UVB more than UVA, see Material and Methods) but did not completely block it, acting in a similar way to a 320 nm cut-off filter by diminishing the UVB effects. Furthermore, photosynthetic recovery in the biofilter treated samples was even slightly better than in the PAR+UVA treated samples after a possible acclimation to the radiation conditions (Fig. 13). Taking the results together it may be assumed that the MAAs mostly screen harmful UVB and only partly UVA, offering a higher degree of protection than the cut-off filters.

Similar results during UVR stress were found in *C. truncatus*. All UV-treated samples were similarly photoinhibited, but the PAR+UVA and biofilter (only p-334)–treated samples recovered significantly better than the PAR+UVA+UVB-treated isolates. The results of these two experiments together show that at least shinorine and p-334 screen

in the UVB range, although their absorption maxima is at 334 nm in the UVA, strongly supporting the photoprotective role of the MAAs.

Furthermore, Adams and Shick (2001) calculated two additional extinction coefficient values from a partly purified shinorine extract, apart from the previously known extinction coefficient (at shinorine's absorption maximum). They occurred at the wavelengths 320 and 310 nm (the absorption maxima of palythine and mycosporine-glycine, respectively), which were still 87 and 60 % of those of palythine and mycosporine-glycine (extinction coefficients in Table 1, Introduction). This indicates that shinorine alone can absorb not only UVA wavelengths but also nearly as much UVB as the most common UVB absorbers in marine organisms (Adams and Shick 2001).

The MAA concentrations used in the biofilter were 0.025 mM for shinorine and 0.026 mM for p-334. Taking into account that the specific contents of MAAs from macroalgal field populations are usually <1 % of the dry weight (Publ. 1, 2, Karsten et al. 1998a,b) then the total MAA average concentration of the cells should be less than 0.03 mM, using an MAA average molecular weight of 300 Da for the calculation. This calculated value fits well with the concentrations used in the biofilters, and thus may indicate that the biofilters may act in a similar way as the intracellular MAAs. External MAAs may also function as photoprotectants for other marine organisms lacking the capacitiy to synthesise internal MAAs.

MAA release into the medium is not only observed in cyanobacteria under osmotic stress (Oren 1997, Portwich and García-Pichel 1999) but also in the water column, due to dinoflagellates such as *Lingulodinium polyedra* during blooming (Vernet and Whitehead 1996, Whitehead and Vernet 2000). It has been supposed that MAAs are part of the DOM production in phytoplankton (Vernet and Whitehead 1996), but they may also appear through cell lysis and grazing (Whitehead and Vernet 2000). The measured MAA concentrations were low, varying from 3.43 to 111.40 nM, being diffused and diluted rapidly in the water and dispersed by currents and turbulence. Furthermore, they might be degraded by bacteria used as energy source. Therefore, MAAs do not persist for a long time in the water column, consequently their protective effectiveness might be inconsequential. On the other hand, MAAs may attenuate the UVR during a short period, but are then confined to the plankton bloom area. Thus, they could protected not only phytoplankton but plankton and other benthic organisms in

general. Besides this the MAAs may also have an allelopathic effect on other plankton organisms, for example by inhibiting growth. The excretion of MAAs by macroalgae should be the subject of further investigation, as it should also be considered that the release may occur through leakage induced by stress, fragmentation, decomposition and grazing of MAA-rich thalli, although the concentrations would probably have no sunscreen effect on other organisms.

Despite that, this indicates that MAAs may not only protect marine organisms as intracellular UV-absorbing compounds, whether synthesised by the organisms itself, by symbionts or by a dietary uptake, but also as external compounds, accumulated by phytoplankton blooms providing protection for other organisms present in the water column (Marchant et al. 1991).

Besides the photoprotective and osmotic role for MAAs in marine organisms, other functions have also been proposed:

(1) as an antioxidant function for at least mycosporine-glycine because it was reactive to peroxyl radicals in an concentration-dependent manner. This has not been proven for imino-MAAs (Dunlap and Yamamoto 1995). Since the enzymatical conversion of some imino-MAAs to mycosporine-glycine has been detected, they may serve as precursors for the latter (Dunlap et al. 1999).

(2) as an early source of amino acids before yolk storage compounds become available for assimilation in eggs of the shrimp *Artemia* sp.. This has been suggested as a result of the rapid decline of MAAs in the first hours of embryological development (Karentz 2001, references therein). In contrast, MAAs remain stable in embryos of sea urchins and almost constant in sea hare spawn over a course of 40 days (Adams and Shick 1996, 2001, Carefoot et al. 2000).

(3) in a regulatory role in algal metabolism with a concentration-dependent retardation effect on growth of *Porphyra yezoensis*. This has been observed if MAAs are added to the culture medium (Sivalingam et al. 1974), which might also be an allelopathic effect.

However, the full range of functions of individual MAAs in macroalgae and other marine organisms is still unknown, but one of their most commonly assumed, although possibly not primary role is that of a sunscreen.

5.5 Conclusions

The MAA distribution patterns in red macroalgae as well as the concentration and inducibility in response to enhanced UVR seems to be very species-specific. Nevertheless, MAA occurrence can be classified into three physiological groups based on algal habitats: I) supra- and (upper) eulittoral species contain always highest MAA concentrations, II) eu- and sublittoral algae adjust their MAA content, III) most deep water and understorey algae lack MAAs. These three groups clearly support the importance of MAAs as sunscreens. Species growing in habitats receiving high solar radiation contain the highest MAA concentrations. Additionally, algal responses to enhanced UVR may also be grouped into three physiological response types: a) highest MAA contents under PAR+UVA+UVB, b) highest MAA contents under PAR+UVA, c) MAA decrease under UVA and/or UVB. Once again this indicates that induction, formation and accumulation of (individual) MAAs is a physiologically very flexible and species-specific process.

There was no temperature effect on MAA accumulation in polar macroalgae. However, the interactive effect between temperature and enhanced PAR, with or without UVA/B, resulted in a species-specific response, i.e. a stimulation of MAA accumulation. This response is probably strongly dependent on the level of adaptation to the environmental temperature and on algal physiological temperature characteristics. Hypo- and hypersalinity have different effects on Arctic macroalgae both with, or without, UVR depending on the physiological properties of the respective species. None of these osmotic stress conditions led to higher MAA concentrations but rather to a decrease in MAAs compared to the samples under the control. In case of polar red algae salinity does not affect MAA pool sizes.

The effectiveness of MAAs as sunscreens in red macroalgae have been shown, because the protection of photosynthesis against enhanced UVR is MAA concentration-dependent; the higher the MAA content the better the sunscreen effect. MAAs applied as external filters also protect algae that naturally do not contain MAAs from enhanced UVR. If present MAAs, however, do not provide complete protection, but reduce the effects of UV-damage, indicating that they are part of a biochemical defence system.

From these results it might be concluded that macroalgae with the capability of forming MAAs gain an adventage compared to MAA-lacking taxa and are able to survive in the

harsh Antarctic and Arctic environments. Even if the ozone depletion over these regions becomes worse, causing the UVR levels to rise further, these algae will still be protected. Furthermore, herbivores that feed on MAA-rich algae and that are able to accumulate these compounds in their tissues and reproductive stages will also be better protected in the case of further UVB increase. This indicates that MAAs not only play an important role in the plants themselves but also in the whole aquatic ecosystem.

Further investigations should be performed to elucidate the complete role of MAAs not only in macroalgae but also in other aquatic organisms. More details are required to answer questions related to how physiological and biochemical processes are protected by the MAAs, and hopefully confirm whether there is a specific protection target. It is also important to discover the location (or distribution) of MAAs in the cell, which might help to improve the understanding of their effectiveness and to calculate a photoprotective (sunscreen) factor. However, as MAA synthesis might be a general stress response, the influence of other abiotic stress conditions such as desiccation, chilling, nutrient deficiency should be revealed based on MAA synthesis, and additionally their interactive effects with further abiotic factors should be investigated.

The PAR and UVA/B fluence rates, being able to stimulate MAA accumulation up to a maximum MAA concentration, which is probably a species-specific threshold as well, should be evaluated by dose response experiments to obtain qualitative and quantitative MAA levels most likely found in algae. It will also be important to get an idea about the interaction of distinct spectral ranges, as the PAR:UVB ratio is subject to alterations, and the possibility of masking effects in physiological processes of the cell.

Overall, the biosynthesis of MAAs and the elucidation of their trigger mechanism(s) need to be worked out. This would provide more detailed knowledge about the algal capacity to cope with the phenomena ´ozone hole` and subsequent UVB increase.

6 REFERENCES

Adams NL, Shick JM. 1996. Mycosporine-like amino acids provide protection against ultraviolet radiation in eggs of the green sea urchin *Strongylocentrotus droebachiensis*. Photochem Photobiol B 64:149-58.

Adams NL, Shick JM. 2001. Mycosporine-like amino acids prevent UVB-induced abnormalities during early development of the green sea urchin *Strongylocentrotus droebachiensis*. Mar Biol 138:267-80.

Adams NL, Shick JM, Dunlap WC. 2001. Selective accumulation of mycosporine-like amino acids in ovaries of the green sea urchin *Strongylocentrotus droebachiensis* is not affected by ultraviolet radiation. Mar Biol 138:281-94.

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 191:109-19.

Aguilera J, Dummermuth A, Karsten U, Schriek R, Wiencke C. 2002 a. Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. Polar Biol 25:432-41.

Aguilera H, Bischof K, Karsten U, Hanelt D, Wiencke C. 2002 b. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defence systems against high light stress. Mar Biol 140:1087-95.

Amsler C, Rowley R, Laur D, Quetin L, Ross R. 1995. Vertical distribution of Antarctic peninsular macroalgae: cover, biomass and species composition. Phycologia 34:424-30.

Arntz W, Gutt J, Klages M. 1997. Antarctic marine biodiversity: an overview. In: Battaglia B, Valencia J, Walton D. Antarctic communities: Proc 6th SCAR Biology Symposium, Venice 1994. Cambridge: Cambridge University. p 3-14.

Atkinson RJ, Matthews WA, Newman PA, Plumb RA. 1989. Evidence of the mid-latitude impact of Antarctic ozone depletion. Nature 340:290-4.

Austin J, Butchard N, Shine KP. 1992. Possibility of an Arctic ozone hole in a double-CO_2 climate. Nature 360:221-4.

Banaszak AT, Lesser MP. 1995. Survey of mycosporine-like amino acids in macrophytes of Kane'ohe Bay. In: Gulko D, Jokiel P, eds. Ultraviolet Radiation and Coral Reefs. Hawaii Institute of Marine Biology Technical Report, Vol. 41. Honolulu: University of Honolulu. p 171-9.

Banaszak AT, Trench RK. 1995. Effects of ultraviolet (UV) radiation on marine microlagal-invertebrate symbioses. II. The synthesis of mycosporine-like amino acids in response to exposure to UV in *Anthopleura elegantissima* and *Cassiopeia xamachana*. J Exp Mar Biol Ecol 194:233-50.

Banaszak AT, Lesser M, Kuffner I, Ondrusek M. 1998. Relationship between ultraviolet (UV) radiation and mycosporine-like amino acids (MAAs) in marine organisms. Bulletin of Marine Science 63:617-28.

Banaszak AT, LaJeunesse TC, Trench RK. 2000. The synthesis of mycrosporine-like acids (MAAs) by cultured, symbiotic dinoflagellates. J Exp Mar Biol Ecol 249:219-33.

Banaszak AT, Neale PJ. 2001. Ultraviolet radiation sensitivity of photosynthesis in phytoplankton from an estuarine environment. Limnol Oceanogr 46:592-603.

Bandaranayake WM. 1998. Mycosporines: are they nature´s sunscreens? Natural Product Reports 15:159-72.

Bäsmann BB, Wiencke C. 1996. Temperature requirements for growth and survival of Antarctic rhodophyta. J Phycol 32:525-35.

Bischof K, Hanelt D, Wiencke C. 2000 a. Effects of ultraviolet radiation on photosynthesis and related enzyme reactions of marine macroalgae. Planta 211:555-62.

Bischof K, Kräbs G, Hanelt D, Wiencke C. 2000 b. 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, Hanelt D, Aguilera J, Karsten U, Vögele B, Sawall T, Wiencke C. 2002. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. I. Sensitivity of photosynthesis to ultraviolet radiation. Mar Biol 140:1097-106.

Bischoff B, Wiencke C. 1993. Temperature requirements for growth and survival of macroalgae from Disko Island (Greenland). Helgol Mar Res 47:167-91

Bischoff-Bäsmann B, Wiencke C. 1996. Temperature requirements for growth and survival of Antarctic Rhodophyta. J Phycol 32:525-35.

Boelen P, de Boer MK, Kraay GW, Veldhuis MJ, Buma AG. 2000. UVBR-induced DNA damage in natural marine picoplankton assemblages in the tropical Atlantic Ocean. Mar Ecol Prog Ser 193:1-9.

Böhm GA, Pfleiderer W, Böger P, Scherer S. 1995. Structure of a novel oligosaccharide-mycosporine-amino acid ultraviolet A/B sunscreen pigment from the terrestrial cyanobacterium *Nostoc commune*. J Biol Chem 270:8536-9.

Bracher A, Wiencke C. 2000. Simulation of the effects of naturally enhanced UV radiation on photosynthesis of Antarctic phytoplankton. Mar Ecol Prog Ser 196:127-41.

Büdel B, Karsten U, Garcia-Pichel F. 1997. Ultraviolet-absorbing scytonemin and mycosporine-like amino acid derivatives in exposed, rock-inhabiting cyanobacterial lichens. Oecologia 112:165-72.

Buma AG, van Oijen T, van de Poll W, Veldhuis MJ, Gieskes WW. 2000. The sensity of *Emiliania huxleyi* (Prymnesiophyaceae) to ultraviolet radiation. J Phycol 36:296-303.

Caldwell M, Teramura AH, Tevini M, Bornman JF, Björn LO, Kulandaivelu G. 1995. Effects of increased solar ultraviolet radiation on human health. Ambio 24:166-73.

Carefoot TH, Karentz D, Pennings SC, Young CL. 2000. Distribution of mycosporine-like amino acids in the sea hare *Aplysia dactylomela*: effect of diet on amounts and types sequestered over time in tissues and spawn. Comparative Biochemistry and Physiology (Part C) 126:91-104.

Caretto JI, De Marco SG, Lutz VA. 1989. UV-absorbing pigments in the dinoflagellates *Alexandrium excavatum* and *Prorocentrum micans*. Effects of light intensities. In: Okaichi T, Anderson DM, Nemoto T, eds. Red tides: biology, environmental science, and toxicology; New York: Elsevier. p 333-336

Caretto 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. Toxic Marine Phytoplankton; New York: Elsevier. p. 275-9.

Caretto 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 12:909-21.

Carroll A, Shick J. 1996. Dietary accumulation of UV-absorbing mycosporine-like amino acids (MAAs) by the green sea urchin (*Strongylocentrotus droebachiensis*). Mar Biol 124:561-9.

Cockell CS, Knowland J. 1999. Ultraviolet radiation screening compounds. Biol Rev 74:311-45.

Cockell CS. 2001. A photobiological history of Earth. In: Cockell CS, Blaustein AR, eds. Ecosystems, evolution, and ultraviolet radiation. New York: Springer. p 1-35.

Collén J, Davison IR. 1999. Reactive oxygen production and damage in intertidal *Fucus* spp. (Phaeophyceae). J Phycol 35:54-61.

Conde FR, Churio MS, Previtali CM. 2000. The photoprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. J Photochem Photobiol B 56:139-44.

Diffey B. 2002. Sources and measurement of ultraviolet radiation. Methods 28:4-13.

Dillon J, Tatsumi C, Tandingan P, Castenholz R. 2002. Effect of environmental factors on the synthesis of scytonemin, a UV-screening pigment, in a cyanobacterium (*Chroococcidiopsis* sp.). Arch Microbiol 177:322-31.

Dionisio-Sese M, Ishikura M, Maruyama T, Miyachi S. 1997. UV-absorbing substances in the tunic of a colonial ascidian protect its symbiont, *Prochloron* sp., from damage by UV-B radiation. Mar Biol 128:455-61.

Drew EA, Hastings RM. 1992. A year-round ecophysiological study of *Himantothallus grandifolius* (Desmarestiales, Phaeophyta) at Signy Island, Antarctica. Phycologia 31:262-77.

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 of three species of *Laminaria* (Phaeophyta). Mar Biol 126:183-91.

Drollet J, Teai T, Faucon M, Martin P. 1997. Field study of compensatory changes in UV-absorbing compounds in the mucus of the solitary coral *Fungia repanda* (Scleractinia: Fungiidae) in relation to solar UV radiation, sea-water temperature, and other coincident physico-chemical parameters. Mar Freshwater Res 48:329-33.

Dunlap WC, Chalker BE. 1986. Identification and quantitation of near-UV absorbing compounds (S-320) in a hermatypic scleractinian. Coral Reefs 5:155-9.

Dunlap WC, Chalker BE, Oliver JK. 1986. Bathymetric adaptions of the reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J Exp Mar Biol Ecol 104:239-48.

Dunlap WC, Yamamoto Y. 1995. Small-molecule antioxidants in marine organisms: antioxidant activity of mycosporine-glycine. Comp Biochem Physiol 112**B**:105-14.

Dunlap WC, Shick MJ. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids in coral reef organisms: a biochemical and environmental perspectice. J Phycol 34:418-30.

Eggert A, Wiencke C. 2000. Adaptation and acclimation of growth and phototsynthesis of five Antarctic red algae to low temperatures. Polar Biol 23:609-18.

Ehling-Schulz M, Bilger W, Scherer S. 1997. UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc*

commune. J Bacteriol 179:1940-5.

Farman J, Gardiner B, Shanklin J. 1985. Large losses of total ozone in Antarctica reveal seasonal ClO_x/NO_x interaction. Nature 315:207-10.

Favre-Bonvin J, Bernillon J, Salin N, Arpin N. 1987. Biosynthesis of mycosporines: mycosporine glutaminol in *Trichothecium roseum*. Phytochemistry 26:2509-14.

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 35:682-93.

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 37:257-70.

Frederick JE, Snell HE. 1988. Ultraviolet radiation levels during the Antarctic spring. Science 241:438-40.

García-Pichel F, Castenholz RW. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. J Phycol 27:395-409.

García-Pichel F, Nelson DS, Castenholz RW. 1992. Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis* sp. Photochem.Photobiol.B. 56:17-23.

García-Pichel F, Castenholz RW. 1993. Occurrence of UV-absorbing, mycosporine-like compounds among cyanobacterial isolates and an estimate of their screening capacity. Appl Environ Microbiol 59:163-9.

García-Pichel F, Wingard CE, Castenholz RW. 1993. Evidence regarding the UV sunscreen role of a mycosporine-like compound in the cyanobacteria *Gloeocapsa* sp. Appl Environ Microbiol 59:170-6.

García-Pichel F. 1994. A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. Limnol Oceanogr 39:1704-17.

Gathen von der P, Rex M, Harris NR, Luscic D, Knudsen BM, Braathen GO, De Backer H, Fabian R, Fast H, Gil M Kyrö E, Mikkelsen S, Bummukainen M, Stähelin J, Varotsos C. 1995. Observational evidence for chemical ozone depletion over the Arctic in winter 1991-92. Nature 375:131-4.

George A, Murray A, Montiel P. 2001. Tolerance of Antarctic cyanobacterial mats to enhanced UV radiation. FEMS Microbiology Ecology 37:91-101.

Gleason DF. 1993. Differential effects of ultraviolet radiation on green and brown morphs of the Carribean coral *Porites asteroides*. Limnol Oceanogr 38:1452-63.

Gleason D. 2001. Ultraviolet radiation and coral communities. In: Cockell C, Blaustein A, eds. Ecosystems, evolution, and ultraviolet radiation. New York: Springer. p 118-49.

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 148:281-93.

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 47:46-57.

Gómez I, Figueroa FL, Sousa-Pinto I, Viñegla B, Pérez-Rodríguez E, Maestre C, Coelho S, Felga

A, Pereira R. 2001. Effects of UV radiation and temperature on photosynthesis as measured by PAM flourescence in the red alga *Gelidium pulchellum* (Turner) Kützing. Botanica Marina 44:9-16.

Gröniger A, Hallier C, Häder D-P. 1999. Influence of UV radiation and visible light on *Porphyra umbilicalis*: photoinhibition and MAA concentration. J Appl Phycol 11:437-45.

Gröniger A, Häder D-P. 2002. Induction of the synthesis of an UV-absorbing substance in the green alga *Prasiola stipitata* . J Photochem Photobiol 66:54-9.

Hanelt D, Jaramillo JM, Nultsch W, Senger S, Westermeier R. 1994. Photoinhibition as a regulative mechanism of photosynthesis in marine algae of Antarctica. Ser. Cient. INACH; Serie Científica Instituto Antártico Chileno 44:67-77.

Hanelt D, Melchersmann B, Wiencke C, Nultsch W. 1997 a. Effects of high light stress on photosynthesis of polar macroalgae in relation to depth distribution. Mar Ecol Prog Ser 149:255-66.

Hanelt D, Wiencke C, Nultsch W. 1997 b. Influence of UV radiation on photosynthesis of Arctic macroalgae in the field. J Photochem Photobiol 38:40-7.

Hanelt D. 1998. Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar Biol 131:361-69

Hanelt D, Tüg H, Bischof K, Groß C, Lippert H, Sawall T, Wiencke C. 2001. Light regime in an Arctic fjord: a study related to stratospheric ozone depletion as a basis for determination of UV effects on algal growth. Mar Biol 138:649-58.

Hanelt D, Nultsch W. 2003. Photoinhibition in seaweeds. In: Heldmaier G, Werner D. eds. Environmental signal, processing and adaptation. New York:Springer. p 142-67 (in press).

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

Heese B. 1996. Investigations of contributions by chemical and dynamical processes to the variability of stratospheric ozone above the Arctic. Reports on Polar Research 196:1-91.

Helbling EW, Chalker BE, Dunlap WC, Holm-Hansen O, Villafañe VE. 1996. Photoacclimation of Antarctic marine diatoms to solar ultraviolet radiation. J Exp Mar Biol Ecol 204:85-101.

Helbling W, Buma A, Boer de K, Villafañe V. 2001. In situ impact of solar ultraviolet radiation on photosynthesis and DNA in temperate marine phytoplankton. Mar Ecol Prog Ser 211:43-9.

Hernando M, Carreto JI, Carignan MO, Ferreyra GA, Groß C. 2002. Effects of solar radiation on growth and mycosporine-like amino acids content in *Thalassiosira* 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.

Hop H, Pearson T, Hegseth Nost E, Kovacs KM, Wiencke C, Kwasniewski S, Eiane K, Mehlum F, Gulliksen B, Wlodarska-Kowalczuk M, Wiktor J, Pltermann M, di Prisco G, Papucci C, Gerland S. 2002. The marin ecosystem of Kongsfjorden, Svalbard. Polar Research 21:167-208.

Iken K. 1996. Trophic relations between macroalgae and herbivores in Potter Cove (King George Island, Antarctica). Reports on Polar Research 201:1-206.

Irvine L. 1983. Seaweeds of the British Isles Volume Vol. 1. London: British Museum. 113 pp.

Ishikura M, Kato C, Maruyama T. 1997. UV-absorbing substances in zooxanthellate and azooxanthellate clams. Mar Biol 128:649-55.

Ito S, Hirata Y. 1977. Isolation and structure of a mycosporine from the zoanthid *Palythoa tuberculosa*. Tetrahedron Letters 28:2429-4230.

Jacob A, Kirst G-O, Wiencke C, Lehmann H. 1991. Physiological responses of the Antarctic green alga *Prasiola crispa* ssp. *antarctica* to salinity stress. J Plant Physiol 139:57-62.

Jeffrey SW, MacTavish HS, Dunlap WC, Vesk M, Groenwoud K. 1999. Occurrence of UVA- and UVB-absorbing compounds in 152 species (206 strains) of marine microalgae. Mar Ecol Prog Ser 189:35-51.

Jensen H, Pedersen L, Burmeister A, Hansen B. 1999. Pelagic primary production during summer along 65 to 72°N off West Greenland. Polar Biol 21:269-78.

Jokiel PL, Lesser MP, Ondrusek ME. 1997. UV-absorbing compounds in the coral *Pocillopora damicornis*: interactive effects of UV radiation, photosynthetically active radiation, and water flow. Limnol Oceanogr 42:1468-73.

Kain J, Norton T. 1990. Marine ecology. In Cole K, Sheath R, eds. Biology of the red algae. Cambridge: Cambridge University. p 377-422.

Karentz D, Lutze LH. 1990. Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. Limnol Oceanogr 35:549-61.

Karentz D, McEuen FS, Land MC, Dunlap WC. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108:157-66.

Karentz D, Bosch I, Dunlap WC. 1992. Distribution of UV-absorbing compounds in the Antarctic limpet *Nacella concinna*. Antarctic J US 27:121-2.

Karentz D, Dunlap W, Bosch I. 1997. Temporal and spatial occurrence of UV-absorbing mycosporine-like amino acids in tissues of the Antarctic sea urchin *Sterechinus neumayeri* during springtime ozone-depletion. Mar Biol 129:343-53.

Karenzt D. 2001. Chemical defenses of marine organisms against solar radiation exposure: UV-absorbing mycosporine-like amino acids and scytonemin. In: McClintock JB, Baker B. eds. Marine chemical ecology. London:CRC. p 481-520

Karsten U, García-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, Franklin LA, Lüning K, Wiencke C. 1998 a. 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, 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 41:443-53.

Karsten U, Sawall T, Wiencke C. 1998 c. A survey of the distribution of UV-absorbing substances in tropical macroalgae. Phycol Res 46:271-9.

Karsten U, Maier J, García-Pichel F. 1998 d. Seasonality in UV-absorbing compounds of

cyanobacterial mat communities from an intertidal mangrove flat. Aquatic Microbial Ecology 16:37-44.

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.

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.

Karsten U, West J. 2000. Living in the intertidal zone - seasonal effects on heterosides and sun-screen compounds in the red alga *Bangia atropurpurea* (Bangiales). J Exp Mar Biol Ecol 254:221-34.

Karsten U, Sawall T, West J, Wiencke C. 2000. Ultraviolet sunscreen compounds in epiphytic red algae from mangroves. Hydrobiologia 432:159-171.

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

Karsten U. 2002. Effects of salinity and ultraviolet radiation on the concentration of mycosporine-like amino acids in various isolates of the benthic cyanobacterium *Microcoleus chthonoplastes*. Phycol Res 50:129-34.

Kirk JT. 1994. Optics of UV-B radiation in natural waters. Stuttgart: Schweizerbart'sche. 509 pp.

Kirst G-O, Wiencke C. 1995. Ecophysiology of polar algae. J Phycol 31:181-99.

Klisch M, Häder D-P. 2002. Wavelength dependence of mycosporine-like amino acid synthesis in *Gyrodinium dorsum*. J Photochem Photobiol 66:60-6.

Klöser H, Ferreyra G, Schloss I, Mercuri G, Laturnus F, Curtosi A. 1993. Seasonal variation of algal growth conditions in sheltered Antarctic bays: the example of Potter Cove (King George Island, South Shetlands). J Mar Sys 4:289-301.

Klöser H, Mercuri G, Laturnus F, Quartino ML, Wiencke C. 1994 a. On the competitive balance of macroalgae at Potter Cove (King George Island, South Shetlands). Polar Biol 14:11-6.

Klöser H, Ferreyra G, Schloss I, Mercuri G, Laturnus F, Curtosi A. 1994 b. Hydrography of Potter Cove, a small fjord-like inlet on King George Island (South Shetlands). Estuarine, Coastal and Shelf Science 38:523-37.

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. Rhodophtya. In: Clayton MN, King RJ. eds. Biology of marine plants. Melbourne: Longman. 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.

Kulunscics Z, Perdiz D, Brulay E, Muel B, Sage E. 1999. Wavelength dependence of ultraviolet-induced DNA damage distribution: involvement of direct or indirect mechanisms and possible artefacts. J Photochem Photobiol 49:71-80.

Langer J. 1999. Measurements of Arctic stratospheric ozone: Comparison of ozone-measurements at Ny-Ålesund, Spitsbergen, in 1997 and 1998. Reports on Polar Research

322:1-222.

Lao K, Glazer AN. 1996. Ultraviolet-B photodestruction of a light-harvesting complex. Proc Nat Acad Sci USA 93:5258-63.

Larkum AW, Wood WF. 1993. The effect of UV-B radiation on photosynthesis and respiration of phytoplankton, benthic macroalgae and seagrass. Photosynthesis Research 36:17-23.

Laurion I, Lami A, Sommaruga R. 2002. Distribution of mycosporine-like amino acids and photoprotective carotenoids among freshwater phytoplankton assemblages. Aquatic Microbial Ecology 26:283-94.

Litchman E, Neale P, Banaszak A. 2002. Increased sensitivity to ultraviolet radiation in nitrogen-limited dinoflagellates: photoprotection and repair. Linmol Oceanogr 47:86-94.

Longstreth J, de Gruijl F, Kripke M, Takizawa Y, van de Leun J. 1995. Effects of increased solar ultraviolet radiation on human health. Ambio 24:153-65.

Lubin D, Frederick JE. 1989. The ultraviolet monitoring program at Palmer Station, spring 1988. Antarctic J US 24:172-4.

Lubin D, Jensen EH, Gies HP. 1998. Global surface ultraviolet radiation climatology from TOMS and ERBE data. J Geophys Res 103:26061-91.

Lüning K. 1990. Seaweeds: their envionment, biogeography, and ecophysiology. New York: Wiley. 527 pp.

Madronich S, McKenzie RL, Caldwell MM, Björn LO. 1995. Changes in ultraviolet radiation reaching the earth's surface. Ambio 24:143-52.

Makarov MV, Voskoboinikov GM. 2001. The influence of ultraviolet-B radiation on spore release and growth of the kelp *Laminaria saccharina*. Botanica Marina 44:89-94.

Marchant HJ, Davidson AT, Kelly GJ. 1991. UV-B protecting compounds in the marine alga *Phaeocystis pouchetii* from Antarctica. Mar Biol 109:391-5.

Mason DS, Schafer F, Shick MJ, Dunlap WC. 1998. Ultraviolet radiation-absorbing mycosporine-like amino acids (MAAs) are acquired from their diet by medaka fish (*Oryzias latipes*) but not by SKH-1 hairless mice. Comparative Biochemistry and Physiology (Part A) 120:587-98.

McClintock JB, Karentz D. 1997. Mycosporine-like amino acids in 38 species of subtidal marine organisms from McMurdo Sound, Antarctica. Antarctic Science 9:392-8.

Menzel D, Kazlauskas R, Reichelt J. 1983. Coumarins in the siphonalean green algal family *Dasycladaceae* Kützing (Chlorophyceae). Botanica Marina 16:23-9.

Mims FM, Frederick JE. 1994. Cumulus clouds and UV-B. Nature 371:291.

Mitchell D, Karentz D. 1993. The induction and repair of DNA photodamage in the environment. In: Young A, Björn L, Moan J, Nultsch W, eds. Environmental UV photobiology. New York: Plenum. p 345-77.

Mobley C. 1994. Light and water. London: Academic. 592 pp.

Moisan TA, Mitchell BG. 2001. UV absorption by mycosporine-like amino acids in *Phaeocystis antarctica* Karsten induced by photosynthetically available radiation. Mar Biol 138:217-27.

Molina X, Montecino V. 1996. Acclimation to UV irradiance in *Gracilaria chilensis* Bird, McLachlan & Oliveira (Gigartinales, Rhodophyta). Hydrobiologia 326/327:415-20.

Neale PJ, Cullen JJ, Davis RF. 1998. Inhibition of marine photosynthesis by ultraviolet radiation: variable sensitivity of phytoplankton in the Weddell-Scotia Confluence during the austral spring. Limnol Oceanogr 43:433-48.

Newman SJ, Dunlap WC, Nicol S, Ritz D. 2000. Antarctic krill (*Euphausia superba*) acquire a UV-absorbing mycosporine-like amino acid from dietary algae. J Exp Mar Biol Ecol 255:93-110.

Norton T, Melkonian M, Andersen R. 1996. Algal biodiversity. Phycologia 35:308-26.

Oren A. 1997. Mycosporine-like amino acids as osmotic solutes in a community of halophilic cyanobacteria. Geomicrobiology Journal 14:231-40.

Pavia H, Cevin G, Lindgren A, Åberg P. 1997. Effects of UV-B radiation and stimulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. Mar Ecol Prog Ser 157:139-46.

Pérez-Rodríguez E, Gómez I, Karsten U, Figueroa F. 1998. Effects of UV radiation on photosynthesis and excretion of UV-absorbing compounds of *Dasycladus vermicularis* (Dasycladales, Chlorophyta) from southern Spain. Phycologia 37:379-87.

Petridou S, Foster K, Kindle K. 1997. Light induces accumulation of isocitrate lyase mRNA in a carotenoid-deficient mutant of *Chlamydomonas reinhardtii*. Plant Molecular Biology 33:381-92.

Poll van de WH, Eggert A, Buma AG, Breeman AM. 2001. Effects of UV-B induced DNA damage and photoinhibition on growth of six temperate marine red macrophytes: distinct habitat related differences in ultraviolet-B tolerance. J Phycol 37:30-7.

Poll van de WH, Eggert A, Buma AG, Breeman AM. 2002. Temperature dependence of UV radiation effects in Arctic and temperate isolates of three red macrophytes. Eur J Phycol 37:59-68.

Portwich A, García-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.

Portwich A, García-Pichel F. 2000. A novel prokaryotic UVB photoreceptor in the cyanobacterium *Chlorogloeopsis* PCC 6912. Photochem Photobiol 74:493-8.

Post A, Larkum AW. 1993. UV-absorbing pigments, photosynthesis and UV exposure in Antarctica: comparison of terrestrial and marine algae. Aquatic Botany 45:231-43.

Quartino M, Klöser H, Zaixso A, Zaixso H. 1998. Communities of benthic marine algae at a sheltered site in Potter Cove, King George Island, South Shetlands, Antarctica. Reports on Polar Research 299:74-81.

Quesada A, Vincent WF. 1997. Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. Eur J Phycol 32:335-42.

Reed R. 1990. Solute accumulation and osmotic adjustment. In Cole K, Sheath R, eds. Biology of the red algae. Cambridge: Cambridge. p 147-70.

Rex M, Harris N, von der Gathen P, Lehmann R, Braathen G, Reimer E, Beck A, Chipperfield M, Alfier R, Allaart M, O´Conner F, Dier H, Derokhov V, Fast J, Gil. M, **Kyrö** E, Litynska Z Mikkelsen I, Molyneux M, Nakane H, Notholt J, Rummukainen M, Viatte P, Wenger I. 1997. Prolonged stratospheric ozone loss in the 1995-96 Arctic winter. Nature 389:835-8.

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

Ries G, Heller W, Puchta H, Sandermann H, Seidlitz HK, Hohn B. 2000. Elevated UV-B radiation reduces genome stabiltiy in plants. Nature 406:98-101.

Roese M, Drabble M. 1998. Wind-driven circulation in Potter Cove. Reports on Polar Research 299:40-6.

Ross JC, Vincent WF. 1998. Temperature dependence of UV radiation effects on Antarctic cyanobacteria. J Phycol 34:118-25.

Roy CR, Gies HP, Elliott G. 1990. Ozone depletion. Nature 347:235-6.

Roy S. 2000. Strategies for the minimisation 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.

Rozema J, van de Staaij J, Björn L, Caldwell M. 1997. UV-B as an environmental factor in plant life: stress and regulation. Tree 12:22-8.

Rozema J, Björn LO, Bornmann JF, Gabersick A, Häder D-P, Trost T, Germ M, Klisch M, Gröniger A, Sinha RP, Lebert M, He Y-Y, Buffoni-Hall R, de Bakker NV, van de Staaij J, Meijkamp BB. 2002. The role of UV-B radiation in aquatic and terrestrial ecosystems - an experimental and functional analysis of the evolution of UV-absorbing compounds. J Photochem Photobiol 66:2-12.

Sagert S, Schubert H. 2000. Acclimation of *Palmaria palmata* (Rhodophyta) to light intensity: comparison between artificial and natural light fields. J Phycol 36:1119-28.

Salawitch R, McElroy M, Yatteau J, Wosfy S, Schoeberl M, Lait L, Newman P, Chan K, Loewenstein M, Podolske J, Strahan S, Proffitt M. 1993. Loss of ozone in the Arctic vortex for the winter of 1989. Geoph Res Lett 17:561-4.

Schloss I, Ferreyra G, Klöser H. 1998. Seasonal variation of the conditions for phytoplankton growth in Potter Cove. Reports on Polar Research 299:59-66.

Schoenwaelder ME. 2002. The occurrence and cellular significance of physodes in brown algae. Phycologia 41:125-39.

Schöne T, Pohl M, Zakrajsek A, Schenke H. 1998. Tide gauge measurements - a contribution for the long term monitoring of the sea level. Reports on Polar Research 299:12-4.

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

Shick M, Lesser M, Stochaj W. 1991. Ultraviolet radiation and photooxidative stress in zooxanthellate anthozoa: the sea anemone *Phyllodiscus semoni* and the octocoral *Clavularia* sp. Symbiosis 10:145-73.

Shick JM, Lesser MP, Dunlap WC, Stochaj WR, Chalker BE, Wu Won J. 1995. Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral *Acropora microthalma*. Mar Biol 122:41-51.

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 population of symbiotic dinoflagellates. Limnol Oceanogr 44:1667-82.

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 radicals in chemistry, biology and medicine. London: OICA Int.. p 215-28.

Shick J, Dunlap W. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation and UV-protective functions in aquatic organisms. Annual Reviews Physiology 64:223-62.

Sinha RP, Klisch M, Helbling EW, Häder D-P. 2001. Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation. J Photochem Photobiol 60:129-35.

Sivalingam PM, Ikawa T, Nisizawa K. 1974. Possible physiological roles of a substance showing characteristic UV-absorbing patterns in some marine algae. Plant and Cell Physiol 15:583-6.

Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D, Ondrusek, M, Wan Z, Waters, KJ. 1992. Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952-9.

Smith S. 1981. Marine macrophytes as global carbon sink. Science 211:838-40.

Solomon S. 1990. Progress towards a quantitative understandig of antarctic ozone depletion. Nature 347:347-54.

Sommaruga R, García-Pichel F. 1999. UV-absorbing mycosporine-like compounds in planktonic and benthic organisms from a high-mountain lake. Arch Hydrobiol 144:259-69.

Springer A, McRoy C. The paradaox of pelagic food webs in the northern Bering Sea - III. Patterns of primary production. Continental Shelf Research 13:575-99.

Stonehouse B. 1989. Polar Ecology. Glasgow: Blackie. 222 pp.

Strutton P, Griffiths B, Waters R, Wright S, Bindoff N. 2000. Primary productivity off the coast of East Antarctica (80-150°E): January to March 1996. Deep Sea Research II 47:2327-62.

Sundbäck K, Odmark S, Wulff A, Nilsson C, Wängberg S-A. 1997. Effects of enhanced UVB radiation on a marine benthic diatom mat. Mar Biol 128:171-9.

Svendsen H, Beszczynska-Møller A, Hagen J, Lefauconnier B, Tverberg V, Gerland S, Ørbæk J, Bischof K, Papucci C, Zajaczkowski M, Azzolini R, Bruland O, Wiencke C, Winther J-G, Dallmann W. 2002. The physical environment of Kongsfjorden-Krossfjorden, an Arctic fjord system in Svalbard. Polar Research 21:133-66.

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: 419-20.

Takano S, Uemura D, Hirata Y. 1978 a. Isolation and structure of a new amino acid, palythine, from the zoanthid *Palythoa tuberculosa*. Tetrahedron Letters 26:2299-300.

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 49:4909-12.

Tartarotti B, Laurion I, Sommaruga R. 2001. Large variability in the concentration of mycosporine-like amino acids among zooplankton from lakes located across an altitude gradient. Limnol Oceanogr 46:1546-52.

Taylor R, Fletcher R, Raven J. 2001. Preliminary studies on the growth of selected 'green tide' algae in laboratory culture: effects of irradiance, temperature, salinity and nutrients on growth rate. Botanica Marina 44:327-36.

Teai T, Drollet JH, Bianchini J-P, Cambon A, Martin PM. 1998. Occurrence of ultraviolet

radiation-absorbing mycosporine-like amino acids in coral mucus and whole corals of French Polynesia. Mar Freshwater Res 49:127-32.

Thiel S, Döhring T, Köfferlein M, Kosak A, Martin P, Seidlitz HK. 1996. A phytotron for plant stress research: How far can artificial lighting compare to natural sunlight? J Plant Physiol 148:456-63.

Thomas DN, Wiencke C. 1991. Photosynthesis, dark respiration and light independent carbon fixation of endemic Antarctic macroalgae. Polar Biol 11:329-37.

Tsujino I, Yabe K, Sekikawa I, Hamanaka N. 1978. Isolation and structure of a mycosporine from the red alga *Chondrus yendoi*. Tetrahedron Letters 16:1401-2.

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.

Varela L. 1998. Hydrology of Matias and Potter creeks. Reports on Polar Research 299:33-9.

Vernet M, Neori A, Haxo FT. 1989. Spectral properties and photosynthetic action in the red-tide populations of *Prorocentrum micans* and *Gonyaulax polyedra*103:365-71.

Vernet M, Whitehead K. 1996. Release of ultraviolet-absorbing compounds by the red-tide dinoflagellate *Lingolidinium polyedra*. Mar Biol 127:35-44.

Villafañe VE, Holm-Hansen O, Chalker BE. 1995. Acclimatization of Antarctic natural phytoplankton assemblages when exposed to solar ultraviolet radiation. J Plankton Res 17:2295-306.

Vincent WF, Neale PJ. 2000. Mechanisms of UV damage to aquatic organisms. In: de Mora S, Demers S, Vernet M, eds. The effects of UV radiation in the marine environment. Cambridge: Cambridge University. p 149-76.

Wängberg S-A, Selmer JS, Eklund NG, Gustavson K. 1996. UV-B effects on nordic marine ecosystem- a literature review. Copenhagen: Nordic Council of Ministers. 45 pp.

Wängberg S-A, Persson A, Karlson B. 1997. Effects of UV-B radiation on synthesis of mycosporine-like amino acid and growth in *Heterocapsa triquetra* (Dinophyceae). J Photochem Photobiol 37:141-6.

Weaver L, Herrmann K. 1997. Dynamics of the shikimate pathway plants. Trends in Plant Science 2:346-51.

Weykam G, Gómez I, Wiencke C, Iken K, Klöser H. 1996. Photosynthetic characteristics and C:N ratios of macroalgae from King George Island (Antarctica). J Exp Mar Biol Ecol 204:1-22.

Whitehead K, Vernet M. 2000. Influence of mycosporine-like amino acids (MAAs) in UV absorption by particulate and dissolved organic matter in La Jolla Bay. Limnol Oceanogr 45:1788-96.

Whitehead K, Karentz D, Hedges J. I. 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 139:1013-9.

Whitehead R, de Mora S, Demers S. 2000. Enhanced UV radiation - a new problem for the marine environment. In: de Mora S, Demers S, Vernet M, eds. The effects of UV radiation in the marine environment. Cambridge: Cambridge University. p 1-34.

Wiencke C, tom Dieck I. 1989. Temperature requirements for growth and temperature tolerance of macroalgae endemic to the Antarctic region. Mar Ecol Prog Ser 54:189-97.

Wiencke C. 1990. Seasonality of red and green macroalgae from Antarctica- a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601-7.

Wiencke C, Fischer G. 1990. Growth and stable carbon isotope composition of cold- water macroalgae in relation to light and temperature. Mar Ecol Prog Ser 65:283-92.

Wiencke C, tom Dieck I. 1990. Temperature requirements for growth and survival of macroalgae from Antarctica and southern Chile. Mar Ecol Prog Ser 59:157-70.

Wiencke C, Bartsch I, Bischoff B, Peters AF, Breeman AM. 1994. Temperature requirements and biogeography of Antarctic, Arctic and amphiequatorial seaweeds. Botanica Mar. 37:247-59.

Wiencke C, Gómez I, Pakker H, Flores-Moya A, Altamirano M, Hanelt D, Bischof K, Figueroa F. 2000. Impact of UV radiation on viability, phototsynthetic characteristics and DNA of brown algal zoospores: Implications for depth zonation. Mar Ecol Prog Ser 197:217-29.

Wiencke C, Clayton M. 2002. Antarctic Seaweeds. In: Wägele J, Sieg J. eds. Synopses of the Antarctic benthos. Volume 9. Königstein:Koeltz Scientific Books. 239 pp.

Winkler J, Schulz F, Kappen J. 1998. Seasonal variation of abiotic factors in terrestrial habitats. Reports on Polar Research 299:28-32.

WMO 1998: World Meteorological Organization. Scientifc assessment of ozone depletion: 1998. World Meteorological Organization Global Ozone Research and Monitoring Project 44.

WMO 2002: World Meteorological Organization. World Climate News 21:1-12.

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

Wulff A, Nilsson C, Sundbäck K, Wängberg S-A, Odmark S. 1999. UV radiation effects on microbenthos - a four month field experiment. Aquatic Microbial Ecology 19:269-78.

Wunderle S, Saurer H, Goßmann H. 1998. Meteorological conditions and snow cover dynamics on the Potter Peninsula, King George Island, Antarctica. Reports on Polar Research 299:15-27.

Xiong F, Kopecky J, Nedbal L. 1999. The occurrence of UV-B absorbing mycosporine-like amino acids in freshwater and terrstrial microalgae (Chloropyta). Aquatic Botany 63:37-49.

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.

Yakovleva IM, Titlyanov EA. 2001. Effect of high visible and UV irradiance on subtidal *Chondrus crispus*: stress, photoinhibition and protective mechanism. Aquatic Botany 71:47-61.

Zudaire L, Roy S. 2001. Photoprotection and long-term acclimation to UV radiation in the marine diatom *Thalassiosira weissflogii*. J Photchem and Photobiol 62:26-34.

ACKNOWLEDGEMENTS

I want to thank my supervisors Prof. Dr. C. Wiencke and Prof. Dr. U. Karsten for their help, valuable discussion, inspiration and corrections during the years, and Prof. Dr. G.-O. Kirst for taking part in the examination of this thesis.

I also thank all the members of the working group "Marine Macroalgae" as well as of the project group "Solar UV radiation" for being supportive colleagues.

I heartly thank Dr. M. Schoenwaelder, who shared the office with me and as a native English speaker always helped me finding the right words.

Heartly thanks are also directed to Dr. M. Schodlok who was helpful at any time.

A big thank you goes to everyone who was in any supportive way involved in my working life.

„Berichte zur Polarforschung"
Eine Titelübersicht der Hefte 1 bis 376 (1981 - 2000) erschien zuletzt im Heft 413 der nachfolgenden Reihe „Berichte zur Polar- und Meeresforschung". Ein Verzeichnis aller Hefte beider Reihen sowie eine Zusammenstellung der Abstracts in englischer Sprache finden Sie im Internet unter der Adresse:
http://www.awi-bremerhaven.de/Resources/publications.html

Ab dem Heft-Nr. 377 erscheint die Reihe unter dem Namen:
„Berichte zur Polar- und Meeresforschung"

Heft-Nr. 377/2000 – „Rekrutierungsmuster ausgewählter Wattfauna nach unterschiedlich strengen Wintern" von Matthias Strasser

Heft-Nr. 378/2001 – „Der Transport von Wärme, Wasser und Salz in den Arktischen Ozean", von Boris Cisewski

Heft-Nr. 379/2001 – „Analyse hydrographischer Schnitte mit Satellitenaltimetrie", von Martin Losch

Heft-Nr. 380/2001 – „Die Expeditionen ANTARKTIS XI/1-2 des Forschungsschiffes POLARSTERN 1998/1999", herausgegeben von Eberhard Fahrbach und Saad El Naggar.

Heft-Nr. 381/2001 – „UV-Schutz- und Reparaturmechanismen bei antarktischen Diatomeen und *Phaeocystis antarctica*", von Lieselotte Riegger.

Heft-Nr. 382/2001 – „Age determination in polar Crustacea using the autofluorescent pigment lipofuscin", by Bodil Bluhm.

Heft-Nr. 383/2001 – „Zeitliche und räumliche Verteilung, Habitatspräferenzen und Populationsdynamik benthischer Copepoda Harpacticoida in der Potter Cove (King George Island, Antarktis)", von Gritta Veit-Köhler.

Heft-Nr. 384/2001 – „Beiträge aus geophysikalischen Messungen in Dronning Maud Land, Antarktis, zur Auffindung eines optimalen Bohrpunktes für eine Eiskerntiefbohrung", von Daniel Steinhage.

Heft-Nr. 385/2001 – „Actinium-227 als Tracer für Advektion und Mischung in der Tiefsee", von Walter Geibert.

Heft-Nr. 386/2001 – „Messung von optischen Eigenschaften troposphärischer Aerosole in der Arktis" von Rolf Schumacher.

Heft-Nr. 387/2001 – „Bestimmung des Ozonabbaus in der arktischen und subarktischen Stratosphäre", von Astrid Schulz.

Heft-Nr. 388/2001 – „Russian-German Cooperation SYSTEM LAPTEV SEA 2000: The Expedition LENA 2000", edited by Volker Rachold and Mikhail N. Grigoriev.

Heft-Nr. 389/2001 – „The Expeditions ARKTIS XVI/1 and ARKTIS XVI/2 of the Research Vessel 'Polarstern' in 2000", edited by Gunther Krause and Ursula Schauer.

Heft-Nr. 390/2001 – „Late Quaternary climate variations recorded in North Atlantic deep-sea ostracodes", by Claudia Didié.

Heft-Nr. 391/2001 – „The polar and subpolar North Atlantic during the last five glacial-interglacial cycles", by Jan. P. Helmke.

Heft-Nr. 392/2000 – „Geochemische Untersuchungen an hydrothermal beeinflußten Sedimenten der Bransfield Straße (Antarktis)", von Anke Dählmann.

Heft-Nr. 393/2001 – „The German-Russian Project on Siberian River Run-off (SIRRO): Scientific Cruise Report of the Kara-Sea Expedition 'SIRRO 2000' of RV 'Boris Petrov' and first results", edited by Ruediger Stein and Oleg Stepanets.

Heft-Nr. 394/2001 – „Untersuchung der Photooxidantien Wasserstoffperoxid, Methylhydroperoxid und Formaldehyd in der Troposphäre der Antarktis", von Katja Riedel.

Heft-Nr. 395/2001 – „Role of benthic cnidarians in the energy transfer processes in the Southern Ocean marine ecosystem (Antarctica)", by Covadonga Orejas Saco del Valle.

Heft-Nr. 396/2001 – „Biogeochemistry of Dissolved Carbohydrates in the Arctic", by Ralph Engbrodt.

Heft-Nr. 397/2001 – „Seasonality of marine algae and grazers of an Antarctic rocky intertidal, with emphasis on the role of the limpet *Nacilla concinna* Strebel (Gastropoda: Patellidae)", by Dohong Kim.

Heft-Nr. 398/2001 – „Polare Stratosphärenwolken und mesoskalige Dynamik am Polarwirbelrand", von Marion Müller.

Heft-Nr. 399/2001 – „North Atlantic Deep Water and Antarctic Bottom Water: Their Interaction and Influence on Modes of the Global Ocean Circulation", by Holger Brix.

Heft-Nr. 400/2001 – „The Expeditions ANTARKTIS XVIII/1-2 of the Research Vessel 'Polarstern' in 2000" edited by Victor Smetacek, Ulrich Bathmann, Saad El Naggar.

Heft-Nr. 401/2001 – „Variabilität von CH_2O (Formaldehyd) - untersucht mit Hilfe der solaren Absorptionsspektroskopie und Modellen" von Torsten Albrecht.

Heft-Nr. 402/2001 – „The Expedition ANTARKTIS XVII/3 (EASIZ III) of RV 'Polarstern' in 2000", edited by Wolf E. Arntz and Thomas Brey.

Heft-Nr. 403/2001 – „Mikrohabitatansprüche benthischer Foraminiferen in Sedimenten des Südatlantiks", von Stefanie Schumacher.

Heft-Nr. 404/2002 – „Die Expedition ANTARKTIS XVII/2 des Forschungsschiffes 'Polarstern' 2000", herausgegeben von Jörn Thiede und Hans Oerter.

Heft-Nr. 405/2002 – „Feeding Ecology of the Arctic Ice-Amphipod *Gammarus wilkitzkii*. Physiological, Morphological and Ecological Studies", by Carolin E. Arndt.

Heft-Nr. 406/2002 – „Radiolarienfauna im Ochotskischen Meer - eine aktuopaläontologische Charakterisierung der Biozönose und Taphozönose", von Anja Nimmergut.

Heft-Nr. 407/2002 – „The Expedition ANTARKTIS XVIII/5b of the Research Vessel 'Polarstern' in 2001, edited by Ulrich Bathmann.

Heft-Nr. 408/2002 – „Siedlungsmuster und Wechselbeziehungen von Seepocken (Cirripedia) auf Muschelbänken (*Mytilus edulis* L.) im Wattenmeer", von Christian Buschbaum.